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Identification and quantification of valuable phenolic compounds from red wines from western part of Romania

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Abstract

The aim of this paper is to identify and quantify different phenolic compounds known and appreciated for their antioxidant action from five red wines from the west side of Romania. For the determinations HPLC methods were used, the samples were done in cycles of three for better results. Phenolic acids that drew the attention have different values that ranging from 1.3007 mg/L (elagic acid) to 19.3315 (t-caftaric acid), some various flavonoids (flavone, flavonoli și flavanoli) the values of which were recorded between 0.9997 mg/L (apigenin) and 56.1617 mg/L (catechin) and the stilbens namely the resveratrol has reached the maximum value of 8.1427 mg/L. The phenolic compounds that were determined lead to the conclusion that autochthonous wines are rich in antioxidant elements, and they are recommended for prophylactic consumption.

Keywords: Red wines; Phenolic compounds; HPLC; Antioxidant action

1. Introduction

Polyphenols are the most important and surely the most numerous of the groups of phytochemicals present in the vegetable kingdom, where there have been identified above 8000 of phenolic structures, and more than 4000 of these are flavonoids [1]. However, it is considered that the total amount of polyphenols from plants is underestimated because a lot of phenolic compounds that are in fruits, vegetables and derivatives were not identified, because the analyzing methods and techniques that were used are still perfectible [2]. Polyphenols from wine were studied both by food and health specialists, the research was conducted especially for the beneficial effects on human health. One of the most certain results is expressed through "The French paradox". The intense investigations that were made on red wines started in 1991, when the experts realized that in France, where the regular and moderate consumption of red wine is traditional, despite food rich in cholesterol, the percentage of cardiovascular diseases is lower and the lifetime expectancy is high. Consumers of red wine are less prone to the cardiovascular diseases, and the life expectancy is higher than in other regions [3]. The most well-known and appreciated phenolic compounds are: caffeic acid, chlorogenic acid, elagic acid, ferulic acid, siringic acid, vanilic acid, apigenin, (+) catechin, (-) epicatechin, kaempferol, luteolin, miricetin, quercetin, resveratrol [4-6].

An upward trend is now emerging in red wine consumption, with increasing demand worldwide and the launch of various studies that demonstrate its beneficial qualities, but also by optimizing manufacturing technologies to potentiate bioactive compounds [7]. Studies have established that there is a direct proportional relationship between the antioxidant effect and the amount of phenolic compounds found in wine [8, 9]. Phenolic compounds, representative of red wines, participate in the formation of its composition, color and stability, inhibiting the growth of free radicals in the body with beneficial effects in limiting cardiovascular disease, cancer or diabetes [10-16].

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The amount of phenolic compounds varies considerably in different types of wine, depending on: grape variety, soil and atmospheric conditions, vineyard environment factors, agronomic techniques used, fruit maturation, grape health, wine processing techniques, altitude [11, 17-20].

Studies have shown that the antioxidant activity of young red wines is greater than that of mature ones, which are able to conserve a higher amount of polyphenols [21]. Red and dry red wines are richer in phenolic compounds than sweet or semi-sweet. Specialists can recommend solutions to polyphenol concentration optimization by modifying production practices with increasing antioxidant content to meet market demands. The most frequent interventions in this regard are the grape processing techniques, the use of maceration enzymes, the optimization of the temperature and the extraction time [22].

2. Material and methods

2.1. Chemicals and reagents

All the chemicals used (acetonitrile, phosphoric acid and methanol) for HPLC were of chromatographic purity, also from the German company Sigma-Aldrich GmbH.

2.2. Samples

Red wines with the Registered Designation of Origin "RECAŞ" (DOC). Burgund mare, Cadarcă, Fetească Neagră, Novac, Syrah year of manufacture 2018. The harvesting conditions of grapes of these varieties require a minimum sugar of 187 g / L, the purity of the variety to be 100% and the percentage of grapes affected should not exceed 10%. The five varieties of wine under processing showed an alcoholic concentration of 13.5% v / v, the maceration-fermentation technology being identical for all the selected samples.

