# Stilbene content in Albanian mono-varietal wines from different vintages

# Anisa PECULI<sup>1</sup>, Sylvain SCHNEE<sup>2</sup>, Elton BASHA<sup>1</sup>, Ágnes DIENES-NAGY<sup>3</sup>

<sup>1</sup>Agricultural University of Tirana/ Rruga "Pajsi Vodica", Faculty of Biotechnology and Food, Tiranë 1029, Albania (e-mail: apeculi@ubt.edu.al)

<sup>2</sup>Agroscope, Strategic Research Division of Plant Protection, 1260 Nyon, Switzerland

<sup>3</sup>Agroscope, Methods Development and Analysis, Group Wine Quality, 1260 Nyon, Switzerland

## Abstract

Albanian wines produced from three most important grape varieties, were analysed for their stilbene content (trans-resveratrol, cis-resveratrol, trans-piceid and cis-piceid), as they are known as important compounds related with positive effects on human health. 22 samples of mono-varietal wines from Albanian varieties "Merlot" (4 samples), "Kallmet" (6 samples) and "Sheshi i Zi" (12 samples) from 2013 to 2016 vintages, were analysed. Stilbene were determine by HPLC-DAD. Results of analysis has shown, that stilbene content ranked in order in Merlot (29.5 mg/L) > Shesh i Zi (12.3 mg/L) > Kallmet (7.27 mg/L). The glycoside form (piceid) was higher in content than the aglycone form (resveratrol) which is a stilbene pattern typical for Mediterranean wine.

Key words: trans-piceid, trans-resveratrol, Albania, wines, health benefit

#### Introduction

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a stilbenoid, a type of natural phenol that belongs to the nonflavonoid class, also known as phytoalexin. It is produced naturally by several plants, but the first plant known for its content is Japanese Knotweed (Burns et al., 2002). It is also present in grape, produced as a defence mechanism against injuries, diseases and fungal attacks. The first study that demonstrates the presence of trans-resveratrol in grapevine as a response to fungal infection and exposure to ultraviolet light were conducted by Langcake and Pryce (Langcake & Pryce, 1976). As it is most concentrated at the skins, it is comprehensible that it is present in higher concentration in red wines than in white wines.

In recent year a lot of studies has arisen attention for the benefit of wine in health prompted from the French Paradox, a term that describes low rate of mortality from cardiovascular disease in French population due to the moderate and regular consumption of wine (Renaud & Gueguen, 1998). During the years the evidence of the role of polyphenols in wine, but mostly of resveratrol, has been increased and it includes the role of resveratrol not only in cardiovascular disease but also its role as anti-carcinogenic, neuroprotector, anti-inflammatory, anti-platelet, obesity and dental health (Arranz et al., 2012; Basli et al., 2012; Bertelli et al., 1995; Gehm et al., 1997)(Zhang et al., 2013). Several studies have tried to understand the mechanisms behind the benefits of resveratrol in our organism. A number of research are conducted both *in vitro* and *in vivo* in order to understand the bioavailability, mechanism and pharmacokinetic of resveratrol that would enable us to know which is the best dose for a better effect (Rotches-Ribalta et al., 2012; Smoliga et al., 2011; Tomé-Carneiro et al., 2013). On the other hand the science of viticulture and winemaking is working in direction to design grape and wines with enhanced health benefits through agricultural practices like exposition to fungal infection or environmental conditions (Panceri et al., 2013) that increase the levels of stilbenes (Bavaresco, 2002). Also the way how winemaking process is directed, affects the concentration of stilbenes in wine (Atanacković et al., 2012). The aim of <u>this</u> study was the investigation of the presence of aglycone (resveratrol) and glycoside (piceide) form and their ratio in the most characteristic varietal wines from Albania.

## Material and methods

#### Wine samples

Twenty-two samples of mono-varietal wines from Albanian varieties "Merlot" (4 samples), "Kallmet" (6 samples) and "Sheshi i Zi" (12 samples) were collected. They were chosen as representatives of all wine grape-growing regions in Albania and various vintages, from 2013 to 2016.

## Stilbene determination

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  $\mu$ 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 delivered at a flow rate of 1 mLmin<sup>-1</sup> and room temperature for HPLC. Consequently, stilbenes are 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  $\mu$ L). UV spectra were made from 200 to 400 nm using the diode array on line detection (Pezet et al., 2003).

