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Short title: Phenolics, Flavors and Texture of Romanian Grapes

ABSTRACT

In this work, the chemical compositions and texture characteristics of three native Romanian wine grape varieties (Fetească regală, Fetească albă and Fetească neagră) were studied. We assessed the distinct characteristics directly linked to their phenolic compositions, volatile profiles and mechanical properties and compared these characteristics with those of Pinot noir grapes. The effect of the growing zone was also evaluated. Various spectrophotometric indices directly related to the phenolic compositions of berry skins and seeds were determined. The detailed phenolic compositions (anthocyanins, hydroxycinnamoyl tartaric acids and stilbenes) of the skins were determined using high-performance liquid chromatography methods. Free and bound volatile compounds in the berries were quantified by gas chromatography/mass spectrometry. The textural properties of the skins and seeds were measured by instrumental texture analysis. The results showed high diversity among the varieties and zones that affected the enological potential. Among the white varieties, Fetească albă grapes could be less susceptible to browning as a consequence of their lower *trans*-caffeoyltartaric acid concentration, whereas Fetească regală grapes from Cluj had the highest concentrations of total free and bound volatile compounds, particularly terpenes and norisoprenoids. Among the red varieties, Fetească neagră was identified as a promising variety to be exploited in the future for its particular phenolic characteristics, particularly those grapes grown in Mica. Nevertheless, Fetească neagră grapes grown in Cluj had the highest total glycosidically bound terpene concentrations. Finally, differences in the mechanical and/or acoustic properties of the skins and seeds could strongly influence the kinetics and completeness of phenolic compound extractions.

Keywords: Anthocyanins, hydroxycinnamoyl tartaric acids, stilbenes, volatile compounds, texture properties, Romanian wine grape varieties

INTRODUCTION

Increasing competition in the wine market is promoting the production of varietal wines with distinctive characteristics that arise from using minor and/or native grape varieties that are historically associated with specific viticultural areas. The preservation of valuable traits genetically linked to endangered grape cultivars requires protecting these cultivars from potential extinction.

Although unknown as a wine-producing country, Romania has a long tradition in viticulture and a large number of wine grape varieties. The first traces of viticultural activity date back to the Neolithic and Bronze Age in Transylvania.[1] Today, wine holds an important place in the national economy. In Romania, a vineyard area of 177,661 hectares and a total grape production of 746,385 tons were reported by the Food and Agriculture Organization of the United Nations in 2012. Although knowledge of the secondary metabolites of these Romanian wine grape varieties is crucial to evaluate their enological potentials and to properly manage the vinification process, to date, there are no detailed scientific studies on this information.

In addition to compounds related to technological ripeness (sugars and acids), phenols extracted from the skin and seeds are the main compounds responsible for the quality of grapes and of corresponding wines, particularly for the red varieties. Each phenolic compound evolves differently during grape ripening, and the concentration is influenced by genetic, climatic and geographical factors, and cultural practices.[2-6] Profiles of different grape varieties at harvest for anthocyanins, flavan-3-ols, flavonols, dihydroxyflavonols, hydroxycinnamoyl tartaric acids (HCTs), hydroxybenzoic acids and hydroxystilbenes in the skins,[7-9] polymeric and oligomeric flavan-3-ols in the skins and seeds,[3,10] and HCTs in the pulp have been reported.[11] Anthocyanins are responsible for the skin colour of red grape varieties,[12] as well as for the colour of young red wines.[13] Furthermore, these compounds can form pigmented polymers with flavan-3-ols, providing long-term colour stability to wine.[14] Flavan-3-ols strongly influence the bitterness and astringency of wine.[15] Flavonols not only directly contribute to the colour of white wines but also affect the colour of red wines by copigmentation.[16] HCTs are the most abundant non-flavonoid phenols in grapes and wines, are involved in the browning reactions of must and wine, and are precursors of volatile phenols.[17] The beneficial effects of biologically active phenolic compounds on human health

due to their antioxidant properties have prompted a growing number of investigations in wine grapes and the resulting monovarietal wines.[10]

The final amount of phenols extracted from grapes into wine depends on the molecular structure and chemical composition of the skin cell wall.[18] Other influencing factors include dehydration of the outer integument, lignification of the medium integument, and oxidative coupling of flavan-3-ol monomers and procyanidins to cross-link wall components of the seeds.[19] Instrumental mechanical and acoustic properties sufficiently explain the resistance of grape skin and seeds to the release of phenolic compounds. Therefore, instrumental texture parameters can be considered as important grape quality markers.[20,21]

Finally, the concentrations and profiles of the free and bound aroma compounds of grapes are other quality parameters involved in varietal characterization because they contribute to the discrimination of wines.[22] In addition to the cultivar, several factors such as the growing location, climate and, particularly the ripening stage, influence the varietal and pre-fermentative volatiles of grapes.[23]

Given the lack of scientific literature on the chemical composition and texture properties of Romanian white and red wine grape cultivars, the purpose of this study was to evaluate the phenolic, volatile and textural characteristics of three native grape varieties (Fetească regală, Fetească albă and Fetească neagră) from different growing zones. These varieties have not been previously characterized. Furthermore, these varieties were compared to one of the most widely grown and recognized varieties (Pinot noir). Total phenols, flavan-3-ols, anthocyanins, HCTs and stilbenes were determined at harvest using spectrophotometric and high-performance liquid chromatography (HPLC) methods. Free and bound volatile compounds were quantified by gas chromatography/mass spectrometry (GC/MS). Texture analysis, which is an analytical technique currently used for the instrumental measurement of mechanical and acoustic properties of fruits, was used to determine the skin and seed textural characteristics.

MATERIALS AND METHODS

Grape samples

Two Romanian white wine grape varieties (Fetească regală and Fetească albă), one Romanian red wine grape variety (Fetească neagră) and one international red wine grape variety (Pinot noir) were collected at technological maturity from three different growing zones of Transylvania (Romania) in 2011. 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 harvested at a commercial vineyard located in Mica; and Fetească regală samples were harvested at another commercial vineyard located in Batoş.

Planted over 25 years ago, the collection vineyard of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca is located at 46° 45' N latitude and 23° 34' E longitude. It has an altitude of 365 m and a northern exposure. The annual average temperature is 9.3 °C, the annual precipitation is approximately 445 mm, the frost-free period lasts 176 days, and the sunshine lasts 1157 h. The soil is a preluvosoil with stagnic properties, a texture ranging from clay loam to silty clay and silt loam, and a pH of 6.09. The 6-years-old Mica vineyard is located at 46° 22' N latitude, 24° 25' E longitude and 370 m altitude on the right side of the Târnava Mică river and has a uniform slope with southern, southeastern and southwestern exposures. The annual average temperature is 10.2 °C, the annual precipitation totals 461 mm, the frost-free period lasts 176 days, and the sunshine lasts 1367 h. The soil, formed during the age of the Sarmatian-Pannonian transition, comprises marly clays and marls with sandy intercalations. The soils formed on these deposits are fine-textured clay and clay loam with weak internal drainage. Batoş, the youngest vineyard, is 4-years-old, is located at 46° 53' N latitude, 24° 40' E longitude and 490 m altitude, and has a temperate continental climate. The annual average temperature is 10.4 °C, the annual precipitation totals 395 mm, the frost-free period lasts 179 days, and the sunshine lasts 1258 h. The Batoş vineyard soil type is argic chernozems with a pH ranging from 5.33 to 5.40.[24] Meteorological data were recorded by automatic weather stations located in the vineyards.

The Fetească albă variety, which resulted from popular selections of Fetească neagră, was stabilized in culture between 1100 and 1150.[25] The Fetească regală variety was selected in

1920 in the village of Daneș from the natural hybridization of Fetească albă and Grasă de Cotnari. Fetească neagră, which is a very old variety dating back to the Dacian period and originating in the village of Uricani, resulted from a popular selection of *Vitis sylvestris*.[25] In the three vineyards, the vines were grafted onto SO_4 rootstock, planted at 1.8 m x 1.2 m, vertical shoot positioned and cane pruned.

For each cultivar and vineyard, five hundred berries were randomly sampled from at least ten plants from different parts of the cluster (shoulders, middle and bottom) and with different solar exposures (shaded and sun-exposed). Various sets of berries were randomly selected to determine texture properties and chemical composition. The remaining berries from each cultivar and vineyard, which were subdivided into three replicates, were used to determine the technological ripeness parameters in the grape must obtained by manual crushing and centrifugation.

Chemical analysis

Reagents and standards: HPLC gradient-grade solvents and all other analytical reagent-grade chemicals were purchased from Sigma (Milan, Italy). The solutions were prepared in deionized water produced by a Purelab Classic system (Elga Labwater, Marlow, UK). Standards for (+)-catechin, cyanidin chloride, anthocyanins and stilbenes were supplied by Extrasynthèse (Genay, France), volatiles were obtained from Sigma (Milan, Italy) and HCTs were purchased from Fluka (Buchs, Switzerland).

