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Identification and Quantification of Valuable Compounds in Red Grape Seeds

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Abstract: Grape seeds are a by-product of the wine industry. They represent 38–52% of grape pomace and about 5% of the weight of grapes. The main objective of this study is to establish some important characteristics of grape seeds from red varieties cultivated in Romania. The analyzed grape varieties were Cabernet Sauvignon, Merlot, Pinot noir, Burgund Mare, Cadarcă, Syrah, Novac. The grape seeds were dried and ground and the following determinations were made: determination of total polyphenol content, antioxidant capacity, antiradical capacity and determination of phenolic compounds. The analyses were performed on the first day after obtaining the grape extract, on the 14th day and the 30th day. The obtained results demonstrate that all the analyzed samples have a high content of polyphenols and show antioxidant and antiradical capacity. The highest values were obtained on the first day after separation, drying, grinding and extraction of the grape seeds and began to decrease almost constantly in time, so that for 30 days from storage the values obtained could ensure good operating yields. The seeds from the Novac grape variety obtained the best results throughout the analysis period. In the case of the total polyphenol content, the average value of the three samples Novac was 394.57 mgGAE/g dry extract and the average value of antioxidant capacity was 284.35 mgAAE/g dry extract. The greatest antiradical capacity was presented by the seeds of the Syrah and Novac varieties. The average value of the three samples from the Syrah variety was 62.1%, and in the case of the Novac variety was 61.33%. The paper demonstrates the opportunity of superior capitalization of seeds from the seven grape varieties cultivated on the territory of Romania due to the characteristics it possesses. At present, there is a major interest of consumers in the most natural products, with a major contribution to increasing the body's immunity. The use of natural compounds in the food and pharmaceutical industry can be an important alternative.

Keywords: antioxidant capacity; antiradical capacity; natural products; biotechnology; polyphenols

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1. Introduction

Wine production is one of the most important agricultural activities, and grapes are a very valuable food product. The by-products of the wine industry are rich in a wide range of bioactive compounds. In recent years, grape by-products have been considered an alternative source for obtaining high value-added materials due to their antioxidant and antimicrobial activities. Grape seeds are a by-product of the wine industry. They represent 38–52% of grape pomace and about 5% of the weight of grapes. Grape seeds have a high content of fiber (40% *w*/*w*), protein (11% *w*/*w*), lipids (16% *w*/*w*), polyphenolic compounds (7% *w*/*w*), carbohydrates and minerals. Due to the benefits they bring to human health, the use of grape seed extracts has increased in recent years [1,2].

1.1. The Benefits of Grape Seeds

Grape seeds contain two main groups of compounds: polyphenols and oils [3]. Grape seeds contain significant concentrations of specific polyphenols such as proanthocyanidins [4], with an antioxidant role that helps lower blood pressure, protecting the heart, blood vessels and brain [5–8]. They form oligomeric proanthocyanidins complexes to protect against free radicals, acting positively in reducing allergies and together with phenolic acids and flavonoids combat oxidative stress [9–11]. It is involved in protecting healthy cells in the body by acting against toxins and stimulating nitric oxide. Studies to date confirm that polyphenols in grape seeds protect the heart and blood vessels leading to lower blood pressure and thrombotic complications, preventing the formation of clots [12,13]. Recent research claims that the use of grape seed extracts leads to an improved cognitive decline being recommended in the pathology of Alzheimer's disease and its prevention. These studies have shown that polyphenols in grape seeds reduce the damage induced by free radicals in the hippocampus by increasing the level of antioxidants, preventing the protein mutations responsible for this disease [14,15]. A beneficial role is also found in the antimicrobial effect of ointments based on grape seed extract, phenolic compounds acting in faster wound healing and infection prevention. Including internal organs can benefit from the beneficial action of grape seed components, such as resveratrol [16]. In this respect, studies carried out with polyphenolic extracts from grape seeds introduced as medicine have led to a decrease in liver enzymes, protecting the liver from the accumulation of fats (fatty liver), toxins, aflatoxins, heavy metals, chemicals or various medicines [17–20]. It was also noted a reduction in inflammation of the kidneys, with a sharp stimulation of renal functions and an increase in protective molecules. The beneficial compounds in these seeds have a positive role in stimulating insulin secretion, in rheumatoid arthritis, arthrosis, osteoarthritis, blood sugar, diabetes [21–24]. The antibacterial action of these polyphenol-rich grape seed extracts is manifested by inhibition and lysis of Gram-negative cells such as *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella*, *Pseudomonas aeruginosa*, bacteria commonly found in human infectious pathology [25–30]. The US Food and Drug Administration (FDA) has awarded the grape seed extract the Certification Generally Recognized As Safe (GRAS) because it can be a potential nutritional supplement. High doses of grape extract (1–4 g/kg) have shown a protective and safe effect in various experiments on the metabolic effect in animals and humans [31]. In 2021, Zhao et.al demonstrated that grape seed extract improved the disturbance and metabolic function of intestinal flora induced by 2-Amino-1-methyl-6-phenylimidazo (4,5-b) pyridine (PhIP) in rats [32].

1.2. The Antioxidant and Antiradical Capacity of Grape Seeds

Most of the compounds with antioxidant effect that can be found in grapes are anthocyanins, catechin, gallic acid and resveratrol [33]. Resveratrol is a polyphenol found in grapes in greater quantities than in other fruits. This compound is found in vines in roots, seeds and stems, but the largest amount is found in the skin of grapes. The content of polyphenols with antioxidant action in wines is variable. It varies depending on the grape variety, the geographical location of the vineyard, the cultivation system, the climate, the type of soil, the harvest time or the oenological methods [34].

Catechin has a higher concentration of resveratrol in grapes. Malvidin 3,5-diglucoside is an anthocyanin compound found in grapes, which has a higher antioxidant activity than alpha-tocopherol. In red wine, anthocyanin compounds increase their antioxidant effect. The antioxidant effects of red wine are 50 times higher than that of white wine, so regular consumption of red wine reduces the risk of cardiovascular disease [34].

The polyphenolic content of wine, responsible for the antiradical activity, consists of two classes of components, flavonoids and non-flavonoids. They depend on the grape variety, the location of the vineyards, the cultivation system, the climate, the type of soil, the cultivation of the vines, the harvest time, the production and ageing process [35].

