

ANTIOXIDANT PROPERTIES OF ROMANIAN GRAPE VARIETIES

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Abstract: This study explores the application of the photochemiluminescence assay in two distinct extracts, hydrophilic and lipophilic, alongside the DPPH method to evaluate the antioxidant potential of three grape varieties (Chasselas, Romanian Muscat, and Muscat Hamburg). The results revealed significant phenolic compound concentrations, ranging from 21.68 to 43.44 mg gallic acid equivalent per 100 g of fresh weight. Flavonoid content was between 4.26 and 8.86 mg rutin equivalent per 100 g of fresh weight, while anthocyanin content varied from 0.16 to 7.02 mg cyanidin-3-glucoside equivalent per 100 g of fresh weight. Among the tested grapes, Muscat Hamburg demonstrated the highest antioxidant activity using DPPH method. In terms of antioxidant capacity, Chasselas exhibited the highest value in the hydrophilic system, while Muscat Hamburg showed the greatest antioxidant capacity in the lipophilic system.

Keywords: *antioxidant capacity, flavonoids, grapes, phenolics, photochemiluminescence*

INTRODUCTION

Grapes (*Vitis* species), belonging to the family Vitaceae, are among the most popular, widely cultivated, and commonly consumed fruits worldwide [1]. According to the FAO [2] statistical database, global grape production reached almost 75 million tons in 2022 (Figure 1).

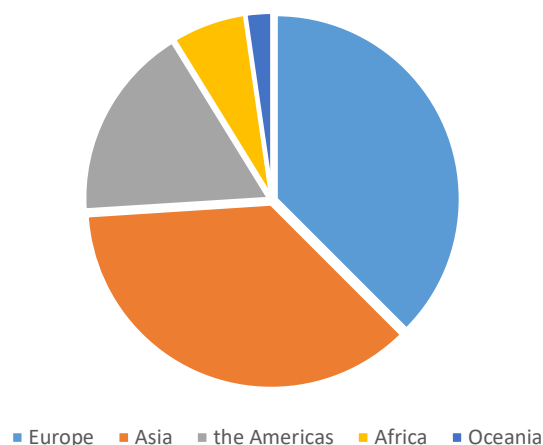


Figure 1. Production of top global grape producers in 2022

The top 10 grape-producing countries are China (12.6 million tons), Italy (12.6 million tons), France (12.6 million tons), Spain (11.25 million tons), the United States (10.24 million tons), Turkey (7.95 million tons), India (6.48 million tons), Chile (4.58 million tons), South Africa (3.95 million tons), and Argentina (3.7 million tons). Romania was ranked 22nd in grape production [2].

Grapes are an excellent source of bioactive compounds, though the concentration of these antioxidants is influenced by various factors. These include grape variety, maturity, post-harvest storage, and environmental conditions such as location, light exposure, temperature, nutrition, water availability, microbial activity, and viticulture practices [4 – 7].

The primary bioactive compounds found in grapes are phenolics, flavonoids, anthocyanins, stilbenes, proanthocyanidins and vitamin E [8]. Most of these compounds may possess antioxidant properties, which are crucial for human health as they may help reduce the oxidation of low-density lipoproteins, thereby lowering the risk of heart disease and contributing to cancer prevention [9].

In the present study, the antioxidant activity of 3 grapes varieties (Chasselas, Romanian Muscat, and Muscat Hamburg) cultivated in Romania were measured, and their total phenolic contents, total flavonoid contents and total anthocyanin content were evaluated. In addition, the antioxidant capacity in hydrophilic and lipophilic system were quantified using Photochem device.

MATERIALS AND METHODS

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), (+)-rutin, gallic acid, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma Chemical Co. (Switzerland). Folin–Ciocalteu’s phenol reagent was purchased from Merck (Germany). The kits for photochemiluminescence in hydrophilic and lipophilic system were from Analytik Jena, Germany. All chemicals used were of analytical grade. Standard solutions were prepared with distilled water.

Fruit material

The selected fruits consisted in three grape varieties: two white grapes (Chasselas and Romanian Muscat) and one dark purple variety, Muscat Hamburg. The samples were cultivated in Romania and were purchased from a local market in Bucharest, in 2021. The edible portions of the fruits (20 - 30 g) were homogenized using a laboratory mixer.

