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# Importance of grape phenols in the human diet

**Urcan Delia Elena [1,2,*], Pop Nastasia [1], Rolle L.[2], Bora F.D.[3]**

[1] University of Agricultural Sciences and Veterinary Medicine, Cluj Napoca, Calea Mănăştur 3-5, 400372 Romania;[2] University of Turin, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy;[3] Research Station for Viticulture and Enology, Târgu Bujor, Galaţi, Romania;

*Corresponding author: delia.urcan@usamvcluj.ro

**Abstract** Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. The phenolic compounds, present in vegetables and fruits and their juices possess antioxidant activity that may have beneficial effects on human health. Moreover, they are responsible for certain organoleptic properties intimately related to wine quality, particularly color, astringency and bitterness. Proanthocyanidins and flavanols are located in the skin and seeds of grapes, and are transferred to the must/wine during the maceration step of winemaking. Numerous studies have shown that long term consumption of diets rich in plant polyphenols offer protection against development of cancers, cardiovascular diseases, osteoporosis, neurodegenerative diseases and diabetes. Red wine is a very rich source of flavonoids, particularly the class called flavanols. Flavonoids represent a large group of low molecular weight compounds with high antioxidant properties.

The purpose of this study was to evaluate the skins and seeds phenolic composition of three native Romanian wine grape varieties (Fetească regală, Fetească albă and Fetească neagră). The grapes were collected at technological maturity from three different growing zones of Transylvania (Romania) in 2011. Spectrophotometric methods were used to measure the absorbance at 280 nm ($A_{280}$), the flavanols reactive to vanillin and the proanthocyanidin indices in the skin and seed extracts. Among white varieties, the proanthocyanidins and flavanols concentrations in the skins were significantly higher in the Fetească regală variety. Furthermore, we assessed the distinct characteristics of Fetească neagră variety linked to their phenolic composition, and compared these characteristics with those of Pinot noir grapes. According to $A_{280}$, the red varieties contained more total skin polyphenols.
Fetească neagră was identified as a promising variety to be exploited in the future for its particular phenolic characteristics.

**Key words**

phenolic composition, proanthocyanidins, Romanian wine grape varieties, flavanols

Grape (*Vitis vinifera* L.) is the world's largest fruit crop with an annual production of more than 67 million tons of berries and more than 80% of the worldwide grape production is used in winemaking (6). They are important sources of antioxidants such as phenolic compounds (7). The composition and properties of grapes have been extensively investigated, and it was reported that grapes contain large amounts of phenolic compounds. The types and concentrations of the phenolic compounds depend on a number of factors: grape variety and ripening stage, soil and climatic conditions, vine cultivation and the treatment to which it is subjected (23). Polyphenols contained in grapes and wine can in general be classified into two main groups: flavonoid (anthocyanins, flavan-3-ols, condensed tannins and flavonols) and non-flavonoid compounds (phenolic acids and stilbenes). Moreover, these compounds have many favourable effects on human health such as the reduction of cancer and cardiovascular diseases (9, 22, 24). Besides the free radical scavenging and antioxidant activity, proanthocyanidins exhibit vasodilatory, anti-allergic, anti-inflammatory, antibacterial, immune-stimulating, anti-viral and estrogenic activities, as well as being inhibitors of the enzymes phospholipase $A_2$, cyclooxygenase and lipooxygenase (19, 15). However, after ingestion dietary phenols are modified and degraded in the gastrointestinal tract and appear in the circulatory system at low concentratio and in different chemical forms (4). The wine industry has a long history, wine being evaluated over time from a pleasure elixir to prestige drink, and product with beneficial effects on human health. Proanthocyanidins, also called condensed tannins, are transferred from the solid parts of the grape (skins, seeds, and stems) into the must during winemaking operations. Furthermore, proanthocyanidins constitute a complex mixture of monomers, oligomers and polymers which generally consists of (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin and their 3-O-gallic acid esters (14). Red wines are considered to have more protective effect than white and rosé wines, due to their higher content in antioxidant substances released from the grape skin and seeds (1, 20, 8). Red wine is typically consumed with food, which is a consideration for their derived health benefits. In addition to their important role as antioxidant, phenolics play a strong role in the organoleptic characteristics of wine, affecting color, bitterness and astringency (12). The grape seeds are a rich source of flavanols that are partially extracted during winemaking (10).

