Polyphenolic profile and stilbene content of Albanian “Kallmet” monovarietal wine

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Abstract

Aim: The aim of this work was to characterize the phenolic content of wines from the most promising autochthonous red grape variety in Albania, the Kallmet.

Methods and results: To make this first survey, 16 commercial monovarietal Kallmet wines were analyzed with special attention to proanthocyanidin (condensed tannins), anthocyanin and stilbene composition. The average content of total proanthocyanidins in these wines was 3877 mgL⁻¹ and the anthocyanin content averaged 242 mgL⁻¹. The mean degree of polymerization (mDP) varied from 8 subunits in young wine to 31 subunits in 6-year-old wine, with a mean value of 14.5 subunits. The total free anthocyanin content ranged from 85 mgL⁻¹ in old wine to 609 mgL⁻¹ in young wine, with a mean value of 242.5 mgL⁻¹. Trans-piceid was the most abundant stilbene (11 mgL⁻¹), followed by trans-resveratrol (1.8 mgL⁻¹). It was evidenced that cis-forms of resveratrol and piceid presented a lower content than their trans-forms. The ratio between piceid (glycoside form) and resveratrol (aglycone form) was 8.2, showing a similar stilbene pattern as in Mediterranean wines.

Conclusions: Kallmet wines show proanthocyanidin polymerization characteristics and stilbene profiles very similar to red wines deriving from other varieties grown under warm climates.

Significance and impact of the study: In spite of the strong interest in Kallmet grape, there is still a need for detailed studies to know the range of the different components of interest found in the produced wine.

Keywords: Kallmet, flavonoids, stilbenes, red wine, anthocyanin, Albania

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Introduction

Albania is a country in South Eastern Europe. It is characterized by a typical Mediterranean climate with mild and wet winters, and sunny and dry summers. Traditionally, viticulture is considered as one of the most important sectors in Albanian agriculture.

Historically, Kallmet is one of the oldest autochthonous grape cultivars; it has been used for centuries as one of the most widespread red wine cultivars, especially in north and north-west Albania. It is often known as Kadarka, Scadarka, Nero di Scutari and Ganzza. Interestingly, Kallmet is native to Albania and has been cultivated since the Roman Empire in Hungary, Croatia, Austria, and Rumania (Susaj L., 2012). Over the next few years, Kallmet is planned to occupy 20% of the red wine cultivars in Albania, due to the high environmental adaptability and quality of the wine.

The grape in the maturation phase is medium size, with a diameter of 10-12 mm, and has a round or elliptical form, with red-purple color of skin and very waxy. The thickness of the skin is medium; the pulp is firm and juicy, with a fair balance of color and taste and a fair amount of seeds (1.3 seeds per berry; Susaj L. et al., 2012). As regards environmental and cultural requirements, it prefers deep, well-drained and ventilated soils (Susaj L. et al., 2013). Cultivation is recommended in areas where the maturity phase does not coincide particularly with rainy periods. Typically, the musts of this variety are characterized by an average sugar content of 21%, a pH of 3.4 and total acidity of 4.5 gL⁻¹. The wines produced from Kallmet are vinified with the traditional method by producing high quality dry wines, with high alcoholic content (13-15%). They have a clear red ruby color, with violet highlights (Kullaj and Çakalli, 2012), an elegant aroma bouquet with scent of cherries, cornel, and hints of violet, and a good structure for aging process. In the last ten years, Kallmet wines have considerably increased their value, representing an attractive high quality alternative to wines with geographical indication in Albania.

Albania has a wide grapevine germplasm collection and in the last years it has been paying great attention to its valorization, mostly the winemaking germplasm like Kallmet (Peçuli and Mane, 2014; Peçuli et al., 2014; Susaj E. et al., 2013). In spite of the strong interest in Kallmet grape, there is still a need for detailed studies to know the range of the different components of interest found in the produced wine. The purpose of our work is the characterization and evaluation of phenolic compounds content of Kallmet involved in the quality of the wine and known for biological activity.

Materials and methods

1. Wine samples

The 16 samples of Kallmet red wine were from several Albanian wineries. They were chosen to represent all viticultural areas in north-west Albania and various vintages, more precisely: 2007 (n=2), 2008 (n=1), 2009 (n=3), 2011 (n=1), 2012 (n=6) and 2013 (n=3).

2. Reagents

All chemicals, unless cited otherwise, were of analytical grade from Sigma-Aldrich.