Extraction procedure

For the determination of bioactive compounds, antioxidant activity through DPPH assay, and antioxidant capacity (using photochemiluminescence), 1.0 g of fresh grapes (skin and pulp) was weighed and combined with 30 mL of 50 % aqueous methanol. The mixtures were subjected to vigorous agitation at 2,000 rpm for one hour using a vortex mixer (Heidolph Instruments Multi Reax) to facilitate compound extraction. Subsequently, the extracts were centrifuged at 10,000 rpm for 10 minutes at 4 °C, following the protocol described by Multescu and Susman [10]. The resulting supernatant was stored at -20 °C until further analysis.

Determination of bioactive compounds

Determination of Total Phenolic Content (TPC)

The determination of TPC was performed using the Folin-Ciocalteu method with slight modifications as outlined by Multescu et al. [10]. Briefly, 500 µL of the grape extracts was mixed with 5 mL Folin-Ciocalteu reagent and 500 µL of saturated sodium carbonate solution. The reaction mixtures were incubated in darkness for 20 minutes to facilitate color development. Absorbance was measured at 765 nm using a Specord 210 UV-VIS spectrophotometer (Analytic Jena, Germany). A calibration curve was constructed using gallic acid at concentrations ranging from 10 to 50 µg·mL⁻¹ under identical experimental conditions ($r^2 = 0.999$). The total phenolic content was expressed as mg of gallic acid equivalent per 1 g of fresh weight (mg GAE/ g f.w.).

Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined using the aluminum chloride (AlCl₃) method as described by Multescu et al. [10]. In brief, 0.1 mL of extract was mixed with 0.1 mL 10 % sodium acetate and 0.12 mL 2.5 % AlCl₃ solution. The final volume was adjusted to 1 mL with ethanol 70 %. The mixtures were vortexed and incubated in the

dark for 45 minutes to ensure reaction completion. The absorbance was measured at 510 nm. A standard curve was plotted using different concentrations (10 - 60 $\mu\text{g}\cdot\text{mL}^{-1}$) of rutin ($r^2 = 0.999$). Total flavonoid content was expressed as mg rutin equivalent $\cdot\text{g}^{-1}$ of fresh weight (mg RE/g f.w.).

Determination of Anthocyanins Content (TAC)

The total anthocyanin content was measured using the pH differential method, following the AOAC (2005) procedure [11] with minor changes. Briefly, 2 mL of extract were mixed with 8 mL of a pH 1.0 buffer (potassium chloride, 0.025 M), and another 2 mL was mixed with 8 mL of a pH 4.5 buffer (sodium acetate, 0.4 M). The absorbance of these mixtures was measured at 520 nm and 700 nm using a Specord 210 UV-VIS spectrophotometer (Analytik Jena, Germany). TAC was calculated using the AOAC formula (1), and the results were expressed as mg cyanidin-3-glucoside equivalents C3G per 100 g f.w.

$$\text{cyanidin-3-glucoside (mg/100g f.w.)} = A \cdot M \cdot FD \cdot 103 / \epsilon \cdot l \quad (1)$$

where $A = (A_{520} - A_{700})_{\text{pH}1} - (A_{520} - A_{700})_{\text{pH}4.5}$; $M = 449.2 \text{ g}\cdot\text{mol}^{-1}$ (molecular weight of cyanidin-3-glucoside); FD = dilution factor; ϵ = molar extinction coefficient of cyanidin-3-glucoside, 26900; l = length of the measuring cuvette.

Determination of Antioxidant Activity through DPPH

The DPPH radical scavenging activity was assessed by measuring the reduction of the DPPH radical, following the method of Horszwald and Andlauer [12] with minor modifications. The reaction mixture consisted of 1 mL of the methanolic extract and 6 mL of a DPPH radical solution. The mixture was incubated in the dark for 20 minutes to ensure reaction completion. Subsequently, the absorbance was recorded at 517 nm using a UV-VIS spectrophotometer. Antioxidant activity was quantified using a calibration curve prepared with Trolox at concentrations ranging from 0.0156 to 0.0625 $\mu\text{g}\cdot\text{mL}^{-1}$ ($r^2 = 0.999$). The results were expressed as micromoles of Trolox equivalents per gram of fresh weight ($\mu\text{mol TE}\cdot\text{g}^{-1}$ f.w.).