In a study conducted in 2016 by Karasu et al., five grape varieties were analyzed, and in all cases, the highest antiradical capacity was identified in seeds [36]. In 2016, Carbone and Fiordiponti analyzed a local variety of wine grown in the Lazio region and concluded that macerated wines showed the highest antiradical capacity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-Azinobis-(3-Ethylbenzthiazolin-6-Sulfonic Acid)) methods [37]. In 2009, Biagi et al. carried out a study in which they wanted to determine the antiradical capacity of Italian red wines. All types of wine analyzed showed high antiradical activity, always greater than 50% and a high antioxidant capacity, over 80%. Following the results obtained, it was concluded that the antiradical properties of the wine depend on the total polyphenolic content, especially on anthocyanins [35].

This research aims to highlight the importance of existing compounds in grape seeds. Most of the seeds left after processing the grapes are considered waste and remain unused. To carry out this study we analyzed seven varieties of grapes grown exclusively in Romania. We aim to highlight natural alternatives obtained from plants for use in the food and pharmaceutical industry.

2. Materials and Methods

2.1. Materials

Seven varieties of red grapes were selected for this study, namely Cabernet Sauvignon, Merlot, Pinot noir, Burgund Mare, Cadarcă, Syrah, Novac from the hilly area of Banat.

Cabernet Sauvignon is a red grape variety with the aroma of berries that have a thick and weather-resistant skinand the wine obtained has a high tannin content [38]. Following several studies and HPLC analysis, it was found that the Cabernet Sauvignon grape variety has a high content of resveratrol (52.3 and 49.6 mg/kgDW) [39].

Merlot is the second most planted variety in the world after Cabernet Sauvignon. The variety adapts to different microclimates, resistant to colder temperatures, in such conditions, it expresses itself better [40]. In the wine obtained from the Merlot grape variety, there is a high content of glycerol, ethyl acetate and succinic acid. These are giving the wine a specific taste and a well-pronounced flavor [41].

The grapes of the Pinot noir variety have thin skin and do not load the grape must. Pinot noir wines are always transparent. This variety likes cooler areas where it draws its freshness and subtle fruitiness that does not overwhelm other flavors [42]. Pinot noir is considered to be a difficult variety of product, being sensitive to the environment, with thin skin, has a high susceptibility to disease and lower concentrations of anthocyanin that often lead to reduced intensity of wine color [43].

The Burgund Mare variety has existed in Romania for over a century, but it has expanded considerably in culture after 1975 and offers high grape production. The wine does not have a specific personality, but it is balanced, with extractive and medium color intensity, reaching an alcoholic strength of 11–12 vol% [44].

"Cadarcă" or "Cadarcă de Miniș" is a traditional grape variety originating from the Miniș–Măderat vineyards, Arad area, where it was first prepared in 1744. The wine obtained is called from ancient times "bull's blood", a reputation that the locals keep even today, defining the red-ruby color, personality and strength. The grapes are medium-sized, cylindrical, sometimes winged, with the berries often placed in clusters and uneven in size. The bean is medium, spherical, slightly ovoid, with thin skin and unevenly colored in bluish-black. The variety is resistant to frost but has low resistance to drought, summer rains in sunny weather with a detrimental effect on plant development and medium resistance to grey rot of grapes [45].

Syrah is a red grape variety from France, from the Rohne Valley. Being a traditional French wine, Syrah expresses floral aromas in its youth, and over time, it develops aromas of black and white pepper, grassy notes, skin and smoke [46].

Novac is a grape variety with red grapes for red wines. It was obtained at SCDVV Drăgășani by sexual hybridization between the varieties Negru vârtos and Saperavi, being approved in 1987. It has medium-sized grains, weighing in the range of 168–315 g/100 grains, ovoid in shape, with black-bluish skin, covered with plum. The flesh is red, juicy, with a pleasant sweet and sour aroma and taste. The main climatic data from the test period were represented by the average temperature, with values between 11.4 and 12.5 °C [47].

Grapes were crushed by hand, the seeds separated by pulp and shell. The seeds were then washed under running water and then dried at room temperature. After drying, they were ground. The powder obtained was subjected to extraction with petroleum ether for 8 h. For each seed sample, 20 g of product was placed in an extraction cartridge and so on degreased, applying the Soxhlet method at a temperature of 50 °C. The samples were then homogenized with 50 mL solvent consisting of nine parts acetone and one part distilled water for the extraction of polyphenols for 12 h. Subsequently, they were centrifuged for 30 min at 3500 revolutions/min, filtered and evaporated in rotavapor—1:10. To obtain the most conclusive results, three samples of each variety selected for the study were used (1, 2, 3). The extracts obtained were stored at a temperature between 0 and 4 °C in dark glass containers.

2.2. Procedure Methods

The analyses were performed after extraction, in three periods, on the first day after their drying, on the 14th day and on the 30th day of storage of the grape seed extract. The purpose of making the determinations in the three periods was to demonstrate the preservation of the characteristics of the grape seed extract and the possibility of preserving it. To eliminate as many errors as possible, such as those related to equipment, human errors, temperature or humidity, we decided to analyze three samples from each grape extract on each day of analysis.

2.2.1. Determination of the Total Polyphenol Content of the Grape Seeds

The determination of the total polyphenol content was carried out by the Folin–Ciocâlteu method. A UV–VIS Cecil 1200 spectrophotometer was used, measuring at the wavelength of 665 nm. The results obtained were compared with the standard curve and expressed in mg gallic acid equivalent—GAE/g dry extract [48].

2.2.2. Determination of the Antioxidant Capacity of the Grape Seeds

The antioxidant capacity was determined by the method of reduction with phosphomolibdenum (Prieto et al. 1999), the reading of the samples being carried at the wavelength length of 695 nm with the spectrophotometer Cecil 1200. The results are expressed in mg ascorbic acid—AAE/g dry extract. In total, 0.1 mL of sample was homogenized in an Eppendorf tube with 1 mL of 0.6 M sulfuric acid, 28 mm sodium phosphate and 4 mm ammonium molybdate. The coated tubes were incubated for 90 min at 95 °C, cooled, then the absorbance was read at a wavelength of 695 nm on the Cecil 1200 spectrophotometer in the presence of the control sample. The control sample consists of the same reagents used and the procedure minus the extract. The results are expressed in mg ascorbic acid (AAE/g dry extract), the calibration line was performed in a range of 50 mg/g–350 mgAAE/g dry extract [49].