Due to their importance for human health as the most powerful natural antioxidants, the aim of this study was to examine the skins and seeds phenolic composition of three autochthonous wine grape varieties (Fetească albă, Fetească regală and Fetească neagră) from different growing zones of Romania. Furthermore, these varieties were compared to one of the most widely grown and recognized varieties, Pinot noir. These data may provide valuable information for the characterization of grape samples. Romania has a large number of wine grape varieties and grapevine cultivation represents a millenary tradition.

## Materials and Methods

### Biological material

Three Romanian wine grape varieties (Fetească regală, Fetească albă and Fetească neagră), and one international red wine grape variety (Pinot noir) were harvested in 2011 at technological maturity from three different growing zones of Transylvania, Romania. Fetească regală, Fetească neagră, and Pinot noir samples were harvested at the collection vineyard of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca; Fetească albă, Fetească regală, Fetească neagră, and Pinot noir samples were provided by a commercial vineyard located in Mica; and Fetească regală samples were harvested at another commercial vineyard located in Batoş. For each cultivar and vineyard, 200 berries were randomly sampled from at least ten plants from different parts of the cluster and with different solar exposures (shaded and sun-exposed). For each variety, the vines were grafted onto $SO_4$ rootstock, planted at 1.8 m × 1.2 m, vertical shoot positioned and cane pruned.

### Chemical analysis

Spectrophotometric methods were used to evaluate the absorbance at 280 nm (1/kg berries; $A_{280}$), as well as to determine the flavanols reactive to vanillin (mg (+)-catechin/kg berries) and the proanthocyanidin (mg cyanidin chloride/kg berries) indices in the skin and seed extracts (Rolle et al. 2011). A UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used. Three replicates of ten berries each were used for the determination of spectrophotometric indices related to the phenolic composition (5). Once the ten berries were weighed, the skins and seeds were carefully separated from the pulp using a laboratory spatula. The skins were weighed and quickly immersed into 25 mL of a hydroalcoholic buffer containing 2 g/L sodium metabisulfite, 5 g/L tartaric acid, and 12% v/v ethanol. The pH was adjusted to 3.2 by adding 1 M sodium hydroxide (Rolle et al., 2011). After homogenization at 8000 rpm for 1 minute with an UltraTurrax T25 (IKA Labortechnik, Staufen, Germany), the extract was centrifuged (3000 *g*, 15 min, 20°C). The supernatant was then used for skin analysis. The seeds were also immersed into 25 mL of the hydroalcoholic buffer and placed in a controlled-temperature room at 25°C for one week (20). The extract was then used for seed analysis.

### Statistical Analysis

Statistical analyses were performed using the statistical software package SPSS, version 19.0 (IBM Corporation, Armonk, NY, USA). The Tukey-b test was used to establish significant differences at $p < 0.05$ by one-way analysis of variance (ANOVA).

## Results and Discussions

The skins and seeds of grapes are known to be rich sources of phenolic compounds, both flavonoids and non-flavonoids (15, 11, 13). In the white grape varieties grown in Mica, shown in Table 1, the proanthocyanidins (1991 mg/kg berries) and flavanols (1387 mg/kg berries) concentrations in the skins were significantly higher in the Fetească regală variety, whereas those in the seeds were lower. In contrast, the Fetească albă variety had higher proanthocyanidins (1657 mg/kg berries) and flavanols (996 mg/kg berries) concentrations in the seeds than in the skins. In Fetească regală, higher proanthocyanidins and flavanols concentrations were observed in the skins, independently of the zone. Compared between zones, Fetească regală berries from the Mica zone had significantly higher total polyphenols, proanthocyanidins and flavanols concentrations in the skin than those from Batoş and Cluj.

The phenolic compositions of the berry skins and seeds of red varieties are shown in Table 2. The red varieties contained more total skin polyphenols than the white varieties, according to $A_{280}$. Significantly higher $A_{280}$ measurements were obtained for Fetească neagră skins, particularly those from the Cluj zone, compared with

Pinot noir skins. The Pinot noir variety was characterized by the highest concentrations of proanthocyanidins in the skins and seeds and of flavanols in the seeds from the two growing zones. However, the differences in proanthocyanidins concentrations among the two red varieties were not significant in the seeds from Cluj. Fetească neagră seeds from Cluj had significantly higher proanthocyanidins and flavanols concentrations than those from Mica. Pinot noir berries from Mica had significantly higher proanthocyanidins concentrations in the skins, whereas those from Cluj had increased flavanols concentrations in the seeds. The concentrations of flavan-3-ol in red grape skins were of the same order as those in white grape skins and these findings coincide with those reported by other authors (2, 3, 16).