Photochemiluminescence Assay – hydrophilic system (PCL-ACW)

The reactions were carried out using kits for the determination of antioxidant capacity of water-soluble substances (Analytik Jena, Jena, Germany), mixing 1500 μL of water (reagent 1), 1000 μL of buffer solution (reagent 2), 25 μL of luminol (reagent 3), and 10 μL of extract. Measurement was performed on a Photochem device with PCL soft (Analytik Jena). Vitamin C was used to prepare the calibration curve. The results are expressed as μmol of vitamin C equivalents per g of fresh weight.

Photochemiluminescence Assay – lipophilic system (PCL-ACL)

The reactions were carried out using kits for the determination of antioxidant capacity of lipid-soluble substances (Analytik Jena, Jena, Germany), mixing 2300 μL of methanol (reagent 1), 200 μL of buffer solution (reagent 2), 25 μL of luminol (reagent 3), and 10 μL of extract. Measurement was performed on a Photochem device with PCL soft (Analytik Jena). Trolox was used to prepare the calibration curve. The results are expressed as μmol of TE per g of fresh weight.

Statistical analysis

All methods were applied for sample characterization with a minimum of three replicates. The results are expressed as means $\pm$ standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by Tukey's test to evaluate differences between means (Minitab software, Minitab Inc., Coventry, UK). Differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION**Antioxidant compounds content of *Vitis* species**

In Table 1 the TPC, TFC and TAC of the analyzed grape varieties from the *Vitis* genus are presented. The TPC ranged between 21.68 - 43.44 mg GAE/g f.w. Among the three grape varieties studied, Muscat Hamburg exhibited the highest TPC value of 43.44 mg GAE/g f.w., whereas Romanian Muscat showed lower TPCs, measuring 21.68 mg GAE/g f.w. The concentration of polyphenols was significantly different ($p < 0.05$) among the grape samples.

Table 1. Bioactive content in the analyzed Romanian grape varieties

Sample	TPC [mg GAE·g ⁻¹ f.w.]	TFC [mg RE·g ⁻¹ f.w.]	TAC [mg C3G/100 g f.w.]
Chasselas	39.06 $\pm$ 0.52 ^b	7.96 $\pm$ 0.25 ^a	0.16 $\pm$ 0.04 ^c
Romanian Muscat	21.68 $\pm$ 0.73 ^c	4.26 $\pm$ 0.05 ^c	0.41 $\pm$ 0.01 ^b
Muscat Hamburg	43.44 $\pm$ 0.41 ^a	8.86 $\pm$ 0.02 ^b	7.02 $\pm$ 0.01 ^a

The values are expressed as means $\pm$ standard deviations (n = 3)

Values followed by different letters in the same column are significantly different ($p < 0.05$)

In a prior investigation, Xu *et al.* [13] determined phenolic content in seeds and skins among 18 grape cultivars belonging to seven species and interspecific hybrids. The results ranged between 15.79 - 99.28 mg GAE·g⁻¹ in seeds and 12.11 - 41.21 mg GAE·g⁻¹ in skins. Pantelić *et al.* [14] quantified phenolic compounds in the skins, pulp, and seeds of 13 grape varieties, including 7 red and 6 white grape cultivars, grown in Serbia. The study has underlined that in red grape seeds, the total phenolic content ranged from 38.02 to 102.98 mg GAE·g⁻¹, while in white grape seeds, it varied between 47.55 and 96.89 mg GAE·g⁻¹. For the skins, the TPC ranged from 7.43 to 12.32 mg GAE·g⁻¹ in red grapes and from 0.39 to 3.71 mg GAE·g⁻¹ in white grapes. In the pulp, the values were much lower, ranging from 0.07 to 0.20 mg GAE·g⁻¹ in red grapes and 0.07 to 0.15 mg GAE·g⁻¹ in white grapes. These results underscore the significant variability of phenolic content across different grape cultivars. Phenolic compounds in skins and seeds of five wild grapes and two hybrids native to Japan were between 1.2 - 13.8 mg GAE·g⁻¹, and 3.6 - 54.9 mg GAE·g⁻¹, respectively [15]. The total phenolic contents of the 7 grape samples from Turkey harvested in 2015 were found to be between 44.063 and 199.063 mg GAE/100 g [9]. These measurements were lower than those in our previous study. Total phenolic contents of 50 different grape seeds growing in Turkey were between 1,120.83 mg GAE/100 g f.w. and 3,485.24 mg GAE/100 g f.w. [16]. Thirteen grape samples were analysed in order to determine total phenolic content. The results ranged between 0.44 - 7.94 mg GAE·g⁻¹ [17]. This data

indicates lower levels compared to our earlier analysis. Vieira da Mota *et al.* [18] analysed 5 grape cultivars from Brazil and they reported that TPC ranged from 110.70 to 356.10 mg GAE/100g. Two grape cultivars in Taiwan, Kyoho (*Vitis labruscana*) and American wild bunch (*Vitis aestivalis*) were analysed in term of TPC. The results were between 0.27 - 0.36 mg GAE·g⁻¹ in Kyoho grape cultivars while for American cultivars ranged from 0.30 to 0.466 mg GAE·g⁻¹ [19]. This data indicates lower levels compared to our earlier analysis.