3. Polyphenols analysis

Total proanthocyanidins were analyzed spectrophotometrically according to the method by Porter (Porter et al., 1985). This method, named acidic butanolysis, is based on the conversion of proanthocyanidins into colored anthocyanidins by oxidative cleavage under drastic acidic conditions (100°C for 30 min in a mixture of chlorhydric acid 32%, n-butanol and iron (III) sulfate Fe₂(SO₄)₃). After the reaction, the absorbance was measured at a fixed wavelength of 550 nm. A non-heated sample prepared with all reagents stored at room temperature in darkness was used as a control.

In order to evaluate free and terminal catechic monomers, DMACA (4-DiMethylAminoCinnam Aldehyde) reaction (Tanner et al., 2003) was carried out. The mean degree of polymerization (mDP; number of catechin units/chain) method used in this work was described elsewhere (Vivas et al., 1994).

The total anthocyanin content was estimated according to Puissant-Leon by the reaction with HCl 1N (Puissant and Léon, 1967). The percentage of color due to copigmentation and to polymerized pigments was calculated according to the Boulton assay (Boulton, 1996). The basis of this method is the treatment of the wine with excess acetaldehyde, and a subsequent dilution with a hydro-alcoholic medium and excess SO₂. The absorbance was measured at 520 nm.

Color was recorded using the CIE-L* a* b* uniform color space (CIE-Lab) (spectrophotometer CM-5 KONICA-MINOLTA, Japan), a three dimensional system where the L* axis indicates lightness, and a* and b* axes indicate chromaticity or color. L*...
(lightness) varies from 0 (black, bottom) to 100 (white, top), a* indicates hue on a green (-) to red (+) axis, and b* indicates hue on a blue (-) to yellow (+) axis. CIE Standard Illuminant D65 was used.

4. Free anthocyanin content

The most important free anthocyanins in wine were measured according to the method OIV-MA-AS315-11 (International Organization of Vine and Wine, 2003), which consists of reverse phase high performance liquid chromatography (RP-HPLC), performed with the Hewlett Packard Agilent 1200 equipped with a UV-VIS detector at 520 nm. Data acquisition and analysis were performed by 7.1.2 Chromeleon Chromatography Data System (Dionex Softron GmbH, Germany). Separation of the components was accomplished with a silicon-based column (Zorbax Eclipse Plus C18, 4.6 mm x 50 mm, 1.8 μm; Agilent). The mobile phases were: solvent A, which consisted of de-ionized water/formic acid/acetonitrile 88:10:2, and solvent B, which consisted of de-ionized water/formic acid/acetonitrile 2:10:88. The flow rate was 2 mLmin⁻¹ and the column temperature 25 °C. The column was equilibrated for 2 min with 100 % solvent A before each analysis. 0.2 min after the injection the percentage of solvent B was increased with a linear gradient from 0 to 40 % in 5 min. Between 5.2 and 6 min the percentage of B was increased from 40 to 100 %. The total run time was 8 min with an injected volume of 10 μL. The retention time of the mean peaks - delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside and malvidin-3-glucoside - was determined using standard solutions. Acetylated and coumarylated anthocyanins were each defined as a group of peaks according to the method of OIV. The anthocyanin profile was expressed in percentage of peak area compared to the total peak area.

5. Stilbene content

To provide a way of quantitative analysis of stilbenes, HPLC was carried out using an Ultimate 3000 from Thermo Scientific including a DAD detector and the software Chromeleon (Dionex). Separation was accomplished on a C18 column (LiCrosphere 100 RP-18, 250 mm x 4 mm, 5 mm; Merck). The solvent system consisted of water with 0.1 % formic acid: acetonitrile with 0.1% formic acid. The separation started with 1 min isocratic 20 % acetonitrile in water, 30 min linear gradient from 20 to 75 % of acetonitrile in water, 2 min linear gradient from 75 to 100 % of acetonitrile, 3 min isocratic 100 % of acetonitrile, 1 min linear gradient from 100 to 20% of acetonitrile in water, and 4 min isocratic 20% of acetonitrile in water at a flow rate of 1 mLmin⁻¹ and room temperature. Consequently, stilbenes were detected at 307 nm, and calibration curves were calculated for each cis- and trans-stilbene on the basis of seven different concentrations from 0.5 to 1000 ng (injection volume 10 μL). UV spectra were made from 200 to 400 nm using the diode array on line detection (Pezet et al., 2003).