Technological ripeness parameters: pH was determined by potentiometry using an InoLab 730 pH meter (WTW, Weilheim, Germany), and titratable acidity (g/L tartaric acid, as TA) was estimated using the OIV method. Reducing sugars (glucose and fructose) and organic acids (citric acid, tartaric acid and malic acid) (g/L) were determined isocratically using an HPLC system equipped with a refractive index detector and a diode array detector (DAD) set to 210 nm, respectively.[26]

Spectrophotometric methods: Three replicates of 10 berries each were used for the determination of spectrophotometric indices related to the phenolic composition.[27] Once the 10 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 5 g/L tartaric acid, 2 g/L sodium metabisulfite and 12% v/v ethanol, and the pH was adjusted to 3.2 by adding 1 M sodium hydroxide.[28] The skins were then homogenized at 8000 rpm for 1 min with an Ultraturrax T25 high-speed homogenizer (IKA Labortechnik, Staufen, Germany) and centrifuged for 15 min at 3000×g at 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.[29] The extract was then used for seed analysis.

Spectrophotometric methods were used to measure the absorbance at 280 nm (1/kg berries; A_{280}) and the total anthocyanin index (mg malvidin-3-O-glucoside chloride/kg berries or g skins; TAI) in the berry skin, as well as to determine the flavanols reactive to vanillin (mg (+)-catechin/kg berries; FRV) and the proanthocyanidin (mg cyanidin chloride/kg berries; PRO) indices in the skin and seed extracts.[28] A UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used.

Anthocyanins: The anthocyanin profile was determined after one aliquot of the berry skin extracts prepared for the spectrophotometric measurements was treated by reverse-phase solid-phase extraction (RP-SPE). A 1-g Sep-Pak C-18 cartridge (Waters Corporation, Milford, MA, USA) was used, with methanol as the eluent.[28] The HPLC-DAD system and chromatographic conditions have been previously reported in the literature.[28] A LiChroCART analytical column (25 cm × 0.4 cm i.d.) purchased from Merck (Darmstadt, Germany), which was packed with LiChrospher 100 RP-18 (5 μm) particles supplied by Alltech (Deerfield, IL, USA), was used. The mobile phases were as follows, with a flow rate of 1 mL/min: A = formic acid/water (10:90, v/v) and B = formic acid/methanol/water (10:50:40, v/v). A linear gradient was used, starting at 72% A and decreasing to 55% A in 15 min, 30% A in 20 min, 10% A in 10 min, and 1% A in 5 min, and then returning to 72% A in 3 min. After the identification, the individual anthocyanin percentages were calculated by comparing the area of the appropriate peak with the total peak area. All analyses were performed in duplicate.

Hydroxycinnamoyl tartaric acids: Three replicates of 10 berries each were processed as described by Ferrandino and Guidoni.[30] Briefly, each replicate was weighed, and the skins were manually separated from the seeds and pulps. The skins were quickly immersed into a hydroalcoholic solution buffered at pH 3.2 containing 2 g/L sodium metabisulfite and 12%

v/v ethanol, and incubated for 72 h at 30 °C. The berry skin extract was then diluted 1.1-fold with 1 M phosphoric acid and filtered through 0.2-µm GHP membrane filters (Pall Corporation, New York, NY, USA).

The HPLC-DAD system and chromatographic conditions have been previously reported in the literature.[7] The above-mentioned analytical column used for anthocyanins was again used to determine the HCTs. The mobile phases were as follows: A = 10^{-3} M phosphoric acid and B = methanol. A linear gradient was established between 5 and 100% B over 49 min at a flow rate of 0.48 mL/min. After the identification, p-coumaroyl- and caffeoyltartaric acids were quantified as p-coumaric acid equivalents, and *trans*-feruloyltartaric acid was quantified as a ferulic acid equivalent. The results were multiplied by the ratio between the molecular weight of each compound and the molecular weight of p-coumaric acid for the p-coumaroyl and caffeoyl derivatives, and of ferulic acid for the feruloyl derivative. The total HCT concentration (mg/kg berries) was calculated as the sum of the concentrations of the individual compounds. The individual HCT percentages were calculated by comparing the concentration of each compound with the total HCT concentration. All analyses were performed in duplicate.

Stilbenes: Two replicates of 20 berries each were used. For each replicate, the skins were manually removed from frozen berries and then weighed and freeze-dried. One gram of freeze-dried skins was treated as reported by Vincenzi et al.[9] Briefly, the skins were immersed into a solution containing 40 mL methanol, 50 µL hydrochloric acid and 250 µL of an internal standard (*trans*-hydroxystilbene, 200 mg/L in ethanol). After homogenization for 1 min with Ultraturrax, the sample was stirred for 48 h at room temperature in the dark. The polyphenol-containing solution and the methanolic washing solutions were recovered by centrifugation (5000×g, 5 min) and then mixed. The extract was almost completely evaporated to dryness using a vacuum rotavapor (Buchi R-210, Switzerland) at 35 °C. The obtained residue was suspended in 20 mL water, and stilbene compounds were extracted twice for 15 min with 10 mL ethyl acetate. The upper organic phase containing stilbenes was carefully recovered. The ethyl acetate fraction was dried by adding anhydrous sodium sulfate, filtered through Whatman 589/3 paper and completely evaporated to dryness under a vacuum at 35 °C. The residue was then dissolved in 2 mL methanol and 50 mM formic acid (1:1, v/v), and the extract was centrifuged at 14000×g for 10 min.

The HPLC-Dual Band UV system and chromatographic conditions have been previously reported in the literature.[9] Stilbenes were separated on the analytical column previously described for the anthocyanins and HCTs. The following mobile phases were used: A = 50 mM formic acid and B = methanol. A linear gradient was used, starting at 0% B and increasing to 10% B in 3 min, 30% B in 5 min, 44% B in 35 min, 55% B in 2 min, 75% B in 15 min, and 100% B in 1 min, and then returning to 0% B in 3 min. The flow rate was 1.0 mL/min, and the column temperature was set to 40 °C. The identification of *cis*-isomers required exposure of the corresponding *trans*-molecules to UV light for 1 min. For the quantification of *cis*-piceid, the same extinction coefficient of the respective *trans* form was assumed. The amounts of individual stilbenes were expressed as concentrations (µg/g skin). All analyses were performed in duplicate.

Free and glycosylated volatile compounds: Two hundred berries were weighed and processed following the procedure proposed by Di Stefano and summarized by Rolle et al.[31] The berries were deseeded, and the pulp was manually separated from the skin. Sodium metabisulfite (50 mg) was added to the pulps, whereas the skins were treated with 20 mL of methanol for 1 h. The pulps and skins were crushed separately under a nitrogen atmosphere with a laboratory blender (Waring Laboratory, Torrington, CT, USA). The skin suspension and pulp homogenate were then combined. The mixture was centrifuged twice (7000×g, 15 min, 4 °C) to wash the solid residue with tartaric acid buffer (pH 3.2). The extract (250 mL) was then clarified with a pectolytic enzyme (100 mg) without secondary glycosidase activity (Rapidase X-Press, DSM, The Netherlands) at room temperature for 2 h. 1-Heptanol was added to the sample as an internal standard (200 µL, 44 mg/L in 10% v/v ethanol). An aliquot (100 mL for white grapes or 50 mL for red grapes; n=2) was then loaded onto a 1-g Sep-Pak C-18 RP-SPE cartridge (Waters Corporation). The free fraction was eluted with 12 mL dichloromethane. The eluate was then dried over anhydrous sodium sulfate and concentrated to approximately 200 µL under a stream of nitrogen. The extract containing free volatile compounds was immediately analyzed by GC-MS.

The glycoconjugates were finally eluted from the cartridge with 20 mL of methanol, and the eluate was concentrated to dryness under a vacuum at 35 °C. The dried glycosidic extract was dissolved in 3 mL of citrate-phosphate buffer (0.2 M, pH 5). Enzymatic hydrolysis was carried out using 50 mg of an AR-2000 commercial preparation with glycosidase side activities (DSM Oenology, The Netherlands) and incubated at 40 °C for 24 h. After adding

200 µL 1-heptanol (44 mg/L in 10% v/v ethanol), glycosylated precursors were extracted following the SPE method, as previously described. The dichloromethane extract obtained was dried over anhydrous sodium sulfate, concentrated to 200 µL under nitrogen and kept at -20° C until analysis.

The GC-MS system and chromatographic conditions have been previously reported by Rolle et al.[31] A DB-WAXETR capillary column (30 m × 0.25 mm, 0.25 µm; J&W Scientific Inc., Folsom, CA, USA) was used. The injection port temperature was 250 °C, the ion source temperature was 240 °C, and the interface temperature was 230 °C (solvent delay of 6.5 min). The detection was carried out by electron impact mass spectrometry in total ion current (TIC) mode using an ionization energy of 70 eV. The mass acquisition range was *m/z* 30-330. Semiquantitative data (µg/kg berries) were obtained by measuring the relative peak area of each identified compound in relation to that of the added internal standard. All analyses were performed in duplicate.