The flavonoid content in romanian grape samples analysed ranged between 4.26 - 8.86 mg RE·g⁻¹ f.w. with higher values observed for Muscat Hamburg followed by Chasselas. The concentration of flavonoids was significantly different ($p < 0.05$) among the grape samples. Other studies reported that TFC varied between 9.11 - 61.70 mg RE·g⁻¹ d.m. in grape seeds and 6.46 - 31.84 mg RE·g⁻¹ d.m. in grape skins [13], 0.70 - 5.50 mg quercetin equivalent QE·g⁻¹ f.w. in seeds and 0.30 - 3.40 mg QE·g⁻¹ f.w. in skins [15]. Özcan *et al.* [9] determined TFC in 7 grape varieties from Turkey. The results ranged from 0.491 to 6.810 mg·g⁻¹ in pulp+skin and between 90.595 - 145.595 mg·g⁻¹ in seeds. In a study conducted by Ghafoor *et al.* [16] the flavonoid content of different grape seeds grown in Turkey were investigated. The results varied from 10,087.13 mg catechin equivalent (CA)/100 g f.w. to 47,013.06 mg CA/100 g f.w.

Sridhar *et al.* [20] determined TFC in Kyoho and American wild bunch grape cultivars of Taiwan. The flavonoid content was between 2.94 - 4.63 mg QE·g⁻¹ for Kyoho grape cultivars, and 2.04 - 2.94 mg QE·g⁻¹ for American grape cultivars, respectively. These findings are lower than those in our study. In a study conducted by Xia *et al.* [21] thirty-one grape cultivars were investigated in terms of flavonoid content. The samples consisted of 7 cultivars of American group, 5 of Euro-American hybrids, 3 of *V. Vinifera* L, 3 of Euro-Asian hybrids, 8 of Oriental group, and 5 of Muscadine grapes. The results showed a TFC ranging between 10.94 - 93.09 mg RE·g⁻¹ d.m. in seeds and 1.58 - 35.13 mg RE·g⁻¹ d.m. in skins. TFC values of 30 grape varieties ranged from 0.176 mg QE·g⁻¹ f.w. to 3.957 mg QE·g⁻¹ f.w. [22]. These measurements are lower compared to our data.

The variations in TPC and TFC were probably due to the results of plant variety and environmental conditions [20].

Total anthocyanins content found out in Chasselas, Romanan Muscat and Muscat Hamburg were between 0.16 - 7.02 mg C3G/100 g f.w. Muscat Hamburg presented the highest concentration in anthocyanins due to its black color, while Chasselas variety showed the lowest TAC (0.16 mg C3G/100 g f.w.). Other studies reported TAC between 26.70 - 190.00 mg malvidin-3-glucoside/100 g [18], 4.09 - 300.37 mg malvidin-3-glucoside/100 g d.m. [19].

Antioxidant activities of grapes cultivars

The values of antioxidant activity using DPPH method of investigated cultivars are presented in Figure 2. The results ranged from 117.06 µmol TE·g⁻¹ f.w. to 257.68 µmol TE·g⁻¹ f.w. Muscat Hamburg grapes showed the highest value of DPPH, while the lowest DPPH activity was found in Romanian Muscat variety.

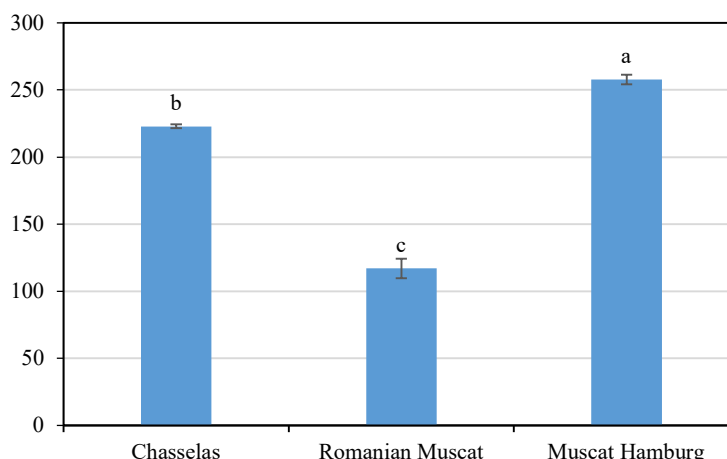


Figure 2. Antioxidant capacity through DPPH of the grape cultivars

Bars with different letters represent a significant difference between samples ($p < 0.05$)