2.3. HPLC analysis

HPLC Agilent 1200 with UV detector was used for the analysis. The separation of the components was performed by the method described by Bonerz et al. [23] on C18 column chromatography using the following solutions: solution A - water / phosphoric acid (99.5 / 0.5); solution B - acetonitrile / water / phosphoric acid (50 / 49.5 / 0.5). Prior to HPLC analysis, all samples were diluted with methanol (99.9%) and filtered through a membrane filter (pore diameter of 0.45 µm Millipore, Bedford, MA). The injected sample volume was of 20 µL, the results being expressed in mg / L. Detection was performed at the following wavelengths: at 280 nm for caffeic acid, t-caftaric acid, chlorogenic acid, elagic acid, gallic acid, siringic acid, vanilic acid, apigenin, catechin, epicatechin, kaempferol, luteolin, miricetin, quercetin, quercetin-3-glucose and 310 nm for resveratrol. The identification of phenolic compounds was performed by comparing the retention time of the standards with the observed compounds according to the software.

Caffeic acid, caffeic acid, chlorogenic acid, elagic acid, gallic acid, siringic acid, vanilic acid, apigenin, catechin, epicatechin, kaempferol, luteolin, miricetin, quercetin, quercetin-3-glucoside and resveratrol standards were purchased at Sigma-Aldrich GmbH (Germany).

3. Results and discussion

3.1. Chemicals used and quantitative detection of phenolic compounds using HPLC

The content of phenolic acids determined in the samples taken at work differs substantially from one wine to another and in particular from one year of production to another. Thus, Table 1 shows that phenolic acids present values ranging from 1.3007 mg / L to elagic acid and reach values of 19.3315 mg / L for t-caftaric acid. Caffeic acid (3,4-dihydroxycinnamic acid) is a synapic acid present in wines with strong antioxidant properties (Gülçin 2006). Caffeic acid is found in a quantity of 5.3423 mg / L in the wine of the Burgund mare, its values increasing to 9.3017 in the wine of the Cadarcă variety. Nearby values presented the wines from the Fetească Neagră and Novac varieties where the caffeic acid was detected in an amount of 15.3423mg / L and 15.9985 mg / L respectively. Syrah wine showed a caffeic acid value 20% lower than previously mentioned.

Table 1 Quantification of phenolic acids in red wines such as Burgund mare, Cadarcă, Fetească Neagră, Novac and Syrah from the western part of Romania

Wine Assortment	Phenolic acids						
	Caffeic acid	T-caftaric acid	Chlorogenic acid	Elagic acid	Gallic acid	Succinic acid	Vanillic acid
Burgund mare	5.34±0.14	12.09±1.43	2.80±0.19	2.30±0.09	11.32±0.19	8.31±0.08	6.98±1.02
Cadarcă	9.30±0.09	15.35±0.10	4.99±0.10	1.44±0.15	14.31±0.05	4.33±0.05	8.10±0.04
Fetească Neagră	15.34±0.14	19.33±0.46	4.77±0.20	1.30±0.07	12.90±0.09	7.31±0.11	9.27±0.09
Novac	15.99±0.14	17.19±1.43	2.88±0.10	1.99±0.07	15.11±0.11	8.31±0.09	8.90±0.11
Syrah	13.09±0.13	16.09±1.22	3.12±0.01	1.99±0.08	12.40±0.09	5.30±0.10	8.30±0.09

T-caftaric acid is the most abundant phenolic non-flavonoid compound in grapes and wine, and ranges from 12.0945 mg / L (Burgund mare) to 19.3315 mg / L (Fetească Neagră). From the results obtained it is found that the acid is found at 15.3553 mg / l for the wine obtained from Cadarcă grapes, 16.0932 mg / L for the wine obtained from Syrah grapes and 17.1943 mg / l for Novac wine.

In the selected red wines, chlorogenic acid and elagic acid were also found which showed values between 2.8093 mg / L (Burgund mare) and 4.9945 mg / L (Cadarcă) and 1.3007 mg / L (Fetească Neagră) and 2.3097 mg / L (Burgund mare). Chlorogenic acid, a potent antioxidant, is a cinnamate ester formed by the condensation of the carboxyl group of t-caffeic acid with the hydroxyl group of quinic acid. It is found in berries, coffee, grapes and related by-products, along with another powerful antioxidant elagic acid.