## Conclusions

The phenolic composition of grapes depends on multiple factors, including climate, grapevine variety, degree of ripeness and berry size. However, it may be concluded that the skins and seeds of white grape varieties present a similar qualitative and quantitative composition to that of red grape varieties in terms of $A_{280}$, proanthocyanidins and flavanols. Romanian white and red grape varieties must therefore be considered a good source of phenols or natural antioxidant compounds of growing industrial importance. This study has also shown that Fetească regală and Fetească neagră grape skins and seeds have a very unique polyphenolic profile, with relatively high concentrations of proanthocyanidins and flavanols, particularly for those grown in Mica zone.

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**Table 1**

Skin and seed phenolic composition of white wine grape varieties

| Variety | Growing zone | Abs$_{280}$ index (l/kg berries) skins | Proanthocyanidin assay (mg/kg berries) skins | Proanthocyanidin assay (mg/kg berries) seeds | Flavanols (mg/kg berries) skins | Flavanols (mg/kg berries) seeds |
|---|---|---|---|---|---|---|
| Fetească albă | Mica | 21.6 ± 1.8 | 1218 ± 104 | 1657 ± 60 | 794 ± 87 | 996 ± 40 |
| Fetească regală | Batoş | 16.9 ± 1.3a | 1178 ± 96a | 1090 ± 139 | 850 ± 84a | 712 ± 74 |
| Fetească regală | Cluj | 19.3 ± 2.5a | 1382 ± 156a | 1170 ± 31 | 946 ± 118a | 737 ± 25 |
| Fetească regală | Mica | 26.8 ± 2.8b | 1991 ± 283b | 1060 ± 75 | 1387 ± 181b | 736 ± 56 |
| | *Sign.*[a] | ** | ** | ns | ** | ns |
| | *Sign.*[b] | ns | * | *** | ** | * |

Values are expressed as average ± standard deviation (n = 3). Different letters within the same column indicate significant differences (Tukey-b test; $p < 0.05$) among different growing zones for the same variety (a), between Fetească albă and Fetească regală varieties grown in the zone of Mica (b): *, **, *** and ns mean significance at $p < 0.05$, $p < 0.01$, $p<0.001$ and not significant, respectively.

**Table 2**

Skin and seed phenolic composition of black wine grape varieties

| Variety | Growing zone | Abs$_{280}$ index (A/kg berries) skins | Proanthocyanidin assay (mg/kg berries) skins | Proanthocyanidin assay (mg/kg berries) seeds | Flavanols (mg/kg berries) skins | Flavanols (mg/kg berries) seeds |
|---|---|---|---|---|---|---|
| Fetească neagră | Cluj | 51.9 ± 1.8 | 1368 ± 24 | 1834 ± 201 | 381 ± 47 | 1573 ± 95 |
| Fetească neagră | Mica | 47.2 ± 1.7 | 1336 ± 90 | 626 ± 80 | 467 ± 78 | 434 ± 43 |
| | *Sign.*[a] | * | ns | *** | ns | *** |
| Pinot noir | Cluj | 41.6 ± 2.8 | 2063 ± 60 | 2386 ± 523 | 771 ± 179 | 3864 ± 241 |
| Pinot noir | Mica | 42.8 ± 2.9 | 2468 ± 153 | 1856 ± 93 | 934 ± 149 | 2183 ± 276 |
| | *Sign.*[a] | ns | * | ns | ns | ** |
| | *Sign.*[b] | **, * | ***, *** | ns, *** | *, ** | ***, *** |

Values are expressed as average ± standard deviation (n = 3). Different letters within the same column indicate significant differences (Tukey-b test; $p < 0.05$) among different growing zones for the same variety (a), between Fetească neagră and Pinot noir varieties grown in the zones of Cluj and Mica (b): *, **, *** and ns mean significance at $p < 0.05$, $p < 0.01$, $p<0.001$ and not significant, respectively.