## **Results and discussion**

Average stilbene content as function of grape variety is shown in Table 1. Results shown have highlighted the variety importance in stilbene content. The highest levels of total stilbene were found in Merlot (29.49 mg/L) followed by Sheshi Zi (12.36 mg/L) and the last was Kallmet (7.27 mg/L). Similar studies on stilbene content as function of variety in Croatia has shown Merlot as the variety with the highest level of free resveratrol monomers content even though the levels were slightly lowers (5.22 mg/L) than Merlot wines in this study (6.58 mg/L) (Pezo et al., 2008). *Cis*-piceid was the most abundant stilbene in all wines, Merlot (12.7 mg/L) > Shesh Zi (5.68 mg/L) > Kallmet (2.9 mg/L) and *cis*-resveratrol the least present, Kallmet (0.81 mg/L) < Shesh Zi (0.85 mg/L) < Merlot (2.89 mg/L). Different studies have shown a predominance of *trans* forms of resveratrol and piceid as opposed to *cis* form (ref). These findings about the ratio of *trans/cis* stand only for resveratrol (>1) in our study (Table 1) but not for piceid (<1).

The level of *trans*-resveratrol in Merlot wines in this study was very similar with Hungarian Merlot wines (Nikfardjam et al., 2006). Kallmet wines have shown lower levels of stilbene content than those shown in other studies (20.3 mg/L) (Peçuli et al., 2018). Differences on stilbene pattern within the same variety can happen as the samples were taken from different regions with different geographical positions and climates and the oenological practices (extraction of skin components, efficient conservation during vinification and storage) which plays also an important role on stilbenes concentration in wine (Bavaresco et al., 2016) could be different from different producer.

	mg/L tr- piceide	mg/L cis- piceide	<i>tr/cis</i> piceid ratio	mg/L tr- resveratrol	mg/L cis- resveratrol	<i>tr/cis</i> resveratrol ratio	Total stilbenes	ratio piceid / resveratrol
Merlot (n=4)								
Mean	10.20	12.71	0.80	3.69	2.89	1.27	29.49	3.48
St. dev.	7.78	10.46		2.19	2.31			
Kallmet (n=6)								
Mean	2.41	2.91	0.83	1.15	0.81	1.41	7.27	2.71
St. dev.	2.04	1.62		0.87	0.59			
Shesh Zi (n=12)								
Mean	4.53	5.68	0.80	1.30	0.85	1.53	12.36	4.76
St. dev.	2.95	4.02		0.66	0.67			

Resveratrol is the aglycon form, which in biosynthetic pathway is glycosylated to piceid. Different studies have shown that the ratio piceid/resveratrol is high in red varieties (Goldberg et al., 1996). The highest ratio piceid/resveratrol was found in Shesh Zi wines 4.76, followed by Merlot wine 3.48 and the last one Kallmet wines 2.71.

High ratio piceid/resveratrol is shown to be characteristic of varieties grown in warm regions as high sugar content can favour the glycosylation of resveratrol to piceid. Albanian varieties have shown the same pattern of piceid/ resveratrol ratio as Mediterranean varieties (Lamuela-Raventos et al., 1995).

Figure 1 shows the stilbene content in function of vintages. Results show that younger wines tend to have higher content of total stilbene compared to aged wines. This can be due to recombination or degradation of stilbene during the wine aging. Even though stilbene are relatively stable compounds they can remain stable for years just if the wines are properly stored avoiding exposure to excess heat (Yasui et al., 2002) and in presence of normal levels of antioxidants such as sulfur dioxide (Mattivi & Nicolini, 1993).

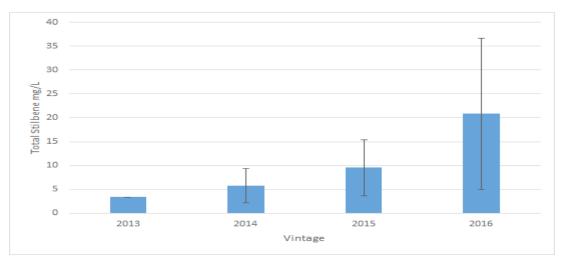


Figure 1. Total stilbene content related to vintage

#### Conclusion

Stilbene content in wines depends on different factors. The grape variety is the main factor as shown in this study, in which Merlot has higher levels of stilbenes, followed by Shesh Zi and Kallmet. Climate, soil, fungal infection of vine, oenological techniques are also decisive factors on the stilbene level in wines. In our study, we could observe the aging effect, while younger wines show higher level of stilbene content in comparison to aged wines. This is probably due to degradation or recombination reaction during the ageing process in or not in proper way. Further research should be conducted for a better understanding of stilbene pattern in Albanian wines related to climate factors and oenological practices, in order to reach the maximum content of stilbene.

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