Gallic acid is one of the acids found in most plants with antifungal and antiviral properties. It also acts as a powerful antioxidant with beneficial effects in treating various diseases such as cancer, internal haemorrhage, psoriasis, etc. The gallic acid values in this paper were at 11.3232 mg / L for wine obtained from the large Burgund mare variety, 30% more for Novac wine, while Fetească Neagră and Syrah showed values very close, namely 12.9005 mg / L and 12.4091 mg / L, respectively. In Cadarcă wine 14.3117 mg / L gallic acid was identified, 15% more than in Burgund mare wine.

Succinic acid and vanillic acid are antioxidants recognized by hepatoprotective effects, which are detected in amounts ranging from 4.3322 mg / L (Cadarcă) to 8.3195 mg / L (Burgund mare), 8.3117 mg / L (Novac) respectively 6.9891 mg / L (Burgund mare) at 9.2798 mg / L (Fetească Neagră). In the case of vanillic acid, the values found were very close to three wines, namely: 8.1053 mg / L, Novac 8.9017 mg / L and Syrah 8.3019 mg / L.

3.2. Chemicals used and quantitative detection flavonoids using HPLC

From this group, the study focused on the identification and quantification of flavonoids (Table 2): apigenin, luteinin; flavonols: kaempferol, myricetin, quercetin and flavanols: catechin, epicatechin.

Both apigenin and luteinin including their glycosidic forms are flavonoids with intense antibacterial, antifungal and antiviral action. They are found in plants, seeds, grapes, fruit, tea, wine, honey with recognized anti-infectious properties. Even if the values found in wines are reduced, their beneficial action on the body is well-known. Thus apigenin was in amounts ranging from 0.9999 mg / L (Cadarcă) to 4.0003 mg / L (Syrah). Other values have been identified for Burgund mare wines (1.3113 mg / L), Fetească neagră (3.3333 mg / L) and Novac (1.7728 mg / L). Luteolin showed the most significant values for Cadarcă wine (5.2223 mg / L), 40% lower for Fetească Neagră and Novac wines and very low for Burgund mare wines (2.0005 mg / L) and Syrah (2.0845 mg / L).

Flavonols (kaempferol, myricetin and quercetin) are flavonoids that are always present in the red wine formulation with beneficial effects on the human body through their antioxidant action. Positive results have been identified in cardiovascular disease, cancer, tumors, metastases, estrogenic activity [19]. In the wines studied, the values found were between 1.0045 mg / L (Cadarcă) and 2.8091 mg / L (Novac) for kaempferol, 2.0097 mg / L (Fetească neagră) and 2.8093 mg / L (Cadarcă) for myricetin and between 2.4432 mg / L (Fetească neagră) and 3.3197 mg / L (Burgund mare) for quercetin.

Table 2 Quantification of flavonoids in Burgund mare, Cadarcă, Black Feteasca, Novac and Sirah from the western part of Romania

Wine Assortment	Flavonoids						
	Flavones		Flavonols			Flavanols	
	Apigenin	Luteolina	Kaempferol	Myricetin	Quercetin	Catechin	Epicatechin
Burgund mare	1.31±0.01	2.00±0.03	1.01±0.01	2.30±0.01	3.31±0.09	32.30±1.09	42.31±0.09
Cadarcă	0.99±0.01	5.22±0.10	1.01±0.40	2.80±0.14	2.70±0.09	45.22±1.01	52.11±1.99
Fetească neagră	3.33±0.04	3.79±1.33	2.01±0.22	2.01±0.01	2.44±0.09	39.20±1.79	47.29±1.79
Novac	1.77±0.22	3.04±0.33	2.80±0.09	2.31±0.07	2.51±0.05	44.10±1.19	48.33±1.29
Syrah	4.00±0.14	2.08±0.43	1.89±0.07	2.30±0.05	3.10±0.08	56.16±1.99	51.80±1.79

The most significant flavonoids values were noted for the catechin and epicatechin flavanols. (+) Catechin and (-) epicatechin are known to be biologically effective antioxidants, being present in human diet, especially in wine and tea. For the wines studied catechin was 32.3097 mg / L (Burgund mare), followed by Fetească neagră with 39.2091 mg / L. Approximate values presented the Cadarcă and Novac wines with 45.2292 mg / L and 44.1007 mg / L, respectively. The most significant amount was recorded for Syrah wine with a value of 56.1617 mg / L. Epicatechin also showed significant values so that Cadarcă wine is noted with a quantum of 52.1117 mg / L followed by Syrah wine with 51.8015 mg / L. Slightly lower were the epicatechin values in Fetească Neagră and Novac wines, where the determined amounts were 47.2981 mg / L and 48.3399 mg / L respectively. The lowest level was found for Burgund mare wine where the epicatechin concentration did not exceed 42.3125 mg / L.