2.2.3. Determination of the Antiradical Capacity of the Grape Seeds

The antiradical capacity was determined by the Brand–Williams et al. method and Yalcin et al. method which involves homogenizing the grape seed extract with 2,2-diphenyl-1-picrilhydrazil in the presence of methanol, keeping in the dark for 30 min and then reading the absorption at 516 nm with the Cecil 1200 spectrophotometer. A blind sample lacking the seed extract was used as the standard sample. The results are expressed by applying the standard formula which implies [50,51]:

$$Ar\ [\%] = 100 \times (1 - Ap{:}Ae) \quad (1)$$

where Ap represents the absorption of the sample; Ae represents the absorption of the standard sample.

2.2.4. Determination of the Phenolic Compounds of the Grape Seeds

The determination of phenolic compounds was carried out by the HPLC (Knauer) a method involving the direct injection of the samples and the separation was carried out on a monolithic column C18. The chromatograph was equipped with a UV/DAD detector, and the solvent had a constant flow rate of 1.2 mL/min at a temperature of 22 °C. Solvent A was composed of distilled water and glacial acetic acid 0.15%, and solvent B of acetonitrile and glacial acetic acid 0.15%. The extraction steps were established according to the protocol as follows: gradient B: 0–4 min 9–12%, 4–10 min 12–15%, 10–20 min 25–70%, 20–30 min 100%. The amount injected was 1 µL and the reading was performed at the wavelengths of the spectra of the identified phenolic compounds: at 225 nm vanilic acid; at 280 nm: gallic acid, catechin, epicatismin, epicatchin gallate, P-hydroxybenzoic acid, M-hydroxybenzoic acid, syringic acid; at 305 nm P-cumaric acid, resveratrol; at 330 nm caffeic acid and chlorogenic acid; at 360 nm rutin and quercetin. Calibration curves were designed on compound concentrations starting from: 0.1, 0.5, 1, 5, 10, 15, 25, 50, 100, and 150 mg/L, all standards being of chromatographic purity—Sigma Aldrich [52].

2.2.5. Statistical Analysis

Results are expressed by mean ± standard deviation (SD) for each group. Graphical representation and statistical processing were performed using the Minitab 14 statistical softwareand $p < 0.05$ was considered significant.

3. Results

3.1. Determination of the Total Polyphenol Content of the Grape Seeds

Figure 1A shows the total polyphenols content in the seeds on the first day of the following grape varieties: Cabernet Sauvignon, Merlot, Pinot noir, Burgund mare, Cadarcă, Syrah, Novac. The highest total polyphenol content was recorded in the case of Novac seeds, the average value of the three samples being 394.57 mgGAE/g dry extract. A high content of polyphenols was also recorded in the case of Syrah seeds. The average value of the three samples is 384.36 mgGAE/g dry extract. Lower values of the total polyphenol content were recorded for the varieties Cadarcă (344.42 mgGAE/g dry extract), Cabernet Sauvignon (340.49 mgGAE/g dry extract), Pinot noir (334.38 mgGAE/g dry extract) and Burgund mare (326.64 mgGAE/g dry extract). Among the analyzed varieties, the lowest level of the total polyphenols content was registered in the case of seeds from the Merlot variety, being registered an average value of 230.45 mgGAE/g dry extract.

Figure 1B shows the total polyphenol content determined from the seeds of the seven grape varieties on the 14th day. Compared to the first day of analysis, we find a slight decrease in the total polyphenol content for all seven grape varieties. On this day of analysis, the Novac variety has the highest polyphenol content, the average value of the three samples being 390.83 mgGAE/g dry extract. The lowest polyphenol content on this day of analysis was recorded in the Merlot variety, the average value of the three samples being 218.42 mgGAE/g dry extract.

Figure 1C shows the total polyphenol content determined from the seeds of the seven grape varieties on the 30th day. The lowest values of polyphenol content of the seven grape varieties were recorded on this day of analysis. Even on this day, the Novac variety recorded the highest polyphenol content, the average value of the three samples being 387.36 mgGAE/g dry extract. The lowest polyphenol content on this day of analysis was recorded in the Merlot variety, the average value of the three samples being 211.21 mgGAE/g dry extract.

3.2. Determination of the Antioxidant Capacity of the Grape Seeds

Figure 2A shows the antioxidant capacity of grape seeds on the first day from the seven varieties studied: Cabernet Sauvignon, Merlot, Pinot noir, Burgund mare, Cadarcă, Syrah, Novac. The highest antioxidant capacity was registered in the case of Novac seeds. The average value of the three samples is 284.35 mgAAE/g dry extract. The Pinot noir seeds reported a fairly high antioxidant capacity, the average value of the three samples being 241.07 mgAAE/g dry extract. The seeds of the Syrah, Cadarcă and Cabernet Sauvignon varieties have antioxidant capacities between 220.58 and 237.52 mgAAE/g dry extract. The lowest antioxidant activities were reported in the Burgund mare and Merlot varieties. The average value of the three samples in the case of the Burgund mare variety is 214.38 mgAAE/g dry extract, and in the case of the Merlot variety, it is 133.91 mgAAE/g dry extract.

Figure 2B shows the antioxidant capacity determined from the seeds of the seven grape varieties on the 14th day. Compared to the first day, there is a slight decrease in the antioxidant capacity of the seven grape varieties. On this day, the highest antioxidant capacity was recorded for the Novac variety, the average value of the three samples being 281.27 mgAAE/g dry extract. The lowest value was obtained in the case of the Merlot variety, the average value of the three samples being 130.37 mgAAE/g dry extract.

Figure 2C shows the antioxidant capacity determined from the seeds of the seven grape varieties on the 30th day. On this day, the lowest values of antioxidant capacity were registered. Additionally, on this day, in the case of the Novac variety the highest values were obtained, the average value of the three samples being 277.04 mgAAE/g dry extract. The Merlot variety recorded the lowest values on this day, the average value of the three samples being 127.46 mgAAE/g dry extract.