Instrumental texture analysis: A Universal Testing Machine (UTM) TA.XTplus texture analyzer (Stable Micro Systems, Godalming, Surrey, UK), equipped with an HDP/90 platform, was used for skin and seed textural analysis. All data were acquired at 500 points per second. Typical deformation curves for the different tests performed on the wine grapes have been shown in detail in previously published studies.[21,32] The mechanical and acoustic properties were calculated from the corresponding curves using the Texture Exponent software package (Stable Micro Systems). Before each test session, the instrument was calibrated for force, distance and acoustic emission.

One set of 20 berries was used to determine the mechanical properties of the skin. Skin hardness was assessed by a puncture test using an SMS P/2N needle probe (Stable Micro Systems), a 5 kg load cell, a test speed of 1 mm/s and an applied penetration of 3 mm.[20] Each berry was individually punctured in the lateral face, and the following three skin parameters were measured: break force (N, as F_{sk}), break energy (mJ, as W_{sk}) and resistance to axial deformation (N/mm, as E_{sk}). A piece of skin (ca. 0.25 cm^2) was then manually separated from the lateral side of each berry with a razor blade, and skin thickness (µm, as Sp_{sk}) was measured by a compression test. The test was carried out using a 2-mm SMS P/2 flat cylindrical probe, a 5 kg load cell and a test speed of 0.2 mm/s.[32]

Another set of 30 berries was used to determine the mechanical and acoustic properties of the seeds. The mechanical properties were determined by a compression test using an SMS P/35 probe and a 50 kg load cell.[21] One seed per berry was carefully separated from the pulp and individually compressed (1 mm/s speed and 50% deformation). The following seed mechanical parameters were measured or calculated: break force (N, as F_s), break energy (mJ, as W_s), resistance to axial deformation (N/mm, as E_s) and deformation index (%, as DI_s). The acoustic emission produced during the compression test was measured using an acoustic envelope detector (AED) (Stable Micro Systems) equipped with a 12.7 mm-diameter Brüel & Kjær 4188-A-021 microphone (Nærum, DK). The recording of the acoustic emission produced was carried out at an instrumental gain SPL value of 0 using a built-in 3.125 KHz high-pass filter. The following acoustic parameters were measured: the acoustic pressure level at breakage (dB), the maximum acoustic pressure level (dB) and the total acoustic energy (dB × mm, as AE).[21]

Statistical analysis

Statistical analyses were performed using SPSS Statistics software, 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

The technological ripeness parameters for the white and red wine grapes from the different zones of Transylvania are shown in Table 1. The Fetească albă and Pinot noir grapes were the richest in sugars and had the highest pH values. The lowest titratable acidity values were found for the Fetească albă and Pinot noir varieties from the Mica zone, whereas the highest values were observed for Fetească neagră from both the Mica and Cluj zones. Regarding acid composition, Fetească albă and Pinot noir exhibited the lowest tartaric acid concentrations. Furthermore, the Pinot noir variety from the Cluj zone showed significantly lower malic acid concentrations than Fetească neagră. The differences in these parameters were also evaluated among the growing zones for each variety. For the Fetească regală variety, the grapes from the Cluj zone had significantly lower sugar concentrations compared with those from Batoş and Mica, and those from Mica had significantly lower malic acid concentrations. The Fetească neagră grapes from Mica had significantly lower tartaric acid concentrations than

those from Cluj, whereas the Pinot noir grapes from Mica were richer in tartaric acid. For each variety, the pH values were zone independent. Furthermore, the citric acid concentrations were not influenced by the zone or variety.

The average berry and berry skin weights influence the concentrations of important skin metabolites. In the white varieties, the smallest berries corresponded to Fetească albă from Mica. Fetească neagră berries from Mica were significantly smaller and had less skin weight than those from Cluj. A possible explanation for the smaller berries produced in the Mica zone may be the weak internal drainage of this vineyard soil. However, this effect was not observed in the Pinot noir berries. Among the red varieties, Pinot noir from Cluj had significantly smaller berries and less skin weight than Fetească neagră. A positive correlation was found between the berry weight and the tartaric and malic acid concentrations (Pearson's correlation coefficients of 0.592 and $p \leq 0.002$; and 0.701 and $p \leq 0.001$, respectively).

The phenolic compositions of the berry skins and seeds 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. This finding may be due to the significantly higher total anthocyanin concentrations in Fetească neagră; additionally, this variety from Cluj exhibited significantly higher total anthocyanin concentrations compared with that from Mica (Table 3). The Pinot noir variety was characterized by the highest concentrations of PRO in the skins and seeds and of FRV in the seeds from the two growing zones. However, the differences in PRO concentrations among the two red varieties were not significant in the seeds from Cluj. In the white grape varieties grown in Mica, the PRO and FRV 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 PRO and FRV concentrations in the seeds than in the skins. In Fetească regală, higher PRO and FRV 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, PRO and FRV concentrations in the skin than those from Batoş and Cluj. Fetească neagră seeds from Cluj had significantly higher PRO and FRV concentrations than those from Mica. Pinot noir berries from Mica had significantly higher PRO concentrations in the skins, whereas those from Cluj had increased FRV concentrations in the seeds.

Because the anthocyanin profile is a chemotaxonomic characteristic of a variety,[4,7] the relative amount of each individual anthocyanin compound significantly differed among the red varieties studied, with very few exceptions (Table 3). Nevertheless, malvidin derivatives were the predominant anthocyanin compounds (53.2-69.3%) for Fetească neagră and Pinot noir. Peonidin derivatives were generally the second most abundant anthocyanins (17.7-27.7%), with the exception of Fetească neagră from Mica (6.9%). Furthermore, these anthocyanin compounds in the Pinot noir variety were observed almost exclusively in their free forms (simple glucosides or unacylated); in the Fetească neagră variety, the free forms of these compounds were highly abundant. In agreement with previous studies,[4] the anthocyanin profile significantly differed as a function of the growing zone for each variety (Table 3). The Fetească neagră and Pinot noir grapes from Mica showed lower percentages of 3′-hydroxylated molecules, such as cyanidin derivatives, but higher proportions of stable forms of anthocyanins, such as malvidin derivatives, than those from Cluj. For each variety, other anthocyanin compounds such as the petunidin and peonidin derivatives of Fetească neagră and the delphinidin and petunidin derivatives of Pinot noir were zone dependent.

The total HCT concentrations and profiles in the skins are shown in Table 4. The total skin HCT concentrations ranged from 94 mg/kg (Fetească albă from Mica) to 252 mg/kg (Fetească neagră from Cluj). The individual HCTs identified were *trans*-caffeoyltartaric acid, *cis*- and *trans*-p-coumaroyltartaric acids and *trans*-feruloyltartaric acid. The main HCTs were *trans*-caffeoyltartaric acid and *trans*-p-coumaroyltartaric acid, which accounted for percentages ranging from 50.3% to 78.5% and from 18.0% to 35.4% of the total HCTs, respectively. With some exceptions, the total skin HCT concentrations and individual compound percentages were dependent on the variety and growing zone. The zone effect was practically negligible for the Pinot noir variety. For each variety grown in Mica, the proportion of *trans*-caffeoyltartaric acid was lower, and the proportions of *trans*-p-coumaroyltartaric acid and *trans*-feruloyltartaric acid were higher than in the other zones studied; however, the differences were not always significant. The Fetească regală variety from Mica had significantly higher total skin HCT concentrations than that from Batoş and Cluj. Nevertheless, the total HCT concentrations were significantly lower in the Fetească neagră grapes from Mica. Furthermore, the percentage of *cis*-p-coumaroyltartaric acid was significantly higher in the Fetească neagră variety from Mica. The ratio of the sum of the p-coumaroyltartaric acids to that of the *trans*-caffeoyltartaric acids was always < 1, despite the variety and zone effects.

The concentrations of resveratrol and some related stilbenes identified in the skins of the different wine grape varieties studied in the various zones of Transylvania are shown in Table 5. *trans*-Resveratrol was the only stilbene detected in all varieties and zones, and was the major compound identified in all varieties, with some exceptions. Although the highest concentrations of *trans*-resveratrol were observed in the skin of the white wine grapes (41.8-74.7 μg/g skin), *trans*-piceatannol was the predominant stilbene in the Fetească regală grapes from Mica, which is the only location where it was detected. The Fetească neagră grapes from Cluj predominantly had *trans*-piceid, whereas this compound was not detected in this same variety grown in Mica and was present in lower amounts in the other varieties studied. *cis*-Piceid was detected only in the red varieties, and its highest concentrations were found in the Fetească neagră and Pinot noir wine grapes from the Cluj zone. However, this stilbene compound was not detected in Pinot noir grown in Mica. Variety and zone effects were not evident for the skin stilbenes.