Antioxidant activity was significantly different ($p < 0.05$) among the grape samples. The average value of antioxidant activity using the DPPH method for individual vine varieties was $9432 \mu\text{g}\cdot\text{g}^{-1}$ GAE in 2015; $10,828 \mu\text{g}\cdot\text{g}^{-1}$ GAE in 2016, and $11,624 \mu\text{g}\cdot\text{g}^{-1}$ GAE in 2017 [23]. Pantelic *et al.* [14] determined DPPH activity in skin, pulp, and seeds in 13 grapevine varieties grown in Serbia. The DPPH values ranged between $406.59 - 1039.92 \mu\text{mol TE}\cdot\text{g}^{-1}$ in seeds, $26.25 - 132.59 \mu\text{mol TE}\cdot\text{g}^{-1}$ in skins, and $12.13 - 18.15 \mu\text{mol TE}\cdot\text{g}^{-1}$ in pulp. In a study conducted by Poudel *et al.* [15] five wild grapes and two hybrids native to Japan were analysed in term of antioxidant activity. The results ranged between $15.70 - 113.30 \text{ mmol TE}\cdot\text{g}^{-1}$ in skin, and $16.80 - 92.20 \text{ mmol TE}\cdot\text{g}^{-1}$ in seeds. Costa *et al.* [24] determined antioxidant activity from 24 grape varieties cultivated in two portuguese wine regions. The results obtained by the DPPH method showed that DPPH values were between $1.78 - 299.99 \mu\text{mol TE}\cdot\text{g}^{-1}$ in skin, $0.18 - 3.13 \mu\text{mol TE}\cdot\text{g}^{-1}$ in pulp, and $75.52 - 363.47 \mu\text{mol TE}\cdot\text{g}^{-1}$ in seeds.

A significant correlation was observed between TPC and DPPH, with a Pearson correlation coefficient of $r = 0.9988$. This correlation explained 99.76 % of the variation in antioxidant activity ($r^2 = 0.9976$), indicating a significant association between total phenolic content and antioxidant activity. Similarly, a high correlation was found between TFC and DPPH activity, with a Pearson correlation coefficient of $r = 0.9985$, accounting for 99.70 % of the variation in antioxidant activity ($r^2 = 0.9970$).

In contrast, a moderate correlation was observed between TAC and DPPH activity, with a Pearson correlation coefficient of $r = 0.6681$, explaining 44.6 % of the variation in antioxidant activity ($r^2 = 0.446$). However, this correlation was not statistically significant ($p = 0.534$), suggesting that the relationship may not be robust or reliable.

Table 2. The correlation coefficients between total phenolic (TPC), flavonoid (TFC) and anthocyanins (TAC) content with antioxidant activity (DPPH)

	TPC	TFC	TAC
DPPH	0.9988	0.9985	0.6681

Antioxidant Capacity of Water Soluble Compounds (ACW) and Lipid Soluble Compounds (ACL)

The results of ACW and ACL antioxidant capacity, determined by the PCL method, are presented in Table 3. The ACW values ranged from 33.80 $\mu\text{mol AA}\cdot\text{g}^{-1}$ f.w. to 70.67 $\mu\text{mol AA}\cdot\text{g}^{-1}$ f.w. Chasselas grapes exhibited the highest ACW value, reaching 70.67 $\mu\text{mol AA}/100$ g f.w. Romanian Muscat had the lowest antioxidant capacity in ACW system.