Phenolic acids, combined with flavonoids, give the wines under study a strong antioxidant character, especially Sirah, Fetească neagră and Novac, followed by Cadarcă and Burgund mare according to Figure 2.

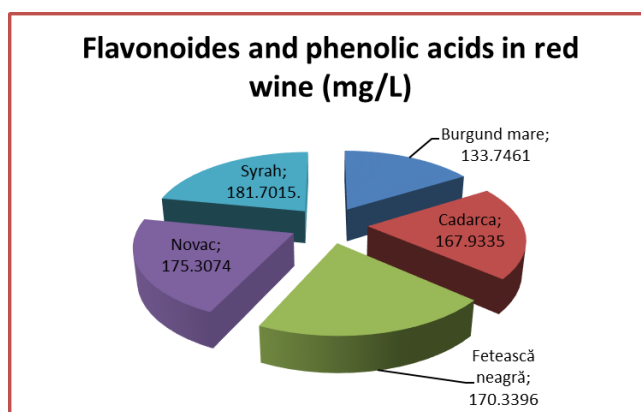


Figure 2 The sum of the phenolic compounds determined in the red wines such as: Burgund mare, Cadarcă, Fetească Neagră, Novac and Sirah from the western part of Romania

3.3. Chemicals used and quantitative detection of stilbenes using HPLC

One of the most important elements in red wine is resveratrol. The numerous studies have shown a number of beneficial effects in the prevention of cardiovascular diseases, chemoprotective, the antioxidant action being used in the case of cancerous diseases, but also in the immunological prophylaxis of the organism [11]. In the five wine varieties, resveratrol values were found and quantified between 5.8791 mg / L (Cadarcă) and 8.1427 mg / L (Fetească neagră), visible in Figure 3.

Novac and Syrah assortments showed values close for resveratrol, namely 6.3423 mg / L and 6.9945 mg / L respectively, and the Burgund mare reached up to 7.9924 mg / L.

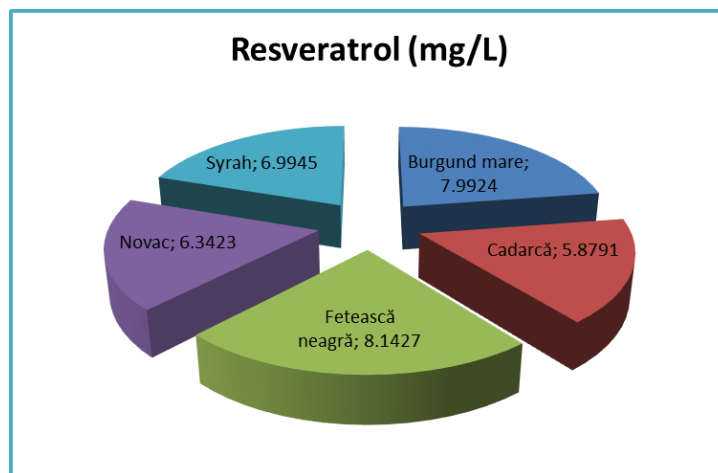


Figure 3 Resveratrol determined in the red wines such as: Burgund mare, Cadarcă, Fetească Neagră, Novac and Syrah from the western part of Romania

4. Conclusions

Following the results, one can say that there is a significant amount of phenolic compounds in red wines in the western part of the country, which can be capitalized to the advantage of the consumer. Phenolic acids were found to be close to those in the literature, especially caffeic acid and t-caftaric acid. The content of flavonoids is appreciable, notably the identified catechin and epicatechin values. Resveratrol, an active styling in red wines, is present in amounts that lead to the conclusion that these wines are suitable for consumption especially for the values of antioxidant elements sufficient for the human body. The ratio of existing compounds shows a balance that gives these wines special qualities and high nutritional value.

Compliance with ethical standards

Disclosure of conflict of interest

None to declare.

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