A total of 24 free and bound volatile compounds were identified and quantified in white and red wine grapes from different zones of Transylvania (Tables 6 and 7, respectively). The free compounds detected in the white varieties were as follows (Table 6): one C6 aldehyde ((*E*)-2-hexenal), four alcohols (1-hexanol, (*E*)-2-hexen-1-ol, benzyl alcohol, and 2-phenyl ethanol), three monoterpenes (linalool, α-terpineol, and 1-hydroxy linalool), two C6 acids (hexanoic acid and (*E*)-2-hexenoic acid) and one phenol (4-vinilguaiacol). Hexanoic acid was the most abundant free volatile compound in the Fetească albă variety, followed by 1-hexanol and 2-phenyl ethanol. Hexanoic acid represented approximately 62% of the total free volatile compound concentration and was significantly more abundant in the Fetească albă grapes than in the Fetească regală grapes from Mica. The Fetească regală grapes were characterized by a prevalence of C6 alcohols and C6 acids (1-hexanol, (*E*)-2-hexen-1-ol and hexanoic acid). However, (*E*)-2-hexenoic acid, representing 31% of the total free volatiles, was the predominant free volatile compound detected in the Fetească regală variety from Batoş and Cluj, whereas 2-phenyl ethanol was the main compound (22%) identified in that from Mica. Furthermore, linalool and 4-vinilguaiacol were also major free compounds found in the Fetească regală grapes from Mica, whereas 2-phenyl ethanol was major in those from Cluj. Some C6 compounds, such as 1-hexanol, hexanoic acid and (*E*)-2-hexenoic acid, permitted the differentiation of the Fetească regală grapes from the various growing zones. Among the

monoterpenes, linalool was the only free compound present in all samples, and α-terpineol and 1-hydroxy linalool were typical of the Fetească regală variety grown in Mica.

With the exception of linalool, α-terpineol and 1-hydroxy linalool, the same free volatile compounds were detected in the red wine grape varieties. However, isoamyl alcohol, (*Z*)-3-hexen-1-ol and *trans*-pyranic linalool oxide were found only in the red varieties (Table 7). Hexanoic acid and 1-hexanol were the predominant free volatile compounds in the Fetească neagră grapes from Cluj, with abundances of 50% and 27% of the total free volatile concentration; in those from Mica, the abundances were 40% and 28%, respectively. Furthermore, (*E*)-2-hexen-1-ol was a major free compound in the Fetească neagră grapes from Mica. The Pinot noir grapes from Cluj and Mica showed a predominance of 1-hexanol (27% and 33%, respectively), although substantial concentrations of 2-phenyl ethanol and hexanoic acid were also detected. (*E*)-2-Hexen-1-ol and benzyl alcohol were also major compounds in the Pinot noir berries from Mica. For the red varieties studied, the only significant zone effect on the free volatile composition corresponded to (*E*)-2-hexenoic acid in the Pinot noir berries. Isoamyl alcohol was typical of the Pinot noir variety. The varietal differences were dependent on the zone studied but were significant for the concentrations of (*Z*)-3-hexen-1-ol in grapes from Mica and of (*E*)-2-hexenal, 1-hexanol, hexanoic acid and 2-phenyl ethanol in those from Cluj. Among the terpenes, *trans*-pyranic linalool oxide was the only compound found in the Fetească neagră variety grown in the Cluj zone.

In the white varieties, a total of 21 bound volatile compounds were identified and quantified (Table 6). Only three compounds of all of the volatiles shown in Table 6 were not detected in their bound form (hexanoic acid, (*E*)-2-hexenoic acid and 1-hydroxy linalool). 2-Phenyl ethanol, diol 1 (2,6-dimethyl-3,7-octadien-2,6-diol) and diol 2 (2,6-dimethyl-1,7-octadien-3,6-diol) were the major glycosidically bound volatile compounds detected; these three compounds represented between 43% and 53% of the total bound volatile compound concentrations. However, the Fetească regală grapes from Cluj were characterized by a very high benzyl alcohol concentration (33%). One bound volatile, (*E*)-2-hexenal, was exclusively found in the Fetească albă grapes, whereas α-terpineol was typical of Fetească regală. Among the terpenes, in addition to diol 1 and diol 2, linalool was the only other bound volatile compound found at high concentrations (> 50 μg/kg) in all samples. Additionally, *trans*-pyranic linalool oxide and nerol were detected at high concentrations in the Fetească regală grapes from Cluj and Batoş, respectively.

In the red varieties, only 17 bound volatile compounds were quantified; (*E*)-2-hexenal, α-terpineol, *cis*-pyranic linalool oxide, hexanoic acid, (*E*)-2-hexenoic acid, 1-hydroxy linalool and dihydro-β-ionone were not detected (Table 7). Benzyl alcohol was the predominant bound volatile, accounting for 25-35% of the total bound volatile compound concentration in Fetească neagră and 47-49% of that in Pinot noir, followed by 2-phenyl ethanol and 1-hexanol in Fetească neagră from Mica and in Pinot noir from Cluj and Mica. The Fetească neagră grapes from Cluj also showed high concentrations of two terpenes (diol 1 and diol 2). The proportion of total norisoprenoids (3-hydroxy-β-damascenone, 3-oxo-α-ionol and dihydro-β-ionone) of the total bound volatiles was low, ranging from 6.3% to 13.2% in the white varieties and from 3.7% to 9.6% in the red varieties.

The concentrations of the different bound volatile compounds were significantly influenced by the variety and/or zone. Taking into account the volatiles detected in all zones studied for each variety, significant differences among zones were found in 55% (11 compounds) for Fetească regală, 36% (5 compounds) for Fetească neagră and 62% (8 compounds) for Pinot noir. Considering the volatiles detected in the two varieties compared in each zone, significant differences were found in 53% (9 compounds) among the white varieties from Mica, 83% (10 compounds) among the red varieties from Cluj and 31% (4 compounds) among the red varieties from Mica. Terpenes are strongly variety dependent; linalool, *trans*-pyranic linalool oxide, nerol and diol 1 permitted the differentiation between the zones and varieties of white wine grapes. However, only diol 1 together with 4-vinilguaiacol permitted the differentiation of red grapes.

Table 8 shows the berry skin and seed texture parameters for the varieties and zones studied. The Fetească albă variety was characterized as having the lowest values of the mechanical properties defining skin hardness (F_{sk}, W_{sk}) and stiffness (E_{sk}), followed by Fetească regală in relation to the F_{sk} and E_{sk} parameters. The skin thickness (Sp_{sk}) ranged from 168 to 208 μm. Particularly for grapes grown in Mica, F_{sk} and W_{sk} permitted the differentiation between varieties with the same skin colour (within the white and red grapes), and significantly higher skin hardness was observed for Fetească regală and Pinot noir, respectively. Among the red varieties grown in Cluj, significantly higher F_{sk}, E_{sk} and Sp_{sk} values were found for Fetească neagră. The zone effect depended on the variety. For the Fetească regală variety, the berry skins from Mica were significantly harder (higher W_{sk}), springier (lower E_{sk}) and thicker

(higher Sp_{sk}) than those from Batoş and Cluj. The Pinot noir berries grown in Mica had significantly harder (higher F_{sk} and W_{sk}) and springier skins than those grown in Cluj. However, the Fetească neagră berries from Cluj were characterized by significantly harder (higher F_{sk} and W_{sk}), stiffer (higher E_{sk}) and thicker skins.

The texture parameters of the seeds were zone dependent, particularly for the red wine grapes (Table 8). Significantly harder (higher F_s and W_s), stiffer (higher E_s) and crunchier (higher acoustic pressure level at breakage and maximum acoustic pressure level) seeds were found for the Fetească neagră variety from Mica compared with that from Cluj. Significantly harder and springier (higher DI_s) seeds were observed in the Pinot noir berries grown in Cluj. Within the same zone, variety effects were also observed. In the Mica vineyard, comparing between white wine grape varieties, the Fetească regală seeds had significantly higher F_s values than the Fetească albă seeds. Among the red wine grape varieties from Mica, Fetească neagră seeds showed significantly higher F_s, W_s and E_s values, acoustic pressure level at breakage and total acoustic energy compared with the Pinot noir seeds. For the Pinot noir seeds from Cluj, the acoustic pressure level at breakage and the maximum acoustic pressure level were significantly higher than the Fetească neagră seeds.

DISCUSSION

The technological ripeness parameters were influenced not only by the variety but also by the growing zone; this finding is in accordance with other studies.[33] Although differences were found in the sugar concentrations and acid compositions of the berries among different varieties or growing zones, all of the varieties achieved a good maturity level.

