

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

Anisa Peçuli<sup>1\*</sup>, Fabrice Lorenzini<sup>2</sup>, Mariglen Angjellari<sup>3</sup>, Sylvain Schnee<sup>4</sup>, Katia Gindro<sup>4</sup>, Ágnes Dienes-Nagy<sup>2</sup>

<sup>1</sup>Faculty of Biotechnology and Food, Agriculture University of Tirana, Tirana, Albania

<sup>2</sup>Agroscope, Methods Development and Analysis, Group Wine Quality, 1260 Nyon, Switzerland

<sup>3</sup>Dipartimento di Scienze e Tecnologie Chimiche Università di Roma ‘Tor Vergata’,  
Via della Ricerca Scientifica 1, 00133, Roma, Italia

<sup>4</sup>Agroscope, Strategic Research Division of Plant Protection, 1260 Nyon, Switzerland

### 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<sup>-1</sup> and the anthocyanin content averaged 242 mgL<sup>-1</sup>. 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<sup>-1</sup> in old wine to 609 mgL<sup>-1</sup> in young wine, with a mean value of 242.5 mgL<sup>-1</sup>. *Trans*-piceid was the most abundant stilbene (11 mgL<sup>-1</sup>), followed by *trans*-resveratrol (1.8 mgL<sup>-1</sup>). 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 Gamza. 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<sup>-1</sup>. 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 Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>). 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 SO<sub>2</sub>. 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  $\mu\text{m}$ ; 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 mL $\text{min}^{-1}$  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  $\mu\text{L}$ . The retention time of the main 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  $\mu\text{m}$ ; 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 mL $\text{min}^{-1}$  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  $\mu\text{L}$ ). 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-flavanil monomers (catechins and gallo catechins, 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

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

Wine Code	KALL_1	KALL_2	KALL_3	KALL_4	KALL_5	KALL_6	KALL_7	KALL_8	KALL_9	KALL_10	KALL_11	KALL_12	KALL_13	KALL_14	KALL_15	KALL_16
Year	2008	2009	2013	2007	2011	2013	2012	2012	2012	2012	2013	2009	2009	2007	2012	2012
Total proanthocyanidins mgL <sup>-1</sup>	2325±0.4	8409±0.6	3531±0.9	2762±0.7	3846±0.6	6066±0.9	3374±0.6	3392±0.1	3601±0.9	3601±0.9	1556±0.4	4248±0.7	3462±0.1	3741±0.7	5105±0.4	3007±0.5
Terminal units (as epicatechin) mgL <sup>-1</sup>	118±0.4	904±0.4	330±0.8	175±0.0	309±0.9	460±0.8	190±0.4	398±0.0	351±0.7	348±0.7	103±0.8	193±0.4	268±0.1	118±0.7	360±0.7	338±0.3
mDP	19±0.6	09±0.2	10±0.6	15±0.7	12±0.4	13±0.1	17±0.7	08±0.5	10±0.2	10±0.3	14±0.9	21±0.9	12±0.9	31±0.5	14±0.1	08±0.8
Total anthocyanins mg/L	144±0.8	287±0.3	609±0.1	142±0.5	149±0.4	174±0.7	342±0.5	285±0.1	204±0.5	248±0.2	452±0.8	195±0.3	121±0.8	85±0.05	216±0.1	220±0.6
Fraction of color due to copigmentation %	-3.7±0.001	14±0.005	42±0.001	9.4±0.001	7±0.002	11±0.005	13±0.001	36±0.002	7±0.003	15±0.001	9±0.001	-5±0.001	3±0.002	-6±0.012	9±0.001	8±0.015
Fraction of color due to polymeric pigments %	92±0.002	54±0.002	39±0.015	69±0.003	72±0.015	56±0.002	52±0.003	32±0.002	63±0.012	48±0.003	63±0.015	93±0.015	81±0.003	87±0.003	64±0.002	61±0.002
L*(D65)	66.8±0.006	64.7±0.006	55.6±0.006	74.9±0.006	68.6±0.006	71.9±0.006	61.4±0.006	70.8±0.006	66.0±0.006	68.5±0.006	48.5±0.006	46.1±0.006	64.2±0.012	71.1±0.006	66.5±0.006	65.2±0.006
a*(D65)	31.1±0.006	35.3±0.010	37.3±0.010	23.6±0.006	31.4±0.006	28.2±0.006	40.1±0.010	31.7±0.006	33.5±0.006	32.4±0.012	50.1±0.015	40.4±0.006	32.4±0.006	24.7±0.006	29.3±0.006	30.9±0.006
b*(D65)	15.4±0.006	6.1±0.006	9.1±0.006	14.2±0.006	12.2±0.006	5.0±0.006	6.5±0.010	2.3±0.006	9.3±0.006	5.9±0.010	11.6±0.015	15.7±0.012	19.1±0.006	22.1±0.006	19.4±0.010	14.3±0.015

in epicatechin equivalent and ranged from 103 to 904 mgL<sup>-1</sup>, with a mean value of 310.7 mgL<sup>-1</sup>. 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, Tricadeira, 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, specially 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 the 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<sup>2</sup>=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<sup>-1</sup>, with a mean value of 242.5 mgL<sup>-1</sup>, 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

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 anthocyanins-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).