6. Statistical analyses

Principal component analysis (PCA) was performed using statistical software ©XLSTAT 2016.01.26633 (Addinsoft, Paris). The analytical data were centered and normalized before being treated by PCA.

Results and discussion

1. Polyphenolic profile of wines

As stated above, 16 monovarietal Kallmet wines were selected, from different years (2007-2013), from the north-west part of Albania. The polyphenolic profile, including the concentration of proanthocyanidins, the determination of the mDP and the total anthocyanin content, was carried out at the same time in 2013. The results are presented in the first part of Table 1.

Generally, color and tannin composition play an important role in the quality of red wines. Wine tannins consist essentially of proanthocyanidins (condensed tannins) made by polymerization of 3-flavanyl monomers (catechins and gallocatechins, epicatechins and epigallocatechins and their galloylated forms) in which two main classes are procyanidins and prodelphinidins (Porter et al., 1985). It was found that they are the major contributors to the body and mouth feel (astringency) of wine (Rébénaque et al., 2015), as well as color stabilizers of wine by forming long-lived polymeric complexes with anthocyanins (Ribéreau-Gayon et al., 2006).

These results revealed that the total proanthocyanidin (tannins) content in Kallmet wines ranged from 1556 to 8409 mgL⁻¹, with a mean value of 3877 mgL⁻¹. However, proanthocyanidin levels in wine depend on different factors, such as grape maturity, maceration process, use of barrel and aging, which can justify the wide range of this parameter in the studied wines.

Another parameter evaluated in this research work is the polymeric structure of proanthocyanidin. This parameter is given by mDP and is calculated after the determination of the terminal units in the polymer chain. In our study, the terminal units were expressed
in epicatechin equivalent and ranged from 103 to 904 mgL⁻¹, with a mean value of 310.7 mgL⁻¹.

Interestingly, compared to other grape varieties the Kallmet wines have a highly polymerized tannin profile, with mDP which ranged from 8 to 31 subunits, with a mean value of 14.5 subunits (Table 1). Actually reports from various authors stated mDP values from 3.1 to 13 subunits in different regions and vine varieties, respectively 3.1-6 subunits in French wines (Souquet et al., 2004); 4.5-6.2 in Lisbon wines from Touriga Nacional, Trincadeira, Cabernet Sauvignon, Castelão and Syrah (Cosme et al., 2009); 4.9-9.8 in Brazilian wines from Cabernet Franc, Merlot, Sangiovese and Syrah (Gris et al., 2011); and 6-13 in Portuguese and 6.9-13 in Spanish red wines from Monastrell (Monagas et al., 2003; Sun et al., 2001). Maury et al. (2001, 2003) reported an average mDP of 9.9 for Syrah and 5.8 for Merlot.

This highly polymerized tannin profile contributes to the mouth feel of Kallmet. The polymeric structure of proanthocyanidin, specifically the mDP, was related by several authors to organoleptic characteristics. A positive correlation between mDP and astringency intensity was reported in Cabernet Sauvignon wines (Chira et al., 2012) and in model wines (Vidal et al., 2003). The same effect was found comparing monomers, dimers and trimers in 1% ethanol solution, where the astringency was increased with mDP, contrary to the bitterness, which was decreased with mDP (Peleg et al., 1999). A positive correlation was observed between the mDP and the age of the wine ($R^2=0.46$).

2. Pigments and chromatic color profile of wines

Color is one of the most important attributes of red wines, and the principal source of red color in wines comes from the anthocyanins or their further derivatives that are extracted or formed during the vinification process and aging (Ginjom et al., 2010). Anthocyanins are water soluble flavonoid pigments that, depending on pH, can contribute diverse colors such as red, purple and blue. They are present in the grape and in the wine as sugar conjugated derivative of aglycone structures (anthocyanidin; He et al., 2012b). The total anthocyanin content in Kallmet samples ranged from 85 to 609 mgL⁻¹, with a mean value of 242.5 mgL⁻¹, which is very similar to the anthocyanin content of Kallmet in other studies (Kullaj and Çakalli, 2012). The important variability between the values can be explained by the fact that total anthocyanin content depends strongly on the winemaking technics (González-Neves et al., 2007).

Unfortunately, no information was available on the total anthocyanin content (Kall and Cizakli, 2012). The important studies on the relationship between the values and the winemaking technics are described in a previous study (González-Neves et al., 2007).