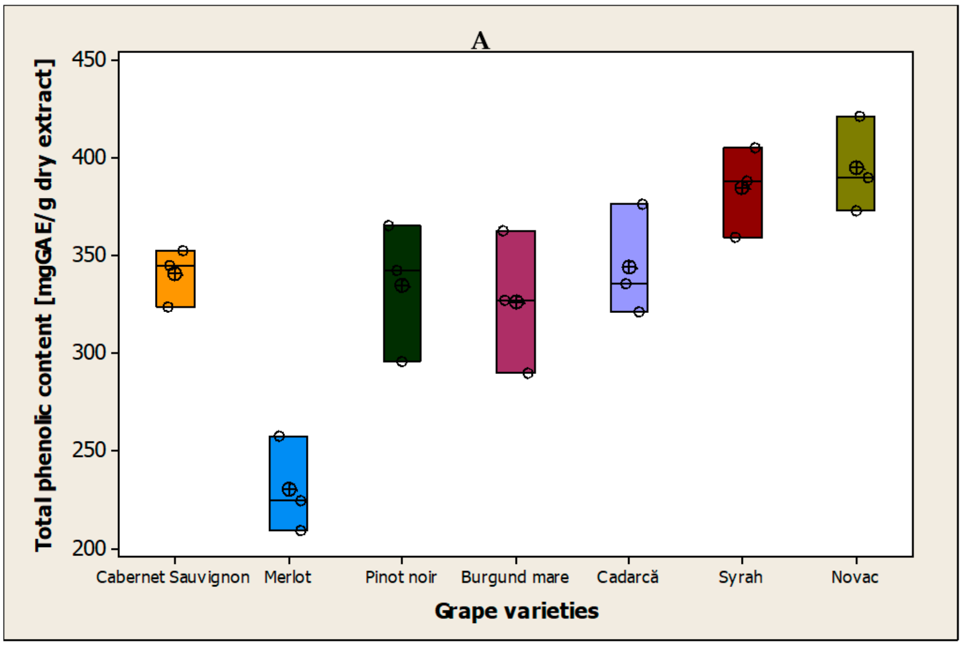
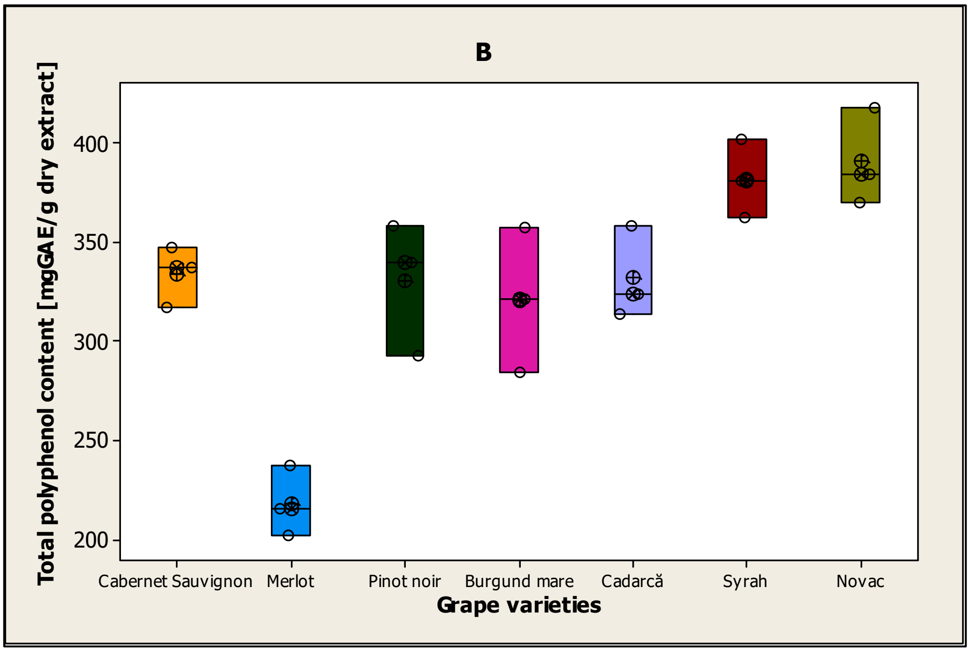


Figure 1. *Cont.*



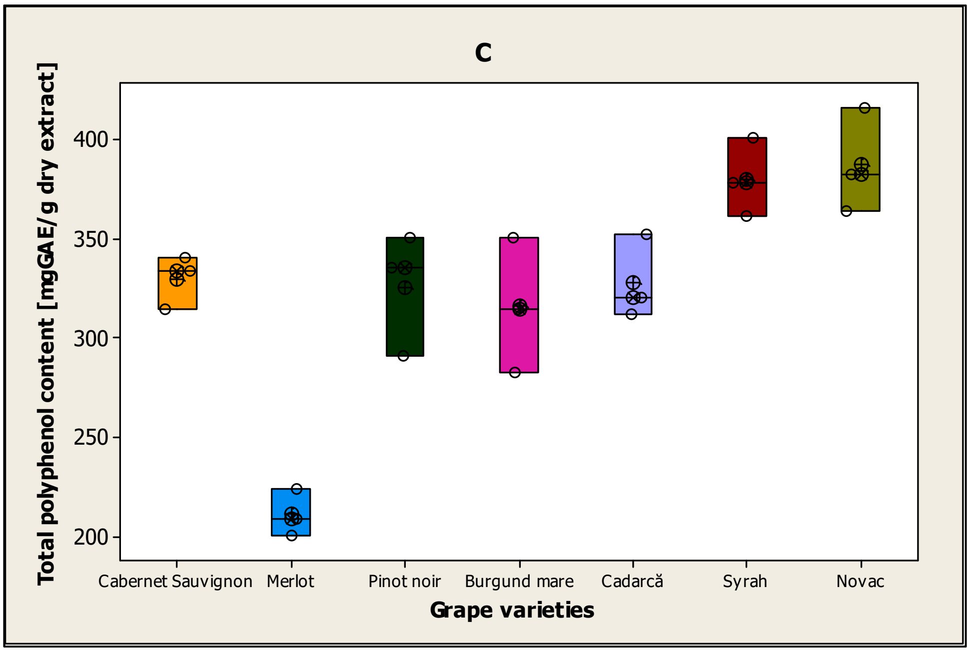


Figure 1. (**A**) The total polyphenol content determined from the seeds of the seven grape varieties on the first day; (**B**) the total polyphenol content determined from the seeds of the seven grape varieties on the 14th day; (**C**) the total polyphenol content determined from the seeds of the seven grape varieties on the 30th day.