The phenolic compositions of berry skins and seeds were strongly influenced by the variety and growing zone.[3,34] The PRO and FRV concentrations found in the skins and seeds of the native Romanian wine grapes (Table 2) were within the ranges published for Italian varieties.[28,34] The PRO and FRV concentrations in the skins and seeds were lower for the native Romanian wine grapes compared with the international Pinot noir variety grown in the same zone, with the exception of FRV in the skins of the Fetească albă and Fetească regală white wine grape varieties. The PRO concentration provides relevant information because this spectrophotometric index is mainly related to the concentration of high-molecular-weight proanthocyanidins (> 5 units). FRV is sensitive to the presence of monomeric flavanols, and

this index is partially related to the concentration of low-molecular-weight proanthocyanidins with a polymerization degree ranging from 2 to 4.[35] Because oligomeric flavanols represent the main phenolic fraction released from the seeds during winemaking, the native Romanian wine grapes may produce wines with lower astringency and bitterness than Pinot noir.[36] In red varieties, flavanols are also of great relevance to the colour of the final product because the formation of flavanol-anthocyanin complexes promotes long-term colour stability. Ristic et al.[37] reported that higher anthocyanin and skin flavanol concentrations, together with a lower seed flavanol concentration, are associated with higher wine quality through pigmented polymer formation. TAI concentrations in the Fetească neagră variety (Table 3) were similar to those in other coloured varieties such as Nebbiolo and Barbera.[7] Thus, Fetească neagră may be a promising variety to be exploited in the future for its particular phenolic characteristics.

As shown in Table 3, despite some differences attributable to the growing zone, the grapevine genome determines the characteristic anthocyanin profile of each variety, which enables the chemotaxonomic differentiation of red wine grape varieties.[4,7] The anthocyanin profile as well as the TAI concentration of the Pinot noir grapes from Transylvania (Table 3) are in agreement with those previously reported for the Pinot noir grapes grown in another country.[7] In both the Fetească neagră and Pinot noir varieties, with the exception of Fetească neagră from Mica, the abundant relative amounts of malvidin and peonidin derivatives (Table 3) could lead to stable red pigmentation. These anthocyanin compounds do not have ortho-hydroxylated groups and can interact with flavanols and ethanal. In fact, the low presence of 3′-hydroxylated molecules (delphinidin, cyanidin and petunidin), which are more prone to oxidation,[38] is important for the production of juices/wines that are less sensitive to colour degradation. As a consequence of the zone effect,[4] the Fetească neagră grapes from Mica presented significantly higher relative amounts of the most stable anthocyanin forms (malvidin derivatives) and lower percentages of the least stable compounds (cyanidin derivatives). Acylated anthocyanins are important because they participate in intramolecular copigmentation processes, thereby protecting the flavylium cation.[39] Therefore, the anthocyanin profile of the Fetească neagră berries, particularly those from Mica, may indicate increased colour stability compared with that of the Pinot noir berries (which contain virtually no acylated forms). The percentages of the acylated forms were significantly lower in the Fetească neagră variety from Cluj, likely because Cluj is colder than Mica.[40]

The total skin HCT concentrations (Table 4) were generally higher than those previously reported for Pinot noir and other white and red grape varieties.[7] Nevertheless, the percentages of individual compounds detected in the Pinot noir variety were similar to those reported by Ferrandino et al.[7] The diversity in the HCT profiles among varieties can have a great impact on winemaking. In white varieties, as soon as the grapes are crushed, the enzymatic oxidation of caffeoyl and p-coumaroyl tartrates by polyphenol oxidase occurs, leading to the browning phenomenon.[41] The oxidative browning intensity is mainly related to the *cis*- and *trans*-caffeoyltartaric acid concentrations, which depend on the variety.[42] The Fetească regală grapes from Mica may be more susceptible to browning during vinification, and the wine produced from these grapes may have a decreased shelf life, as a consequence of their higher *trans*-caffeoyltartaric acid concentration (87.0 mg/kg). Grape HCTs are also linked to the formation of volatile phenols during the aging of red wines in wood by the action of enzymes with cinnamoyl esterase activity.[43] Therefore, the red wines produced from the Fetească neagră grapes from Cluj, which are characterized by a high HCT concentration, may be more prone to off-odour characteristics, particularly in the presence of *Brettanomyces/Dekkera* contamination.

Because of the low stilbene concentrations detected, they were expressed in μg/g of skins (fresh weight) to better compare the varietal responses and to avoid the dilution effect due to different berry sizes (Table 5). The skin *trans*-resveratrol concentration was not significantly different among the red varieties.[44] This finding may be due to the high berry-to-berry variabilities of these compounds[9] whose syntheses depend on a large number of factors. Nevertheless, some stilbenes were absent in some varieties and zones but were detected in others. Therefore, the stilbene compositions of the berry skins varied considerably, depending on the grapevine variety and zone. The stilbene compound concentrations detected were within the range that has been previously reported for red wine grape varieties.[9,44]

Among the white varieties, the Fetească regală grapes from Cluj showed the highest total free and bound volatile compound concentrations (Table 6). Although Pinot noir was the red variety with the highest total free volatile compound concentration, the highest total bound precursor concentration was observed in Fetească neagră from Cluj (Table 7). Furthermore, the total bound volatile compound concentration was much higher than the total free volatile concentration. Although glycosylated compounds do not directly contribute to aroma, they are odourless precursors of flavour. In agreement with previous findings,[45] C6 compounds

represented the major class of free volatiles in the grapes at harvest, independently of the variety and cultivation zone. C6 aldehydes and alcohols, which are formed after crushing due to berry constitutive lipoxygenase activity,[46] provide green and grassy notes.[23] The 1-hexanol concentration is a key factor for the characterization of grapevines because it is variety dependent. According to Tables 6 and 7, the highest concentrations of this volatile compound in the free and bound forms were found in Fetească regală from Cluj and in Pinot noir from Mica for the white and red varieties, respectively. Terpenes, which are mainly present in the bound form,[46] are also closely linked to variety and are largely responsible for fruity (citrus) and floral notes.[23] The Fetească regală and Fetească neagră grapes from Cluj accounted for the highest total concentrations of glycosidically bound terpenes for the white and red varieties, respectively. The importance of norisoprenoids to grape aroma is well known; their glycosides have been intensively studied in many varieties as components of pleasant varietal and pre-fermentative flavours.[23,45,47] The highest abundance of bound norisoprenoids was observed in Fetească regală from Cluj and in Pinot noir from Mica for the white and red varieties, respectively. The volatile concentration and profile were also influenced by the growing location; this finding was in agreement with other previously published studies, which indicated that the concentrations of varietal and pre-fermentative volatiles in wine grapes permitted the effective differentiation of growing zones.[23]

The skin and seed texture properties of the native Romanian wine grape varieties and Pinot noir grapes grown in the Romanian zones (Table 8) were within the ranges published for different varieties grown in Italy.[21,29,48] Instrumental skin texture parameters have been proposed as variety markers, zone discriminators and anthocyanin extractability indices. F_{sk} and W_{sk} represent meaningful skin mechanical properties for the characterization and differentiation of wine grape varieties.[34,48] This differentiating potential was also observed in the present work. As demonstrated for the varieties evaluated in this study, skin texture parameters are effective tools for the discrimination of production areas and even vineyards. However, the relationship between skin texture properties and water regimes was recently demonstrated.[49,50] With regard to the anthocyanin extractability, a higher F_{sk} value facilitates more rapid and complete anthocyanin release from the skin,[20] whereas a lower Sp_{sk} value suggests a higher red pigment extraction yield.[32] However, the chemical composition of grape skin cell walls may determine the mechanical resistance of berry skin to anthocyanin release.[18] Skin hardness and thickness can also affect the extractability of other phenolic compounds such as flavanols.[28] According to the results obtained in the present work, among

the red varieties grown in Cluj, Fetească neagră skins would be able to release anthocyanins more rapidly compared with Pinot noir skins. Although the anthocyanin extraction yield would be slightly lower for Fetească neagră skins, this difference may be compensated by the higher total anthocyanin concentration. Furthermore, the higher skin flavanol extractability would likely result in wines with an improved colour intensity, smoother taste and lower astringency. Pinot noir skins from Mica may have a greater capacity for anthocyanin release.