Table 1. Polyphenolic profile and chromatic color parameters of red wines produced from Kallmet variety.

<table>
<thead>
<tr>
<th>Wine Code</th>
<th>KALL_1</th>
<th>KALL_2</th>
<th>KALL_3</th>
<th>KALL_4</th>
<th>KALL_5</th>
<th>KALL_6</th>
<th>KALL_7</th>
<th>KALL_8</th>
<th>KALL_9</th>
<th>KALL_10</th>
<th>KALL_11</th>
<th>KALL_12</th>
<th>KALL_13</th>
<th>KALL_14</th>
<th>KALL_15</th>
<th>KALL_16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total proanthocyanidins mgL⁻¹</td>
<td>2325±0.4</td>
<td>8409±0.6</td>
<td>3531±0.9</td>
<td>1750±0.0</td>
<td>3846±0.6</td>
<td>6066±0.9</td>
<td>3747±0.0</td>
<td>392±0.1</td>
<td>3601±0.9</td>
<td>3601±0.9</td>
<td>1556±0.4</td>
<td>4386±0.7</td>
<td>3426±0.1</td>
<td>3741±0.7</td>
<td>5935±0.4</td>
<td>3007±0.5</td>
</tr>
<tr>
<td>Terminal units [as epicatechin] mgL⁻¹</td>
<td>1180±0.4</td>
<td>904±0.4</td>
<td>330±0.8</td>
<td>175±0.0</td>
<td>359±0.9</td>
<td>460±0.8</td>
<td>190±0.4</td>
<td>398±0.0</td>
<td>351±0.7</td>
<td>348±0.7</td>
<td>103±0.8</td>
<td>18±0.4</td>
<td>21±0.9</td>
<td>31±0.5</td>
<td>14±0.1</td>
<td>26±0.0</td>
</tr>
<tr>
<td>mDP</td>
<td>19±0.6</td>
<td>09±0.2</td>
<td>10±0.6</td>
<td>15±0.7</td>
<td>12±0.4</td>
<td>13±0.1</td>
<td>17±0.7</td>
<td>08±0.5</td>
<td>06±0.2</td>
<td>06±0.3</td>
<td>14±0.9</td>
<td>19±0.4</td>
<td>10±0.9</td>
<td>2±0.9</td>
<td>11±0.4</td>
<td></td>
</tr>
<tr>
<td>Total anthocyanins mgL⁻¹</td>
<td>14.4±0.3</td>
<td>28±0.3</td>
<td>69±0.1</td>
<td>14±0.5</td>
<td>149±0.4</td>
<td>174±0.7</td>
<td>342±0.3</td>
<td>285±0.1</td>
<td>204±0.5</td>
<td>248±0.2</td>
<td>195±0.3</td>
<td>12±0.4</td>
<td>39±0.2</td>
<td>13±0.4</td>
<td>85±0.5</td>
<td></td>
</tr>
<tr>
<td>Fraction of color due to copigmentation %</td>
<td>92±0.002</td>
<td>54±0.002</td>
<td>39±0.015</td>
<td>69±0.000</td>
<td>72±0.015</td>
<td>56±0.002</td>
<td>52±0.003</td>
<td>32±0.002</td>
<td>63±0.012</td>
<td>48±0.003</td>
<td>63±0.015</td>
<td>93±0.015</td>
<td>81±0.003</td>
<td>87±0.003</td>
<td>64±0.002</td>
<td>61±0.002</td>
</tr>
<tr>
<td>L*(D65)</td>
<td>66.8±0.006</td>
<td>64.7±0.006</td>
<td>55.6±0.006</td>
<td>74.9±0.006</td>
<td>68.6±0.000</td>
<td>71.9±0.000</td>
<td>61.4±0.006</td>
<td>70.8±0.000</td>
<td>66.0±0.006</td>
<td>68.5±0.006</td>
<td>48.5±0.006</td>
<td>46.1±0.006</td>
<td>64.2±0.012</td>
<td>71.1±0.000</td>
<td>66.5±0.006</td>
<td>65.2±0.000</td>
</tr>
<tr>
<td>a*(D65)</td>
<td>31.1±0.006</td>
<td>35.3±0.000</td>
<td>37.3±0.000</td>
<td>23.6±0.006</td>
<td>31.4±0.006</td>
<td>28.2±0.000</td>
<td>40.1±0.000</td>
<td>31.7±0.006</td>
<td>33.5±0.006</td>
<td>324±0.012</td>
<td>501±0.015</td>
<td>404±0.006</td>
<td>324±0.006</td>
<td>247±0.006</td>
<td>293±0.006</td>
<td>309±0.006</td>
</tr>
<tr>
<td>b*(D65)</td>
<td>15.4±0.000</td>
<td>6.1±0.006</td>
<td>9.1±0.000</td>
<td>12±0.006</td>
<td>5.6±0.006</td>
<td>6.5±0.000</td>
<td>23.4±0.006</td>
<td>9.3±0.006</td>
<td>5.9±0.010</td>
<td>11.6±0.015</td>
<td>15±0.012</td>
<td>19±0.006</td>
<td>22.1±0.000</td>
<td>19±0.006</td>
<td>14±0.011</td>
<td></td>
</tr>
</tbody>
</table>
concerning this parameter. However, a negative correlation was observed between the total anthocyanin content and the age of the wine ($R^2=0.58$), due to the polymerization and diverse chemical reactions during aging. In red grapes and young red wines, anthocyanins exist primarily as weak complexes; either with themselves, termed self-association, or with other compounds, termed cofactors or copigments (mostly noncolored organic compounds) resulting in copigmentation phenomena in wines. Copigmentation processes are characteristic mostly in young wines as a way to protect the free anthocyanins from oxidation reactions. But the free anthocyanin-cofactors complexes are weak and with aging they are replaced by anthocyanidin-tannin compounds (polymerization) which stabilize the color of wine. So after several years, although the wines remain red, there are almost no monomeric anthocyanins present (Boulton, 2001). The distribution of colored spaces, namely free anthocyanins, copigments and polymeric pigments, was measured using the Boulton assay. As it can be seen from Table 1, the color of Kallmet wines comes mostly from polymerized pigments (refer to Table 1) ranging from 32 to 92 %, with a mean value of 64 %.