3.3. Determination of the Antiradical Capacity of the Grape Seeds

The antiradical capacity of extracts obtained from the red grape seed is an important indicator in assessing their quality for their widespread use in various contexts.

Figure 3A shows the antiradical capacity of grape seeds on the first day from the seven varieties studied: Cabernet Sauvignon, Merlot, Pinot noir, Burgund mare, Cadarcă, Syrah, Novac. The greatest antiradical capacity is presented by the seeds of the Syrah and Novac varieties. The average value of the three samples from the Syrah variety is 62.1%, and in the case of the Novac variety, it is 61.33%. The antiradical capacities of Pinor noir and Cadarcă varieties are quite close, the average values being 47.45% and 45.36%. The lowest values were obtained for the Cabernet Sauvignon, Merlot and Burgund Mare varieties. The average values of their antiradical capacities are 33.75%, 33.55% and 33.3%.

Figure 3B shows the antiradical capacity determined from the seeds of the seven grape varieties on the 14th day. Compared to the first, there is a slight decrease in antiradical capacity. The highest values of antiradical capacity were recorded for Syrah and Novac varieties. The average value of the three Syrah samples was 59.84% and the average value of the three Novac samples was 59.64%. The lowest value of the antiradical capacity was registered in the case of the Burgund Mare variety, the average value of the three samples being 31.61%.