For each red variety, the seed mechanical properties permitted the differentiation of berries belonging to different growing zones in Transylvania, as has been previously reported for Barbera grapes grown in Italy.[34] Variety discrimination according to the mechanical traits of the seeds was possible only for those from Mica. Zone differentiation based on seed acoustic properties was observed only for the Fetească neagră berries, and within the same zone, the discrimination among red varieties was also possible. The maximum acoustic pressure level measured at gain 0 has been suggested to be a poor method of screening for the extractable FRV concentration in seeds.[21]

CONCLUSIONS

In this work, three native Romanian wine grape varieties were characterized for the first time. The results obtained provide important information for oenologists to exploit the enological potential of grapes and to optimize winemaking techniques to produce wines with specific sensory attributes. According to the phenolic compositions of the skins and seeds, native Romanian wine grapes may produce wines with lower astringency and bitterness than Pinot noir grapes grown in the same zones. The effects of the variety and growing location on the phenolic composition, free and bound volatile compound concentrations and texture properties were studied. Significant differences for the same genotype grown in different zones may indicate that not only the cultivar but also the growing location induce differences in the accumulation of several classes of compounds and in the texture characteristics of berry skins and seeds. The main advantage of Fetească albă over Fetească regală may be its lower susceptibility to browning during vinification and its increased wine shelf life as a consequence of its lower *trans*-caffeoyltartaric acid concentration. However, the Fetească regală berries from Cluj showed the highest total concentrations of varietal and pre-fermentative volatile compounds, particularly glycosidically bound terpenes and norisoprenoids. Furthermore, Fetească neagră is a promising red variety to be exploited in the

future for its particular phenolic characteristics; the Fetească neagră grapes from Mica may have increased colour stability due to their anthocyanin profile. Among the red varieties, the Fetească neagră grapes from Cluj had the highest concentration of total bound precursors with positive aromatic notes, particularly terpenes. Nevertheless, these grapes were also characterized by a high HCT concentration, which contributes to the off-odour characteristics of red wines due to volatile phenol formation. According to the skin texture parameters, the Fetească neagră grapes from Cluj may produce wines with an increased colour intensity, smoother taste and lower astringency because of the more complete anthocyanin extraction and the higher skin flavanol extractability.

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Table 1. Technological ripeness parameters of wine grape varieties.

Variety	Growing zone	Skin color	Sugars (g/L)	pH	TA (g/L tartaric acid)	Citric acid (g/L)	Tartaric acid (g/L)	Malic acid (g/L)	Average berry weight (g)	Average berry skin weight (mg)
Fetească albă	Mica	White	258 ± 9	3.41 ± 0.08	5.07 ± 0.22	0.11 ± 0.01	3.37 ± 0.35	1.10 ± 0.08	1.31 ± 0.04	277 ± 18
Fetească regală	Batoş	White	237 ± 5b	3.08 ± 0.03	8.95 ± 0.28b	0.11 ± 0.03	4.43 ± 0.33	2.98 ± 0.16b	1.91 ± 0.06b	315 ± 10
Fetească regală	Cluj	White	200 ± 11a	3.08 ± 0.06	8.82 ± 0.70b	0.15 ± 0.03	4.80 ± 0.46	3.01 ± 0.01b	1.66 ± 0.13ab	312 ± 58
Fetească regală	Mica	White	230 ± 6b	3.05 ± 0.03	7.02 ± 0.38a	0.09 ± 0.05	4.51 ± 0.23	1.31 ± 0.21a	1.60 ± 0.14a	292 ± 15
	Sign.[a]		**	ns	**	ns	ns	***	*	ns
	Sign.[b]		*	**	**	ns	**	ns	*	ns
Fetească neagră	Cluj	Red	229 ± 7	3.20 ± 0.03	9.87 ± 0.28	0.15 ± 0.04	4.12 ± 0.21	3.19 ± 0.15	2.16 ± 0.08	395 ± 8
Fetească neagră	Mica	Red	196 ± 38	3.16 ± 0.07	9.30 ± 0.47	0.12 ± 0.05	3.65 ± 0.09	2.95 ± 0.99	1.67 ± 0.07	268 ± 16
	Sign.[a]		ns	ns	ns	ns	*	ns	**	***
Pinot noir	Cluj	Red	247 ± 28	3.26 ± 0.08	7.64 ± 1.16	0.19 ± 0.04	2.64 ± 0.15	2.20 ± 0.57	1.34 ± 0.14	301 ± 23
Pinot noir	Mica	Red	276 ± 11	3.32 ± 0.05	5.85 ± 0.46	0.21 ± 0.03	3.19 ± 0.22	1.56 ± 0.15	1.44 ± 0.19	258 ± 18
	Sign.[a]		ns	ns	ns	ns	*	ns	ns	ns
	Sign.[c]		ns, *	ns, *	*, ***	ns, ns	***, *	*, ns	**, ns	**, ns

Values are expressed as average ± standard deviation (n = 30 for average berry and berry skin weight, n = 3 for all other parameters). 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]), between Fetească neagră and Pinot noir varieties grown in the zones of Cluj and Mica ([c]): *, **, *** and ns mean significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively. TA = titratable acidity.

Table 2. Skin and seed phenolic composition of wine grape varieties.

Variety	Growing zone	A_{280} (1/kg berries)	PRO (mg/kg berries)		FRV (mg/kg berries)	
		skins	skins	seeds	skins	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	*	***	**	*
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.[c]	**, *	***, ***	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]), between Fetească neagră and Pinot noir varieties grown in the zones of Cluj and Mica ([c]): *, **, *** and ns mean significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively. A_{280} = absorbance measured at 280 nm, PRO = proanthocyanidins, FRV = flavanols reactive to vanillin.

Table 3. Skin anthocyanin concentration and profile of red wine grape varieties.

Variety	Growing zone	TAI		Percentage of anthocyanin forms (%)							
		mg/kg berries	mg/g skins	∑ delphinidin	∑ cyanidin	∑ petunidin	∑ peonidin	∑ malvidin	∑ glucosides	∑ acetylglucosides	∑ cinnamoyglucosides
Fetească neagră	Cluj	1152 ± 65	6.28 ± 0.09	9.3 ± 0.5	1.9 ± 0.0	9.4 ± 0.4	17.7 ± 0.1	61.7 ± 0.8	91.9 ± 0.4	2.0 ± 0.1	6.1 ± 0.3
Fetească neagră	Mica	929 ± 33	5.82 ± 0.50	10.7 ± 0.8	1.2 ± 0.1	11.9 ± 0.7	6.9 ± 0.3	69.3 ± 1.8	85.3 ± 0.6	2.9 ± 0.1	11.7 ± 0.7
	Sign.[a]	**	ns	ns	***	**	***	**	***	***	***
Pinot noir	Cluj	762 ± 66	3.37 ± 0.23	7.2 ± 0.8	4.3 ± 0.6	7.6 ± 0.6	27.7 ± 4.7	53.2 ± 4.9	> 99.9	traces	traces
Pinot noir	Mica	694 ± 84	3.85 ± 0.24	4.7 ± 0.4	2.7 ± 0.5	5.8 ± 0.3	23.9 ± 4.1	63.0 ± 4.0	> 99.9	traces	traces
	Sign.[a]	ns	ns	**	*	**	ns	ns	n/a	n/a	n/a
	Sign.[b]	**, *	***, **	*, ***	**, **	**, ***	*, **	*, ns	n/a	n/a	n/a

Values are expressed as average ± standard deviation (n = 3). Significance between growing zones for the same variety ([a]), between Fetească neagră and Pinot noir varieties grown in the zones of Cluj and Mica ([b]): *, **, ***, ns and n/a mean significance at $p < 0.05$, $p < 0.01$, $p < 0.001$, not significant and not applicable, respectively. TAI = total anthocyanins index.

Table 4. Skin hydroxycinnamoyl tartaric acid concentration and profile of wine grape varieties.

Variety	Growing zone	Total HCTs (mg/kg berries)	*trans*-caffeoylT (%)	*cis*-p-coumaroylT (%)	*trans*-p-coumaroylT (%)	*trans*-feruloylT (%)	p-coumaroylT / caffeoylT ratio
Fetească albă	Mica	94 ± 10	70.5 ± 1.2	8.2 ± 0.3	18.4 ± 0.5	2.95 ± 0.40	0.38
Fetească regală	Batoş	118 ± 3a	55.3 ± 0.7b	11.3 ± 0.6	32.3 ± 0.2a	1.13 ± 0.07a	0.79a
Fetească regală	Cluj	128 ± 19a	54.1 ± 0.3b	11.0 ± 0.7	34.2 ± 0.3b	0.73 ± 0.20a	0.84a
Fetească regală	Mica	173 ± 16b	50.3 ± 1.5a	12.0 ± 1.4	35.4 ± 0.6c	2.30 ± 0.25b	0.94b
	Sign.[a]	**	**	ns	***	***	**
	Sign.[b]	**	***	*	***	ns	***
Fetească neagră	Cluj	252 ± 15	78.5 ± 0.9	2.9 ± 0.1	18.0 ± 0.7	0.55 ± 0.02	0.27
Fetească neagră	Mica	156 ± 16	62.2 ± 0.4	5.6 ± 0.2	31.6 ± 0.3	0.60 ± 0.07	0.60
	Sign.[a]	**	***	***	***	ns	***
Pinot noir	Cluj	209 ± 22	65.2 ± 0.6	7.3 ± 0.2	26.7 ± 0.4	0.80 ± 0.06	0.52
Pinot noir	Mica	210 ± 20	64.7 ± 2.0	5.7 ± 1.1	28.7 ± 1.0	0.96 ± 0.15	0.53
	Sign.[a]	ns	ns	ns	*	ns	ns
	Sign.[c]	*, *	***, ns	***, ns	***, **	**, *	***, 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]), between Fetească neagră and Pinot noir varieties grown in the zones of Cluj and Mica ([c]): *, **, *** and ns mean significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively. HCTs = hydroxycinnamoyl tartaric acids, T = tartaric acid.