On average, the copigmentation phenomena were little present, with a mean value of 10.4 %. It was found that the negative value comes especially as result of the aging of the wine during which little or no copigmentation remained. Also, it could come from the exposure to excess oxygen which leads to depletion of cofactors, especially those with dihydroxy patterns (Boulton, 2001).

For all the samples, the color descriptor lightness varied from 46 to 75, $a^*$ varied from 24 to 50, and $b^*$ varied from 2.3 to 22 (Table 1). The samples with a high content of the yellow color component had a higher color tint and $L^*$ (lightness) so they were lighter in color.

3. Principal component analysis

PCA of the analytical variables from Table 1 using all wine samples explained more than 70 % of the variation in the data in the first two dimensions, with 45.4 % and 26 % explained by F1 and F2, respectively (Figure 1).

On the PCA score plot, samples are presented with different colors according to their age. Wines appear to be separated along the first axis according to their age. This separation is based essentially on the variables mDP, fraction of color due to polymeric pigmentation % and $b^*$ values, which are positively correlated with age. As it can be seen, these variables are strongly correlated to each other, as it is shown by the loading plot, and they are well known to be related to the wine age. On the other hand, the variables referring to the fraction of color due to free anthocyanin and copigmentation are negatively correlated with the wine age. One wine is completely out of this classification, namely KALL_2, produced in 2009; it is a 4-year-old wine which is placed on the score plot with the young wines. In spite of its age this wine has one of the lowest mDP values (9 subunits) and the highest total proanthocyanidin concentration (8409 mgL$^{-1}$).

On the second axis, a separation is observed corresponding to the free anthocyanin level (high value KALL_11; 3; 7) and to the color descriptors. It
is noteworthy to mention the important intragroup variability of young wines on this axis. Evidently, this can be explained by the fact that with age the free anthocyanin content is decreasing. In the loading plot, a notable positive correlation (0.667) can be observed between the content of total free anthocyanins and a* (the degree of redness). On the other hand, L* (lightness) is negatively correlated to the free anthocyanins (-0.558).

### 4. Anthocyanin profile

Table 2 demonstrates the anthocyanin profile of the young Kallmet wines. In general, the anthocyanin profile of grape varieties is determined in the grape extract or in young wine. With aging, anthocyanins are undergoing chemical changes in the wine to form diverse derivatives and colored polymers (He et al., 2012b). Dipalmo et al. (2016) identified more than 50 anthocyanin-derived pigments in 2-year-old Primitivo wines. The big number of the derived compounds and the formation of polymers, which increases the baseline of the chromatogram, make the characterization of individual anthocyanins very difficult with HPLC-DAD. This is the reason why in this study we report only the profile of wines from 2013 and 2012, which shows a chromatogram without important formation of polymers.