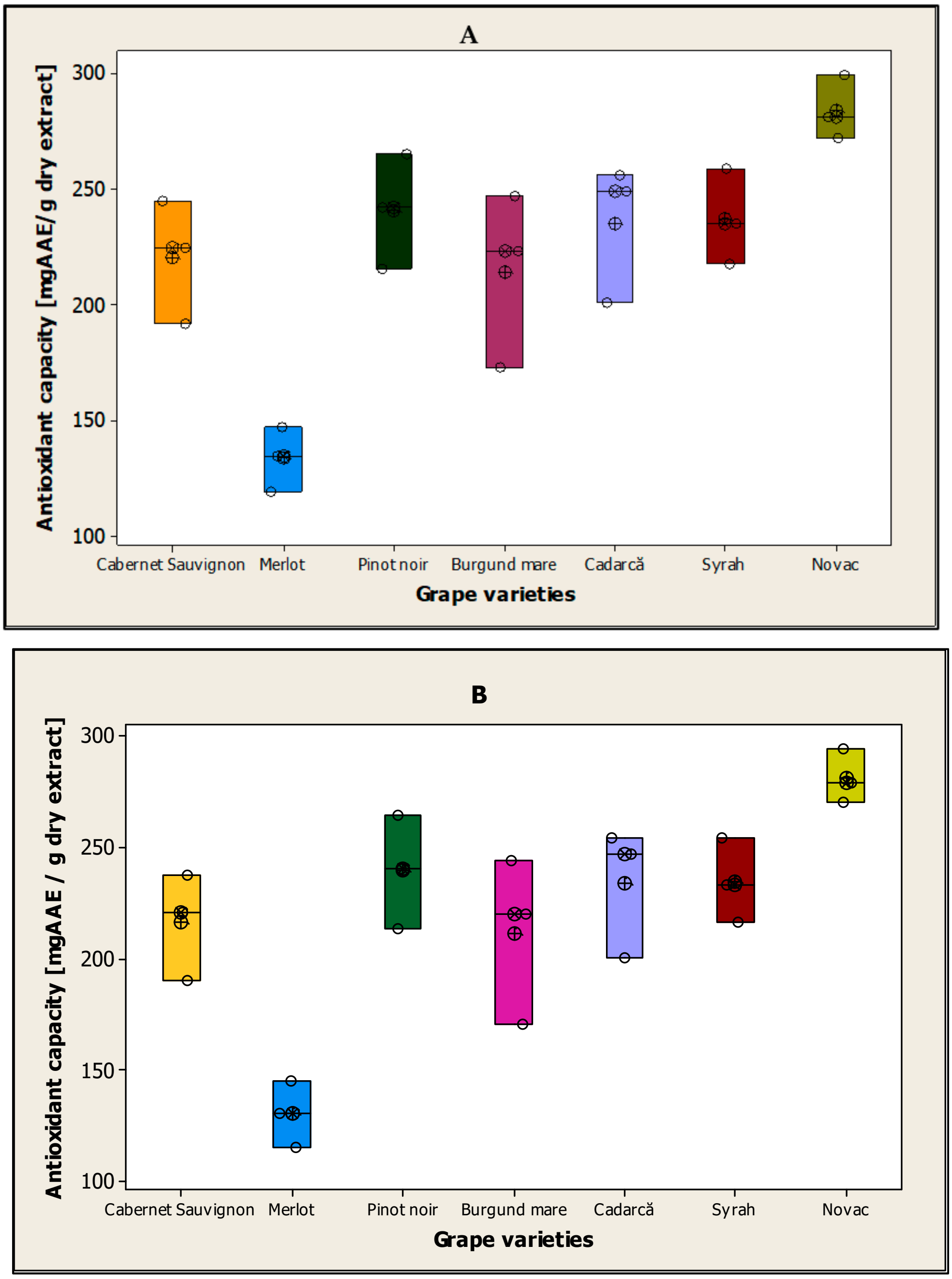


Figure 2. *Cont.*

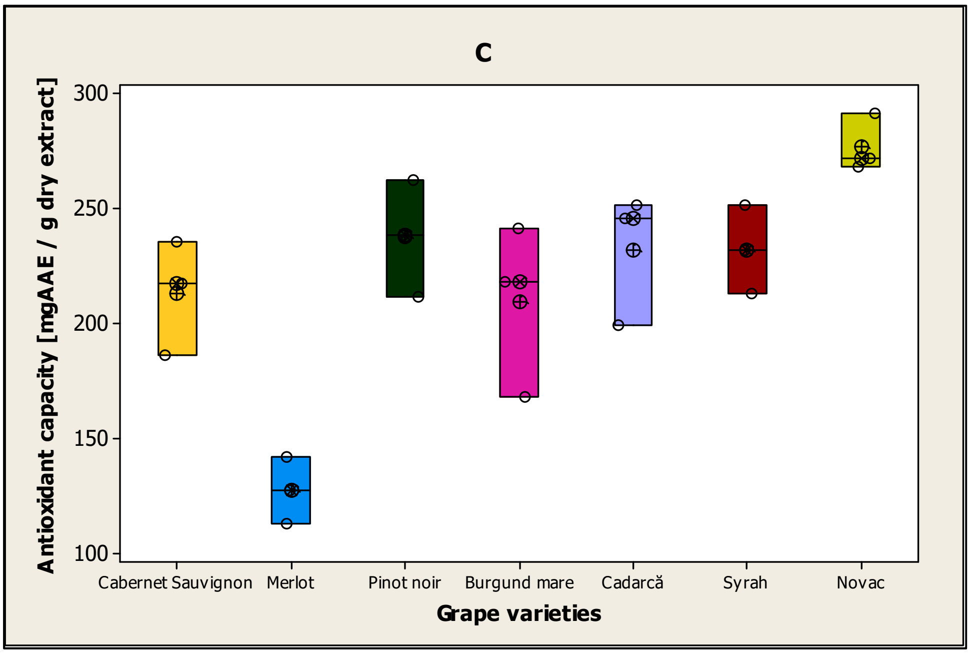
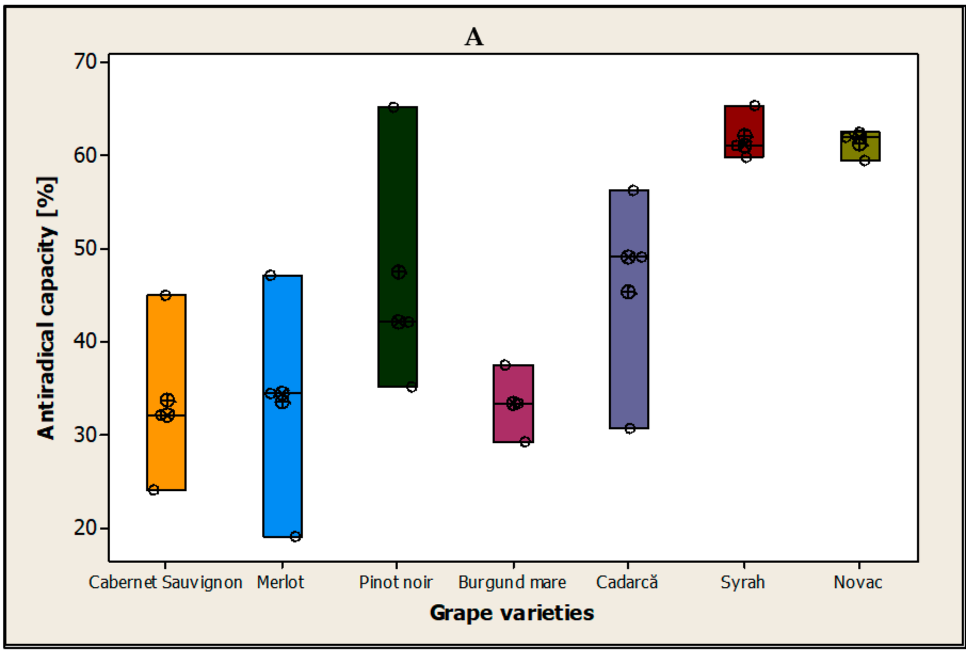


Figure 2. (**A**) Antioxidant capacity determined from the seeds of the seven grape varieties on the first day; (**B**) antioxidant capacity determined from the seeds of the seven grape varieties on the 14th day; (**C**) antioxidant capacity determined from the seeds of the seven grape varieties on the 30th day.

Figure 3C shows the antiradical capacity determined from the seeds of the seven grape varieties on the 30th day. The lowest values were recorded on this day of analysis. The highest values were recorded for Syrah and Novac varieties. The average value of the three Syrah samples was 58.38% and the average value of the three Novac samples was 58.33%. The lowest values were obtained in the case of the Burgund Mare variety, the average value of the three samples being 29.92%.



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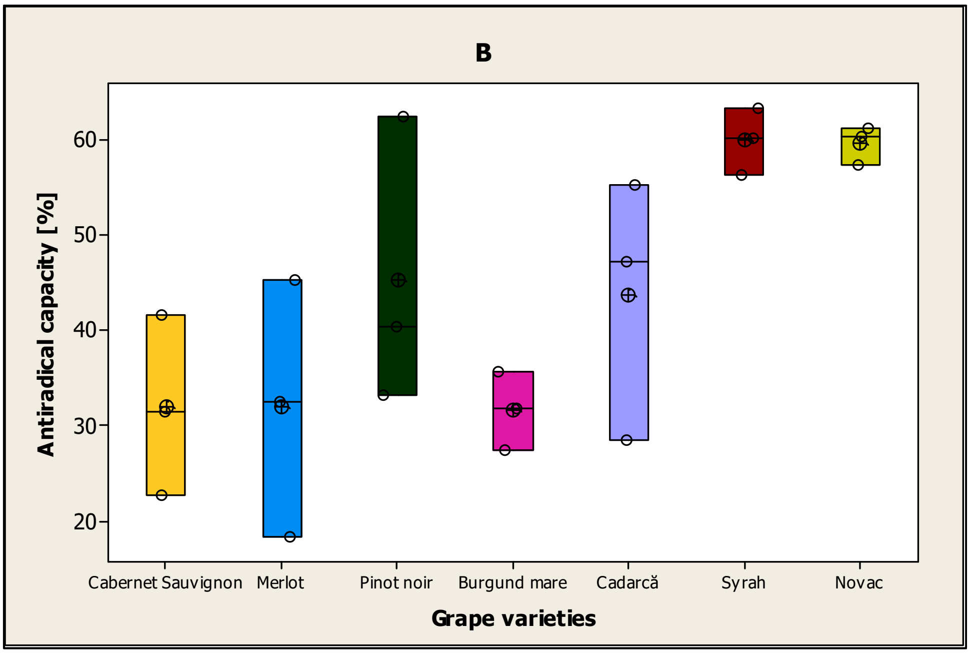