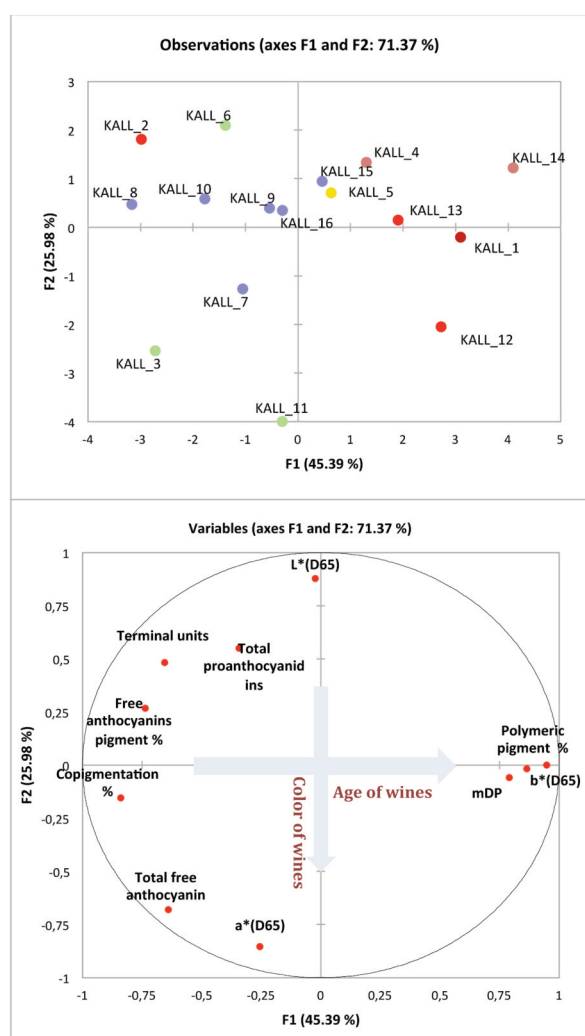
For all the samples, the color descriptor lightness varied from 46 to 75,  $a^*$  varied from 24 to 50, and  $b^*$  varied from 2.3 to 22 (Table 1). The samples with a high content of the yellow color component had a higher color tint and  $L^*$  (lightness) so they were lighter in color.

### 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 ( $8409 \text{ mgL}^{-1}$ ).

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



**Figure 1. Principal component analysis of the analytical determinations of Kallmet wine.**

In the score plot the colors correspond with the year mentioned on the bottle. Deep red - 2007, 2008; red - 2009; yellow - 2011; blue - 2012; green - 2013.

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

Wine code	Year	Delphinidin-3-glucoside	Cyanidin-3-glucoside	Petunidin-3-glucoside	Peonidin-3-glucoside	Malvidin-3-glucoside	Acetylated	Coumarylated	Ratio Acet/Coum
KALL_3	2013	5.2±0.66	0.9±0.44	12.5±0.93	6.0±1.07	58.5±0.43	7.8±0.03	9.2±0.16	0.8±0.02
KALL_6	2013	3.7±0.51	1.2±0.14	9.0±1.37	5.6±0.24	60.9±2.80	12.3±0.29	7.3±0.4	1.6±0.07
KALL_7	2012	3.2±0.17	0.2±0.03	7.0±0.91	3.2±0.23	66.5±2.38	13.5±0.11	6.4±0.03	2.1±0.01
KALL_8	2012	6.5±0.24	0.6±0.22	8.0±0.59	4.7±0.70	57.6±1.35	11.7±0.69	10.9±0.34	1.1±0.09
KALL_9	2012	6.3±0.11	1.9±0.20	14.0±1.24	5.6±0.66	54±4.7	12.6±1.85	5.6±0.31	2.2±0.52
KALL_10	2012	4.5±0.44	1.5±0.18	10.1±0.44	4.6±0.46	60.1±3.49	11.6±1.02	7.6±0.26	1.5±0.35
KALL_15	2012	1.7±0.08	0.3±0.11	20.6±0.18	3.3±0.16	61.8±0.69	6.6±1.00	6.0±0.47	1.1±0.18

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

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 anthocyanin content of aged wine 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 petunidin-3-glucoside, ranging from 7 to 20.6%, followed by peonidin-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).