The predominant anthocyanin in the young Kallmet wines was malvidin-3-glucoside, which ranged from 54 to 66.5%, with a mean value of 60%. Among these monomeric anthocyanins, malvidin-3-glucoside is usually the most abundant and is the source of most of the red color in very young red wines. It was found that malvidin-3-glucoside varied from more than 90% in Grenache, less than 50% in Sangiovese, and no more than 42% of the total anthocyanins in a Merlot wine at the end of alcoholic fermentation (He et al., 2012a).

The content of malvidin-3-glucoside of Kallmet wines and the ratio between acetylated and coumarylated anthocyanins, which has a mean of 1.4, are very similar to those of Vranac variety grown in Macedonia (Ivanova-Petropulos et al., 2015). The second most found anthocyanin was peonidin-3-glucoside, ranging from 7 to 20.6%. Followed by petunidin-3-glucoside (4.7%) and delphinidin-3-glucoside (4.4%). Cyanidin-3-glucoside had the lowest content found in Kallmet wine. Each anthocyanin has a particular hue, ranging from red to blue, and the combination of anthocyanin quantities and profiles contributes to the intensity and hue of the color of fruit and wines (Sarni et al., 1995).

### Table 2. Relative amount (%) of the most abundant free anthocyanins in red wines produced from Kallmet variety.

<table>
<thead>
<tr>
<th>Wine code</th>
<th>Year</th>
<th>Delphinidin-3-glucoside</th>
<th>Cyanidin-3-glucoside</th>
<th>Petunidin-3-glucoside</th>
<th>Peonidin-3-glucoside</th>
<th>Malvidin-3-glucoside</th>
<th>Acetylated</th>
<th>Coumarylated</th>
<th>Acet/Coum</th>
</tr>
</thead>
<tbody>
<tr>
<td>KALL_3</td>
<td>2013</td>
<td>5.2±0.66</td>
<td>0.9±0.44</td>
<td>12.5±0.93</td>
<td>6.0±1.07</td>
<td>58.5±0.43</td>
<td>7.8±0.03</td>
<td>9.2±0.16</td>
<td>0.8±0.02</td>
</tr>
<tr>
<td>KALL_6</td>
<td>2013</td>
<td>3.7±0.51</td>
<td>1.2±0.14</td>
<td>9.0±1.37</td>
<td>5.6±0.24</td>
<td>60.9±2.80</td>
<td>12.3±0.29</td>
<td>7.3±0.4</td>
<td>1.6±0.07</td>
</tr>
<tr>
<td>KALL_7</td>
<td>2012</td>
<td>3.2±0.17</td>
<td>0.2±0.03</td>
<td>7.0±0.91</td>
<td>3.2±0.23</td>
<td>66.5±2.38</td>
<td>13.5±0.11</td>
<td>6.4±0.03</td>
<td>2.1±0.01</td>
</tr>
<tr>
<td>KALL_8</td>
<td>2012</td>
<td>6.5±0.24</td>
<td>0.6±0.22</td>
<td>8.0±0.59</td>
<td>4.7±0.70</td>
<td>57.6±1.35</td>
<td>11.7±0.69</td>
<td>10.9±0.34</td>
<td>1.1±0.09</td>
</tr>
<tr>
<td>KALL_9</td>
<td>2012</td>
<td>6.3±0.11</td>
<td>1.9±0.20</td>
<td>14.0±1.24</td>
<td>5.6±0.66</td>
<td>54.7±4.7</td>
<td>12.6±1.85</td>
<td>5.6±0.31</td>
<td>2.2±0.52</td>
</tr>
<tr>
<td>KALL_10</td>
<td>2012</td>
<td>4.5±0.44</td>
<td>1.5±0.18</td>
<td>10.1±0.44</td>
<td>4.6±0.46</td>
<td>60.1±3.49</td>
<td>11.6±1.02</td>
<td>7.6±0.26</td>
<td>1.5±0.35</td>
</tr>
<tr>
<td>KALL_15</td>
<td>2012</td>
<td>1.7±0.08</td>
<td>0.3±0.11</td>
<td>20.6±0.18</td>
<td>3.3±0.16</td>
<td>61.8±0.69</td>
<td>6.6±1.00</td>
<td>6.0±0.47</td>
<td>1.1±0.18</td>
</tr>
</tbody>
</table>