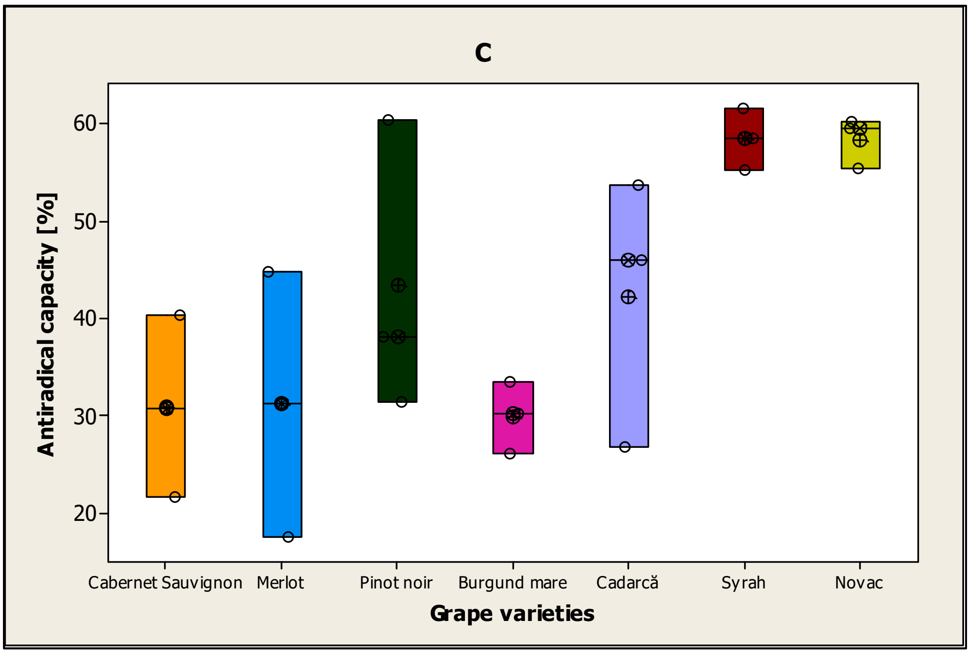

Figure 3. (**A**) Antiradical capacity determined from the seeds of the seven grape varieties on the first day; (**B**) antiradical capacity determined from the seeds of the seven grape varieties on the 14th day; (**C**) antiradical capacity determined from the seeds of the seven grape varieties on the 30th day.

3.4. Determination of the Phenolic Compounds of the Grape Seeds

Table 1 shows the phenolic compounds identified in the seven grape varieties studied: Cabernet Sauvignon, Merlot, Pinot noir, Burgund mare, Cadarcă, Syrah, Novac. The phenolic compounds identified and quantified by the HPLC (high performance liquid chromatography) method have values that vary greatly depending on the nature of the compound but also on the variety from which the seeds come. It is noted that the most significant values are syringic acid, being between 121.22 and 136.66 mg/L. The richest varieties in syringic acid are Syrah and Novac, followed by Burgund Mare and Cadarcă. The lowest values are observed in the case of Cabernet Sauvignon and Merlot extracts. Gallic acid and vanillic acid are found in amounts of tens of mg/L, the most significant values being present in the Novac variety—39.22 and 20.91 mg/L. Catechin has values between 6.05 and 9.01 mg/L and chlorogenic acid has values between 4.82 and 8.02 mg/L. M-hydroxybenzoic acid is about 2–3 times more significant than p-hydroxybenzoic acid, with values ranging from 1.2 to 5.62 mg/L. Epicatechin and p-coumaric acid are around five with oscillations of 1–2 units up or down. Epicatechin gallate varies from 1.84 to 2.56 mg/L, the most significant values being in the case of seed extract from the Pinot noir variety. Caffeic acid is present with values starting from 0.94 mg/L and reaching a maximum of 1.56 mg/L, specific to the Novac variety. Subunit values are observed in the case of ferulic acid with a maximum of 0.96 mg/L for Pinot noir, quercetin with a maximum of 0.96 mg/L for Syrah and 0.41 mg/L for Cadarcă. Resveratrol is found in the seeds of all varieties with values between 1.91 and 2.92 mg/L for Cabernet Sauvignon, 1.41 and 2.23 mg/L for Merlot, 1.93 and 2.37 mg/L for Pinot noir, 1.66 and 1.88 mg/L for Burgund Mare, 1.71 and 2.46 mg/L for Cadarcă, 2.12 and 2.34 mg/L for Syrah and 2.16 and 2.38 mg/L for Novac variety.

Table 1. Identification and quantification of valuable phenolic compounds in the seeds of the seven grape varieties.

Compound	Composition (mg/L ppm (Parts per Million))						
	Cabernet Sauvignon	Merlot	Pinot noir	Burgund Mare	Cadarcă	Syrah	Novac
Catechin	8.17 ± 0.01	7.28 ± 0.01	6.98 ± 0.01	7.15 ± 0.01	8.12 ± 0.01	8.46 ± 0.01	8.78 ± 0.01
Epicatismin	5.11 ± 0.01	5.12 ± 0.01	5.16 ± 0.01	4.82 ± 0.01	4.43 ± 0.01	5.98 ± 0.01	6.17 ± 0.01
Epicatechin gallate	2.15 ± 0.01	2.18 ± 0.01	2.26 ± 0.01	1.95 ± 0.01	2.24 ± 0.01	2.02 ± 0.01	2.46 ± 0.01
Gallic acid	26.34 ± 0.05	0.71 ± 0.01	31.46 ± 0.05	31.02 ± 0.05	32.46 ± 0.05	33.80 ± 0.05	35.46 ± 0.05
P-hydroxybenzoic acid	1.85 ± 0.05	1.23 ± 0.05	1.77 ± 0.05	2.04 ± 0.05	2.09 ± 0.05	2.22 ± 0.05	2.30 ± 0.05
Vanilic acid	18.02 ± 0.01	15.57 ± 0.01	16.15 ± 0.01	13.24 ± 0.01	18.65 ± 0.01	18.45 ± 0.01	20.08 ± 0.01
Syringic acid	122.87 ± 0.25	130.13 ± 0.25	129.40 ± 0.25	129.81 ± 0.25	133.20 ± 0.25	134.26 ± 0.25	134.12 ± 0.25
M-hydroxybenzoic acid	4.58 ± 0.01	4.60 ± 0.01	4.20 ± 0.01	3.69 ± 0.01	3.44 ± 0.01	5.32 ± 0.01	5.26 ± 0.01
Caffeic acid	1.01 ± 0.01	1.08 ± 0.01	0.96 ± 0.01	1.19 ± 0.01	1.27 ± 0.01	1.33 ± 0.01	1.35 ± 0.01
Ferulic acid	0.59 ± 0.01	0.47 ± 0.01	0.90 ± 0.01	0.61 ± 0.01	0.70 ± 0.01	0.62 ± 0.01	0.54 ± 0.01
Chlorogenic acid	7.67 ± 0.01	6.95 ± 0.01	8.24 ± 0.01	5.32 ± 0.01	6.54 ± 0.01	6.67 ± 0.01	7.90 ± 0.01
P-coumaric acid	5.22 ± 0.01	3.50 ± 0.01	4.49 ± 0.01	4.68 ± 0.01	4.45 ± 0.01	5.27 ± 0.01	6.01 ± 0.01
Resveratrol	2.27 ± 0.01	1.22 ± 0.01	0.84 ± 0.01	1.74 ± 0.01	2.01 ± 0.01	2.28 ± 0.01	2.41 ± 0.01
Rutin	0.15 ± 0.01	0.41 ± 0.01	0.21 ± 0.01	0.36 ± 0.01	0.37 ± 0.01	0.36 ± 0.01	0.31 ± 0.01
Quercetin	0.82 ± 0.01	32.43 ± 0.05	2.14 ± 0.01	0.47 ± 0.01	5.84 ± 0.01	2.33 ± 0.01	1.06 ± 0.01