Table 5. Skin stilbene concentration of wine grape varieties.

Variety	Growing zone	*cis*-piceid (µg/g skin)	*trans*-piceid (µg/g skin)	*trans*-piceatannol (µg/g skin)	*trans*-resveratrol (µg/g skin)
Fetească albă	Mica	nd	nd	nd	74.7 ± 25.0
Fetească regală	Batoş	nd	nd	nd	41.8 ± 7.1
Fetească regală	Cluj	nd	nd	nd	59.8 ± 15.1
Fetească regală	Mica	nd	9.1 ± 0.8	66.4 ± 12.4	44.6 ± 6.9
	Sign.[a]	n/a	n/a	n/a	ns
	Sign.[b]	n/a	n/a	n/a	ns
Fetească neagră	Cluj	13.7 ± 1.3	30.1 ± 7.6	nd	18.7 ± 2.1
Fetească neagră	Mica	7.2 ± 10.2	nd	nd	27.2 ± 2.4
	Sign.[a]	ns	n/a	n/a	ns
Pinot noir	Cluj	16.3 ± 3.1	12.3 ± 2.6	nd	37.7 ± 6.2
Pinot noir	Mica	nd	12.3 ± 4.3	nd	30.1 ± 4.5
	Sign.[a]	n/a	ns	n/a	ns
	Sign.[c]	ns, n/a	ns, n/a	n/a, n/a	ns, ns

Values are expressed as average ± standard deviation (n = 2). 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]), between Fetească neagră and Pinot noir varieties grown in the zones of Cluj and Mica ([c]): *, **, *** and ns mean significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively. nd = not detected.

Table 6. Free and bound volatile compounds of white wine grape varieties.

	Free compounds (µg/kg berries)				Bound compounds (µg/kg berries)					
Compound	Fetească albă Mica	Fetească regală Batoş	Fetească regală Cluj	Fetească regală Mica	Fetească albă Mica	Fetească regală Batoş	Fetească regală Cluj	Fetească regală Mica	*Sign.* [a]	*Sign.* [b]
isoamyl alcohol	nd	nd	nd	nd	36.7 ± 1.7	15.6 ± 4.3a	73.1 ± 0.7b	24.7 ± 5.0a	**	ns
(*E*)-2-hexenal	9.4 ± 1.0	nd	nd	nd	0.5 ± 0.5	nd	nd	nd	n/a	n/a
1-hexanol	45.8 ± 4.2	36.7 ± 2.0a	97.0 ± 0.4b	48.8 ± 6.3a	62.0 ± 1.9	46.2 ± 3.5a	189.4 ± 7.6b	48.3 ± 7.0a	***	ns
(Z)-3-hexen-1-ol	nd	nd	nd	nd	nd	6.9 ± 0.2a	15.4 ± 1.3b	8.9 ± 2.0a	*	n/a
(E)-2-hexen-1-ol	nd	37.5 ± 2.3	46.0 ± 1.6	75.1 ± 67.2	25.9 ± 0.5	28.8 ± 3.6a	42.0 ± 0.8b	24.9 ± 3.8a	*	ns
cis-furanic linalool oxide	nd	nd	nd	nd	nd	2.8 ± 3.6a	14.6 ± 0.8b	4.9 ± 0.0a	*	n/a
linalool	3.2 ± 0.8	16.6 ± 0.1	18.9 ± 2.0	86.3 ± 77.7	83.5 ± 1.9	151.1 ± 2.8b	50.8 ± 13.6a	141.6 ± 0.5b	**	***
α-terpineol	nd	nd	nd	4.5 ± 6.2	nd	6.4 ± 0.2ab	2.5 ± 2.0a	9.2 ± 0.5b	*	n/a
trans-pyranic linalool oxide	nd	nd	nd	nd	5.3 ± 0.1	15.8 ± 3.4a	79.3 ± 5.5b	16.7 ± 0.4a	***	***
cis-pyranic linalool oxide	nd	nd	nd	nd	4.2 ± 3.0	6.4 ± 1.0	4.6 ± 4.6	0.3 ± 0.1	ns	ns
nerol	nd	nd	nd	nd	6.3 ± 1.4	70.7 ± 23.8b	7.0 ± 2.9a	22.1 ± 0.1ab	*	**
geraniol	nd	nd	nd	nd	31.6 ± 4.6	44.4 ± 7.7	34.0 ± 7.8	51.9 ± 4.2	ns	*
hexanoic acid	134.4 ± 1.5	76.3 ± 0.9b	77.2 ± 6.7b	56.1 ± 1.0a	*	***	nd	nd	n/a	n/a
benzyl alcohol	4.1 ± 3.8	nd	nd	nd	78.7 ± 43.7	75.6 ± 8.9a	1077.6 ± 19.4b	89.9 ± 37.7a	***	ns
2-phenyl ethanol	21.3 ± 0.2	6.7 ± 0.7	52.1 ± 5.4	103.2 ± 140.0	ns	ns	115.9 ± 3.5	288.0 ± 43.4	271.8 ± 20.9	196.7 ± 7.9	ns	**
(E)-2-hexenoic acid	nd	86.7 ± 4.9b	142.3 ± 17.3c	12.0 ± 16.8a	**	n/a	nd	nd	nd	nd	n/a	n/a
4-vinilguaiacol	nd	17.2 ± 3.1	19.1 ± 1.9	77.2 ± 80.9	ns	n/a	56.6 ± 11.5	22.5 ± 8.4	44.5 ± 3.5	88.1 ± 65.5	ns	ns
diol 1	nd	nd	nd	nd	n/a	n/a	125.1 ± 4.8	230.0 ± 49.2a	781.9 ± 37.1b	259.0 ± 32.0a	**	*
1-hydroxy linalool	nd	nd	nd	6.6 ± 9.3	n/a	n/a	nd	nd	nd	nd	n/a	n/a
diol 2	nd	nd	nd	nd	n/a	n/a	138.7 ± 6.3	211.6 ± 44.3	344.1 ± 21.5	235.7 ± 27.5	ns	*
geranic acid	nd	nd	nd	nd	n/a	n/a	0.4 ± 0.4	8.5 ± 10.9	2.7 ± 0.3	17.2 ± 0.7	ns	**
3-hydroxy-β-damascenone	nd	nd	nd	nd	n/a	n/a	29.3 ± 0.6	35.4 ± 10.4	59.4 ± 6.0	34.5 ± 3.1	ns	ns
3-oxo-α-ionol	nd	nd	nd	nd	n/a	n/a	38.7 ± 2.5	52.8 ± 8.4	51.8 ± 1.6	49.3 ± 1.9	ns	*
dihydro-β-ionone	nd	nd	nd	nd	n/a	n/a	49.8 ± 3.1	56.7 ± 8.2	93.8 ± 2.7	64.9 ± 13.4	ns	ns

Volatile compounds are ordered by their retention time. Values are expressed as average ± standard deviation (n = 2). Different letters within the same column indicate significant differences (Tukey-b test; $p < 0.05$) among different growing zones for Fetească regală 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 7. Free and bound volatile compounds of red wine grape varieties.