Individual anthocyanins are expressed as percentage of combined levels of all anthocyanins. Values are mean of triplicate determination (n=3).
5. Stilbene content

Stilbenes form an important group of polyphenols in wine, especially from the point of view of their health benefits. As it can be seen in Table 3, the most abundant stilbene in Kallmet wine was trans-piceid, which ranged from 3.7 to 19.2 mgL$^{-1}$, with a mean value of 11 mgL$^{-1}$. The cis-form of trans-resveratrol was detected in the range from 0.3 to 4.3 mgL$^{-1}$, with a mean value of 1.8 mgL$^{-1}$. No pterostilbene and viniferin were detected in the wines. The total level of resveratrol derivatives in Kallmet wines ranged from 6.4 to 31.8 mgL$^{-1}$, with a mean value of 20.3 mgL$^{-1}$, a level very similar to the total amounts of resveratrol derivatives found in Portuguese monovarietal red wines (20.2 mgL$^{-1}$; Ribeiro-de-Lima et al., 1999). Stilbenes content and their biosynthesis appears to depend on some genetic control pathways, where the grape variety is the main factor (Bavaresco, 2003; Bavaresco et al., 2007) but other factors such as climate, soil, fungal diseases of grapevine, etc. are also determinant (Bavaresco et al., 2008; Gatto et al., 2008). Enological methods are another decisive factor of the stilbene level in wines (Threlfall et al., 1999).

Our results are in line with most studies, in which levels of cis-forms of resveratrol and piceid were lower than their trans-forms in most cases (Moreno-Labanda et al., 2004; Ribeiro-de-Lima et al., 1999; Vitrac et al., 2005). The average ratio of trans/cis for piceid was 2.3, and for resveratrol 1.8.

Figure 2 illustrates the total content of trans/cis forms of piceid and resveratrol. In the biosynthetic pathway, resveratrol is formed at first and glycosylated into piceid.

The ratio glycoside/aglycone varied from 1.95 in Kall_14 to 25.4 in Kall_9, with a mean value of 8.2. Kallmet wines can be aligned with wines from warm regions which show the same stilbene patterns; the glycoside form is in high concentration compared to aglycone. Goldberg et al. (1996) suggest that the high sugar content of grapes cultivated in warm climate can favor the glycosylation of resveratrol. Wines from Monastrell (Spain) have an average ratio of glycoside/aglycone of 7, Grenache and Tempranillo 1.5, monovarietal and blended red Portuguese wines 4, and Chianti wine (Italy) 1.5 (Moreno-Labanda et al., 2004; Ribeiro-de-Lima et al., 1999). On the contrary, the same author found higher amounts of aglycone compared to glycoside.