4. Discussion

All four ascertainments, the determination of the total content of polyphenols, the determination of antioxidant and antiradical activity, as well as the determination of phenolic compounds were performed on all seven grape varieties. The grapes came from plantations in Romania, more precisely from the Banat area. The analyses were performed after extraction, in three periods, on the first day after their drying, on the 14th day and on the 30th day of storage of the grape seed extract. To eliminate as many errors as possible, such as those related to equipment, human errors, temperature or humidity, we decided to analyze three samples from each grape extract on each day of analysis.

According to the obtained results, in the case of determining the total content of polyphenols, antioxidant and antiradical capacity, a slight decrease of these contents was found in the three days of analysis. This decrease was slight in all seven grape samples. The highest values were obtained in the case of the Novac variety. This is also demonstrated by the analysis of phenolic compounds because this variety has high values for all identified components. Merlot seeds have the lowest values in all three determinations, analysis of total polyphenol compounds, antioxidant and antiradical analysis.

Using the HPLC method, we identified several phenolic compounds contained in the analyzed seeds. In all seven samples, the following proportion has the highest proportion: syringic acid, gallic acid and vanilic acid. Syringic acid is an excellent compound to be used as a therapeutic agent and possesses antioxidant, antimicrobial, anti-inflammatory and antiendotoxic capabilities [53]. Gallic acid has various properties, including antifungal, antimicrobial, and anticancer capabilities [54]. Vanillic acid reduced collagen accumulation and hydroxyproline content [55]. According to the results obtained from this determination, we notice that the seeds of the Novac variety contain the highest amounts of syringic acid, gallic acid and vanillic acid, and the seeds of the Merlot variety have a low content of vanillic acid and gallic acid. These values justify the results obtained in the case of determining the antioxidant and antiradical capacity. In the case of both determinations, the best results were obtained in the case of seeds of the Novac variety, and the lowest in the case of seeds of the Merlot variety. In addition to the three compounds, HPLC analysis showed others that possessed antioxidant properties, such as catechin, caffeic acid, ferulic acid, coumaric acid, resveratrol and quercetin, and antiradical properties, such as chlorogenic acid and rutin [56–63]. Extraction yields differ depending on the quality of the solvent and the working conditions. The obtained results attest to the fact that grape seeds contain significant amounts of polyphenols with strong antioxidant activity.

Antioxidants are widely used as food additives to prevent food degradation. Antioxidants also play an important role in preventing a variety of lifestyle-related diseases and ageing, as they are closely linked to active oxygen and lipid peroxidation. Finding viable solutions for the realization of basic products in food, with the widest possible destination, which in addition to a longer life cycle to ensure at the same time a healthy lifestyle, is of utmost importance. Making foods that increase the body's immunity and bring several benefits to the consumer is an effective alternative to ensuring physical and mental health. In addition, it can be a sustainable and efficient activity [64].

5. Conclusions

Grape seeds are an important source of valuable compounds for human health. Grape seeds mainly contain phenolic compounds, such as proanthocyanidins with an antioxidant capacity 20 times higher than vitamin E and 50 times higher than vitamin C. Due to their antioxidant capacity, grape seeds have antiallergic, anti-inflammatory, anticancer, immune boosting action, as well as beneficial effects in cardiovascular diseases. They are a source of bioactive substances and can currently be an important solution in obtaining new medicines of plant origin.

Several very valuable products have been identified qualitatively and quantitatively such as catechin, epicatismin, epicatechin gallate, gallic acid, P-hydroxybenzoic acid, vanilic acid, syringic acid, M-hydroxybenzoic acid, caffeic acid, ferulic acid, chlorogenic acid, P-coumaric acid, resveratrol, rutin and quercetin with special importance demonstrated for antioxidant and antiradical activity.

The analyses performed at 30 days showed a reduction in the content of useful substances contained in grape seeds, a reduction due to transformations that take place during the storage period of the seeds under analysis, oxidation and degradation. Under the required storage conditions, it is clear that the extraction yields for the active compounds justify the interest. It is more than certain that more adequate special storage conditions—protection against oxidative processes or seed storage humidity in the range of 10–12%—can significantly extend the storage period of yields, on the extraction of useful products on a longer period.

The superior recovery of these by-products and the obtaining of high value added bioproducts for use in the food, pharmaceutical and cosmetics industries are becoming an increasingly important practice. Bioproducts will have a complex biochemical composition and high antioxidant potential, is intended for the prevention and diet therapy of diseases caused by oxidative stress. The orientation towards the use of natural products with bioactive compounds must be a priority for the processors in the food and pharmaceutical industry both from a sustainable and economic point of view. Grape seeds fit perfectly into these product categories and represent an important alternative.

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