Table 3. Values of antioxidant capacity of the water soluble (ACW) and lipid soluble compounds (ACL)

Sample	ACW [$\mu\text{mol AA}\cdot\text{g}^{-1}$ f.w.]	ACL [$\mu\text{mol TE}\cdot\text{g}^{-1}$ f.w.]
Chasselas	70.67 $\pm$ 1.03 ^a	312.95 $\pm$ 1.62 ^b
Romanian Muscat	33.80 $\pm$ 0.42 ^c	250.96 $\pm$ 0.96 ^c
Muscat Hamburg	53.50 $\pm$ 0.97 ^b	396.80 $\pm$ 1.86 ^a

The values are expressed as means $\pm$ standard deviations (n =3)

Values followed by different letters in the same column are significantly different (p <0.05)

Lipid soluble antioxidant capacity data of the 3 grapes samples were between 250.96 - 396.80 $\mu\text{mol TE}\cdot\text{g}^{-1}$ f.w. (Table 3). Muscat Hamburg showed the highest value of 396.80 $\mu\text{mol TE}/100$ g f.w. followed by Chasselas (312.95 $\mu\text{mol TE}\cdot\text{g}^{-1}$ f.w.) and Romanian Muscat (250.96 $\mu\text{mol TE}\cdot\text{g}^{-1}$ f.w.).

A strong correlation was observed between TPC and TFC with PCL-ACW and PCL-ACL methods. The correlation coefficients (r) for these relationships were presented in Table 4.

Table 4. The correlation coefficients between TPC, TFC and TAC with PCL-ACW and PCL-ACL

	TPC	TFC	TAC
PCL-ACW	0.7804	0.7840	0.0075
PCL-ACL	0.9137	0.9113	0.8918

Calculation of the Relative Antioxidant Capacity Index (RACI)

As outlined by Sun and Tanumihardjo [25], RACI was computed as a statistical measure by combining antioxidant capacity data derived from multiple analytical methods. The RACI of each sample was calculated as the mean of the standard scores transformed from the raw data generated with different chemical methods. This process ensures that differences in units and data variability do not affect the results. RACI is a simple way to combine results from different methods, making it easy to compare antioxidant capacities across samples [25]. The ranking of the samples based on their antioxidant capacities using RACI is shown in Figure 3.

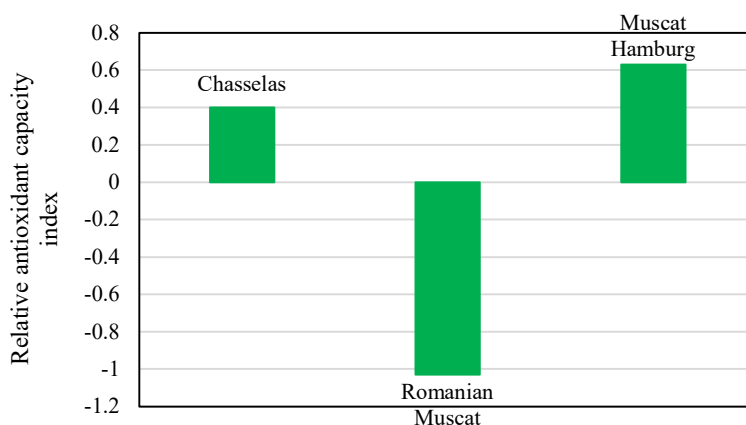


Figure 3. Relative antioxidant capacity index in the grape varieties

In terms of RACI values, Muscat Hamburg presented the highest value followed by Chasselas. Romanian Muscat showed the lowest value of RACI. Furthermore, the RACI value was correlated with the antioxidant capacity assays.

The RACI value was highly correlated with DPPH ($r = 0.9938$; $p < 0.05$), ACL ($r = 0.8863$; $p < 0.05$), and ACW ($r = 0.8183$; $p < 0.05$). This procedure is a simple and effective way to measure food antioxidant capacity, as it covers different chemical methods.

CONCLUSIONS

Grapes are valuable dietary sources of bioactive compounds, contributing significantly to human health. The present study evaluated the phenolic, flavonoid, and anthocyanin content, along with the antioxidant activity by DPPH method and antioxidant capacity in both hydrophilic and lipophilic systems, for three Romanian grape varieties. The results showed that the analysed grapes are particularly rich in phenolic compounds, which play a crucial role in their antioxidant properties. The strong correlation between antioxidant activity, photochemiluminescence analysis, and total phenolic, flavonoid, and anthocyanin content underscores the significant presence of bioactive compounds in these fruits. Additionally, the observed variation in bioactive composition and antioxidant capacity among the analyzed varieties highlights the influence of genetic factors. Notably, Muscat Hamburg exhibited the highest antioxidant capacity. Future research will focus on assessing the total antioxidant capacity of widely consumed fruits in Romania and establishing a comprehensive database of these findings.

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