### Table 3. Amount of resveratrol and piceid isomers in red wine produced from Kallmet variety.

<table>
<thead>
<tr>
<th>Wine code</th>
<th>trans-piceid mg/L</th>
<th>cis-piceid mg/L</th>
<th>trans/cis piceid ratio</th>
<th>trans-resveratrol mg/L</th>
<th>cis-resveratrol mg/L</th>
<th>trans/cis resveratrol ratio</th>
<th>total mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>KALL_1</td>
<td>16.0±0.40</td>
<td>4.7±0.35</td>
<td>3.4</td>
<td>1.5±0.09</td>
<td>0.7±0.03</td>
<td>2.1</td>
<td>22.9</td>
</tr>
<tr>
<td>KALL_2</td>
<td>13.1±0.33</td>
<td>12.4±0.62</td>
<td>1</td>
<td>2.4±0.15</td>
<td>2.5±0.08</td>
<td>0.96</td>
<td>30.4</td>
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<tr>
<td>KALL_3</td>
<td>12.9±0.32</td>
<td>10.4±0.52</td>
<td>1.2</td>
<td>2.2±0.13</td>
<td>1.7±0.05</td>
<td>1.3</td>
<td>27.2</td>
</tr>
<tr>
<td>KALL_4</td>
<td>14.2±0.35</td>
<td>5.8±0.44</td>
<td>2.4</td>
<td>1.7±0.10</td>
<td>0.6±0.03</td>
<td>2.8</td>
<td>22.3</td>
</tr>
<tr>
<td>KALL_5</td>
<td>3.7±0.27</td>
<td>1.6±0.24</td>
<td>2.3</td>
<td>0.7±0.04</td>
<td>0.4±0.02</td>
<td>1.7</td>
<td>6.4</td>
</tr>
<tr>
<td>KALL_6</td>
<td>6.8±0.51</td>
<td>7.2±0.54</td>
<td>0.9</td>
<td>2.3±0.14</td>
<td>1.4±0.04</td>
<td>1.6</td>
<td>17.7</td>
</tr>
<tr>
<td>KALL_7</td>
<td>6.1 ± 0.45</td>
<td>9.0 ± 0.45</td>
<td>0.67</td>
<td>1.2 ± 0.07</td>
<td>1.3 ± 0.04</td>
<td>0.92</td>
<td>17.6</td>
</tr>
<tr>
<td>KALL_8</td>
<td>19.2±0.48</td>
<td>9.9±0.49</td>
<td>1.9</td>
<td>1.9±0.11</td>
<td>0.8±0.04</td>
<td>2.4</td>
<td>31.8</td>
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<tr>
<td>KALL_9</td>
<td>10.8±0.81</td>
<td>1.9±0.29</td>
<td>5.6</td>
<td>0.4±0.02</td>
<td>0.1±0.02</td>
<td>4</td>
<td>13.2</td>
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<td>KALL_10</td>
<td>12.1±0.90</td>
<td>8.7±0.43</td>
<td>1.4</td>
<td>3.1±0.19</td>
<td>2.1±0.06</td>
<td>1.5</td>
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<tr>
<td>KALL_11</td>
<td>16.1±0.40</td>
<td>2.5±0.19</td>
<td>6.4</td>
<td>1.9±0.12</td>
<td>1.0±0.05</td>
<td>1.9</td>
<td>21.5</td>
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<tr>
<td>KALL_12</td>
<td>8.0±0.60</td>
<td>5.7±0.43</td>
<td>1.4</td>
<td>0.3±0.02</td>
<td>0.3±0.01</td>
<td>1</td>
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<td>KALL_13</td>
<td>12.3±0.31</td>
<td>4.8±0.73</td>
<td>2.5</td>
<td>1.5±0.09</td>
<td>0.9±0.05</td>
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<td>19.5</td>
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<tr>
<td>KALL_14</td>
<td>5.7±0.43</td>
<td>2.3±0.34</td>
<td>2.5</td>
<td>2.6±0.15</td>
<td>1.5±0.05</td>
<td>1.7</td>
<td>12.1</td>
</tr>
<tr>
<td>KALL_15</td>
<td>11.3±0.85</td>
<td>5.0±0.75</td>
<td>2.3</td>
<td>1.5±0.09</td>
<td>0.9±0.04</td>
<td>1.6</td>
<td>18.7</td>
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<tr>
<td>KALL_16</td>
<td>8.3±0.62</td>
<td>8.6±0.64</td>
<td>0.97</td>
<td>4.3±0.13</td>
<td>2.8±0.08</td>
<td>1.5</td>
<td>24.0</td>
</tr>
</tbody>
</table>

Values are mean of triplicate determination (n=3)
form in wines of Burgundy (1.1), Bordeaux (6), Châteauneuf (1.2) and Beaujolais (1.1) from the varieties Merlot, Pinot Noir and Cabernet-Sauvignon (Goldberg et al., 1996; Lamuela-Raventos et al., 1995).

Conclusion

Chemical analysis and phenolic pattern of Kallmet wines are important as it is one of the most promising autochthonous grape varieties in Albania. It is the first time that anthocyanin and stilbene profiles of Kallmet are reported. These data will serve as a database to characterize the wines from native grapes and for developing different winemaking protocols with the aim of quality improvement. Kallmet wines were characterized by satisfactory anthocyanin content and a relatively high mDP. The color of the wine is mostly due to polymerized pigments, which lead to good color stability during wine storage. The levels of all the available resveratrol derivatives in Kallmet wines were considerable (20.3 mgL⁻¹). The ratio between the glucoside form and aglycone form of stilbene shows a characteristic of grape variety and climatic conditions, typical for wines from a warm climate country (Mediterranean). These results can constitute a basis for further investigation to study the effect of winemaking process and different agricultural factors in the case of Kallmet grape variety.

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