	Free compounds (µg/kg berries)															Bound compounds (µg/kg berries)												
Compound	Fetească neagră Cluj			Fetească neagră Mica			*Sign.*[a]	Pinot noir Cluj			Pinot noir Mica			*Sign.*[a]	*Sign.*[b]	*Sign.*[c]	Fetească neagră Cluj			Fetească neagră Mica			*Sign.*[a]	Pinot noir Cluj			Pinot noir Mica			*Sign.*[a]	*Sign.*[b]	*Sign.*[c]
isoamyl alcohol	nd			nd			n/a	41.6	±	11.6	48.9	±	6.0	ns	n/a	n/a	71.5	±	0.0	29.5	±	15.2	ns	56.0	±	2.5	68.6	±	7.0	ns	*	ns
(E)-2-hexenal	5.3	±	0.0	nd			n/a	15.5	±	2.6	18.3	±	4.0	ns	*	n/a	nd			nd			n/a	nd			nd			n/a	n/a	n/a
1-hexanol	69.4	±	3.2	76.4	±	4.6	ns	126.0	±	3.4	163.4	±	73.2	ns	**	ns	185.5	±	9.3	167.3	±	67.6	ns	89.2	±	7.9	215.8	±	11.1	**	**	ns
(Z)-3-hexen-1-ol	nd			21.7	±	1.5	n/a	8.0	±	0.2	5.5	±	2.1	ns	n/a	*	15.1	±	1.4	99.2	±	41.0	ns	7.6	±	1.6	13.8	±	0.4	*	*	ns
(E)-2-hexen-1-ol	nd			63.1	±	0.3	n/a	40.3	±	1.9	54.6	±	28.2	ns	ns	ns	41.1	±	1.2	128.0	±	53.0	ns	17.1	±	0.7	34.4	±	1.8	**	**	ns
cis-furanic linalool oxide	nd			nd			n/a	nd			nd			n/a	n/a	n/a	nd			6.9	±	2.1	n/a	nd			nd			n/a	n/a	n/a
linalool	nd			nd			n/a	nd			nd			n/a	n/a	n/a	49.8	±	13.8	7.4	±	1.6	*	nd			nd			n/a	n/a	n/a
α-terpineol	nd			nd			n/a	nd			nd			n/a	n/a	n/a	nd			nd			n/a	nd			nd			n/a	n/a	n/a
trans-pyranic linalool oxide	5.6	±	0.7	nd			n/a	nd			nd			n/a	n/a	n/a	77.7	±	6.2	9.9	±	1.8	**	nd			nd			n/a	n/a	n/a
cis-pyranic linalool oxide	nd			nd			n/a	nd			nd			n/a	n/a	n/a	nd			nd			n/a	nd			nd			n/a	n/a	n/a
nerol	nd			nd			n/a	nd			nd			n/a	n/a	n/a	6.8	±	2.9	nd			n/a	nd			nd			n/a	n/a	n/a
geraniol	nd			nd			n/a	nd			nd			n/a	n/a	n/a	33.1	±	8.3	46.7	±	5.8	ns	9.6	±	2.1	8.8	±	7.6	ns	ns	*
hexanoic acid	129.6	±	0.3	106.5	±	9.2	ns	67.7	±	3.8	50.9	±	16.8	ns	**	ns	nd			nd			n/a	nd			nd			n/a	n/a	n/a
benzyl alcohol	23.0	±	0.9	nd			n/a	25.3	±	3.6	53.4	±	22.8	ns	ns	n/a	1058.9	±	24.2	335.8	±	133.2	*	415.0	±	20.6	707.9	±	56.9	*	**	ns
2-phenyl ethanol	24.0	±	1.0	nd			n/a	86.0	±	1.5	59.0	±	22.6	ns	***	n/a	266.2	±	23.1	294.1	±	72.4	ns	165.3	±	4.1	163.2	±	4.6	ns	*	ns
(E)-2-hexenoic acid	nd			nd			n/a	46.0	±	0.5	15.1	±	6.9	*	n/a	n/a	nd			nd			n/a	nd			nd			n/a	n/a	n/a
4-vinilguaiacol	nd			1.5	±	1.4	n/a	13.0	±	2.6	19.6	±	12.7	ns	n/a	ns	43.6	±	3.9	20.9	±	9.4	ns	8.3	±	5.7	69.0	±	4.8	**	*	*
diol 1	nd			nd			n/a	nd			nd			n/a	n/a	n/a	765.7	±	43.9	52.6	±	8.2	**	10.5	±	2.5	20.0	±	1.7	*	**	*
1-hydroxy linalool	nd			nd			n/a	nd			nd			n/a	n/a	n/a	nd			nd			n/a	nd			nd			n/a	n/a	n/a
diol 2	nd			nd			n/a	nd			nd			n/a	n/a	n/a	337.0	±	24.4	19.0	±	2.4	**	8.7	±	1.8	23.2	±	1.8	*	*	ns
geranic acid	nd			nd			n/a	nd			nd			n/a	n/a	n/a	nd			34.7	±	0.2	n/a	11.4	±	1.9	5.8	±	4.5	ns	n/a	*
3-hydroxy-β-damascenone	nd			nd			n/a	nd			nd			n/a	n/a	n/a	58.2	±	6.5	52.7	±	11.7	ns	24.8	±	2.7	37.7	±	0.6	*	*	ns
3-oxo-α-ionol	nd			nd			n/a	nd			nd			n/a	n/a	n/a	53.7	±	2.1	46.6	±	6.6	ns	60.2	±	10.4	90.4	±	17.7	ns	ns	ns
dihydro-β-ionone	nd			nd			n/a	nd			nd			n/a	n/a	n/a	nd			nd			n/a	nd			nd			n/a	n/a	n/a

Volatile compounds are ordered by their retention time. Values are expressed as average ± standard deviation (n = 2). 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 ([b]) and Mica ([c]): *, **, *** and ns mean significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively.

Table 8. Skin and seed instrumental texture properties of wine grape varieties.

Variety	Growing zone	Skin mechanical parameters				Seed mechanical and acoustic parameters						
		F_{sk} (N)	W_{sk} (mJ)	E_{sk} (N/mm)	Sp_{sk} (µm)	F_s (N)	W_s (mJ)	E_s (N/mm)	DI_s (%)	SPL at breakage (dB)	Maximum SPL (dB)	AE (dB × mm)
Fetească albă	Mica	0.365 ± 0.065	0.241 ± 0.069	0.253 ± 0.051	200 ± 29	25.8 ± 8.0	5.26 ± 2.54	59.4 ± 18.7	21.0 ± 11.9	83.7 ± 10.2	91.7 ± 6.3	8.48 ± 4.65
Fetească regală	Batoş	0.468 ± 0.072	0.336 ± 0.089a	0.302 ± 0.051b	173 ± 29a	29.8 ± 10.5	7.20 ± 3.19	52.5 ± 18.6a	20.5 ± 5.5	76.4 ± 16.4	89.0 ± 8.3	8.13 ± 3.35
Fetească regală	Cluj	0.467 ± 0.073	0.319 ± 0.095a	0.319 ± 0.053b	177 ± 45a	35.6 ± 8.0	7.61 ± 2.60	70.2 ± 18.6b	19.4 ± 5.3	84.1 ± 12.6	90.6 ± 5.9	9.09 ± 2.53
Fetească regală	Mica	0.474 ± 0.128	0.417 ± 0.125b	0.264 ± 0.038a	208 ± 40b	31.3 ± 8.7	7.02 ± 2.96	63.8 ± 16.5ab	19.0 ± 2.8	84.9 ± 12.8	93.4 ± 5.5	9.34 ± 2.26
	Sign.[a]	ns	**	**	*	ns	ns	*	ns	ns	ns	ns
	Sign.[b]	**	***	ns	ns	*	ns	ns	ns	ns	ns	ns
Fetească neagră	Cluj	0.713 ± 0.092	0.365 ± 0.086	0.664 ± 0.105	202 ± 42	24.8 ± 9.1	5.94 ± 1.94	41.8 ± 18.0	24.0 ± 8.2	70.3 ± 11.6	83.3 ± 8.7	7.15 ± 3.11
Fetească neagră	Mica	0.541 ± 0.095	0.303 ± 0.081	0.456 ± 0.116	168 ± 47	41.5 ± 12.5	9.78 ± 4.02	71.1 ± 17.6	20.8 ± 3.3	92.6 ± 8.4	93.5 ± 6.4	8.28 ± 2.53
	Sign.[a]	***	*	***	*	***	***	***	ns	***	***	ns
Pinot noir	Cluj	0.603 ± 0.101	0.337 ± 0.119	0.517 ± 0.100	171 ± 38	28.7 ± 5.0	6.77 ± 1.94	51.3 ± 14.6	22.3 ± 4.6	83.0 ± 12.3	88.5 ± 7.0	8.29 ± 5.14
Pinot noir	Mica	0.675 ± 0.118	0.482 ± 0.180	0.427 ± 0.108	171 ± 25	23.6 ± 4.5	5.27 ± 1.34	45.2 ± 8.4	19.4 ± 3.7	83.4 ± 13.6	89.0 ± 7.7	6.34 ± 2.29
	Sign.[a]	*	**	**	ns	**	**	ns	*	ns	ns	ns
	Sign.[c]	***, ***	ns, ***	***, ns	*, ns	ns, ***	ns, ***	ns, ***	ns, ns	**, *	*, ns	ns, *

Values are expressed as average ± standard deviation (n = 20 for berry skins, n = 30 for berry seeds). 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]), between Fetească neagră and Pinot noir varieties grown in the zones of Cluj and Mica ([c]): *, **, *** and ns mean significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively. F_{sk} = berry skin break force, W_{sk} = berry skin break energy, E_{sk} = berry skin resistance to the axial deformation, Sp_{sk} = berry skin thickness. F_s = berry seed break force, W_s = berry seed break energy, E_s = berry seed resistance to the axial deformation, DI_s = seed deformation index, SPL = acoustic pressure level, AE = acoustic energy.