## 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<sup>-1</sup>, with a mean value 11 mgL<sup>-1</sup>, followed by its *cis*-form. *Trans*-resveratrol was detected in the range from 0.3 to 4.3 mgL<sup>-1</sup>, with a mean value of 1.8 mgL<sup>-1</sup>. 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<sup>-1</sup>, with a mean value of 20.3 mgL<sup>-1</sup>, a level very similar to the total amounts of resveratrol derivatives found in Portuguese monovarietal red wines (20.2 mgL<sup>-1</sup>; 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.

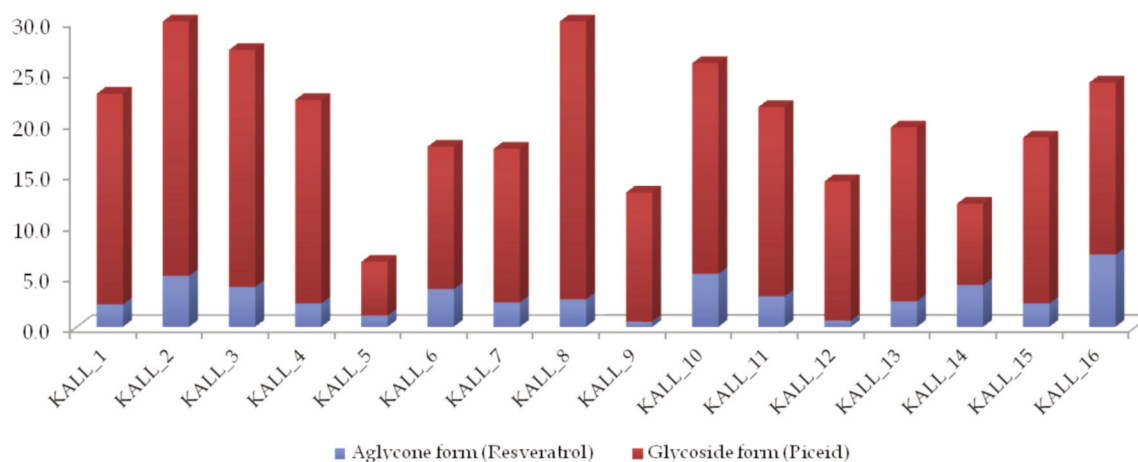
Figure 2 illustrates the total content of *trans/cis* forms of piceid and resveratrol. In the biosynthetic pathway, resveratrol is formed at first and glycosylated into piceid.

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.**

Wine code	<i>trans</i> -piceid mg/L	<i>cis</i> -piceid mg/L	<i>trans/cis</i> piceid ratio	<i>trans</i> - resveratrol mg/L	<i>cis</i> - resveratrol mg/L	<i>trans/cis</i> resveratrol ratio	total mg/L
KALL_1	16.0±0.40	4.7±0.35	3.4	1.5±0.09	0.7±0.03	2.1	22.9
KALL_2	13.1±0.33	12.4±0.62	1	2.4±0.15	2.5±0.08	0.96	30.4
KALL_3	12.9±0.32	10.4±0.52	1.2	2.2±0.13	1.7±0.05	1.3	27.2
KALL_4	14.2±0.35	5.8±0.44	2.4	1.7±0.10	0.6±0.03	2.8	22.3
KALL_5	3.7±0.27	1.6±0.24	2.3	0.7±0.04	0.4±0.02	1.7	6.4
KALL_6	6.8±0.51	7.2±0.54	0.9	2.3±0.14	1.4±0.04	1.6	17.7
KALL_7	6.1 ± 0.45	9.0 ± 0.45	0.67	1.2 ± 0.07	1.3 ± 0.04	0.92	17.6
KALL_8	19.2±0.48	9.9±0.49	1.9	1.9±0.11	0.8±0.04	2.4	31.8
KALL_9	10.8±0.81	1.9±0.29	5.6	0.4±0.02	0.1±0.02	4	13.2
KALL_10	12.1±0.90	8.7±0.43	1.4	3.1±0.19	2.1±0.06	1.5	26.0
KALL_11	16.1±0.40	2.5±0.19	6.4	1.9±0.12	1.0±0.05	1.9	21.5
KALL_12	8.0±0.60	5.7±0.43	1.4	0.3±0.02	0.3±0.01	1	14.3
KALL_13	12.3±0.31	4.8±0.73	2.5	1.5±0.09	0.9±0.05	1.6	19.5
KALL_14	5.7±0.43	2.3±0.34	2.5	2.6±0.15	1.5±0.05	1.7	12.1
KALL_15	11.3±0.85	5.0±0.75	2.3	1.5±0.09	0.9±0.04	1.6	18.7
KALL_16	8.3±0.62	8.6±0.64	0.97	4.3±0.13	2.8±0.08	1.5	24.0

Values are mean of triplicate determination (n=3)



**Figure 2.** Concentration of total glycoside and total aglycone forms of resveratrol in Kallmet wines.

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<sup>-1</sup>). 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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