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Original Article

Comparative Polyphenolic Content of Grape Pomace Flours from 'Fetească neagră' and 'Italian Riesling' Cultivars

Maria L. MUNCACIU[1], Fernando ZAMORA MARÍN[2], Nastasia POP[1], Anca C. BABEŞ[1*]

*[1]University of Agricultural Sciences and Veterinary Medicine, Faculty of Horticulture, Department of Horticulture and Landscaping, 3-5 Calea Mănăştur Str., 400372, Cluj-Napoca, Romania; maria.muncaciu@gmail.com; nastpop@yahoo.com; ancababes@usamvcluj.ro (*corresponding author)*

[2]Rovira i Virgili University, Faculty of Oenology, Department of Biochemistry and Biotechnology, C/Marcel.li Domingo s/n Str., 43007, Tarragona, Spain; fernando.zamora@urv.cat

Abstract

Grape pomace is a waste from wine industry which pollutes the environment. This by-product is considered a potential source of polyphenols, which were proven to be powerful antioxidants or natural coloring agents. The aim of this study was to quantify total polyphenols, total anthocyanins content and some stilbenes in grape pomace seed, skin and mixed flours from 'Fetească neagră' (cultivar for red wines) and 'Italian Riesling' (cultivar for white wines), grown in Miniş-Măderat vineyard, Romania. Flours were obtained by natural drying and ulterior grinding. One-way ANOVA and Tukey's post-hoc test were used.anthocyanins content was high in all the flours from 'Fetească neagră', with the highest concentration in skin flour (35.98 mg malvidin-3-O-glucoside/g dry weight). Stilbenes were also determined through HPLC methods. *Cis*-resveratrol was present in all forms of flour (0.90 to 10.14 µg/g dry weight), compared to its *trans*-isomer, which was not determined in 'Italian Riesling' seed and mixed flour. *Trans*-piceid was the most abundant stilbene and varied between 12.35 µg/g dry weight ('Italian Riesling' mixed flour) and 29.01 µg/g dry weight ('Fetească neagră' seed flour). The concentration of *cis*-piceid was similar among all forms of flour from 'Italian Riesling', while in 'Fetească neagră' seed flour (9.40 µg/g dry weight) it was present at a higher rate. Thus, results proved that 'Fetească neagră' flours are a better source of stilbenes than 'Italian Riesling' flours and seed flours from both varieties are richer in polyphenols than skin flours. Also, the concentration of anthocyanins in 'Fetească neagră' skin flour was high.

Keywords: skin, seeds, total polyphenols, total anthocyanins, stilbenes

Abbreviations: DW: Dry Weight; TAC: Total Anthocyanins Content; TPI: Total Polyphenols Index

Introduction

Grapes are widely cultivated around the globe with vineyards occupying around 7.124.512 ha and producing around 74.499.859 tons according to FAO, 2014. Around 80% of grapes are used in winemaking industry (Kammerer *et al.*, 2005; Jackson, 2008). This leads to the disposing of a huge quantity of residues (including grape pomace), creating both ecologically and economically issues (Nerantzis and Tataridis, 2006; Fontana *et al.*, 2013). Discharging grape pomaces were shown to have a negative impact on flora and fauna due to their high pollution load (Lafka *et al.*, 2007). Using grape pomace as fertilizer inhibits seeds germination, and the presence of lignin prevents the waste to be used as animal feed, reducing digestibility (Fontana *et al.*, 2013). These hindrances impose finding new ways of valorization. Thus, grape pomace is considered a good source of polyphenols with powerful antioxidant capacity (Antoniolli *et al.*, 2015; Iora *et al.*, 2015). Besides exhibiting their antioxidant capacity polyphenols have other bioactivities such as cardioprotective, anticancer, anti-inflammation, antiaging and antimicrobial properties (Torres *et al.*, 2002; Xia *et al.*, 2010; Yu and Ahmedna, 2013).

Different factors such as grape variety, maturity level, vintage, climate, tissue part and winemaking techniques can influence their concentration in grape pomaces (Mildner-Szkudlarz *et al.*, 2013). Recently consumers' demands concentrated on naturally processed, additive-free and safe products. To satisfy consumers' requests, grape pomace flours could be introduced in processed food (Gül *et al.*, 2013; Seo *et al.*, 2015). According to Castaneda-

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Ovando *et al.* (2009), anthocyanins are important natural polyphenolic pigments that constitute an alternative to artificial colorants, possessing also benefic health properties. Resveratrol (3,5,4'-trihydroxystilbene) and piceid (3,5,4'-trihydroxystilbene-3-β-D-glucoside) are two of the major stilbene phytoalexins produced by *Vitaceae* (Moreno-Labanda *et al.,* 2004). They aroused a huge interest in scientists after being identified in wine and linked to the "French paradox" and are mainly present in grape skin, but also in seeds, clusters, and stems (Katalinić *et al.*, 2010). Thus, a big number of reviews confirming resveratrol's biological activities are present in the literature (Athar *et al.*, 2007; Baxter, 2008; Smoliga *et al.*, 2011; Pangeni *et al.*, 2014). Due to their biological and organoleptic characteristics, anthocyanins and stilbenes play a key role in wine quality, and grape extracts are used as sources of natural compounds in the pharmaceutical, food and nutraceutical industries (Flamini *et al.*, 2013).

In the past few years, Romanian research has been concentrated more on polyphenols from fresh grapes or wines and very few on the quantity of compounds that remains in grape pomace with perspective of using it as raw material in other industries (Chedea *et al.*, 2011; Hosu *et al.*, 2011; Bunea *et al.*, 2012a; Bunea *et al.*, 2012b; Lung *et al.*, 2013; Urcan *et al.*, 2016).

The objectives of this study were: (1) to evaluate the TPI (Total polyphenols index) and the TAC (Total anthocyanins content), (2) to separate and characterize stilbenes present in grape pomaces processed in the form of 6 different flours obtained from 'Fetească neagră' and 'Italian Riesling', in order to determine the best polyphenol sources, with prospects of using them as antioxidant agents in food, nutraceutical or pharmaceutical industries.

Materials and Methods

Biological material

The grape pomace of two *Vitis vinifera* cultivars was used to conduct the experiment: one black Romanian variety, 'Fetească neagră' (Variety number VIVC: 4120; VIVC, 2017) and one white variety, 'Italian Riesling' (Variety number VIVC: 13217; VIVC, 2017). Both cultivars were grown in Pâncota as part of the Miniș-Măderat vineyard (46°05' and 46°35', latitude and 21°15' and 22°00', longitude), in Western Romania. All grapes were harvested at technological maturity, in 2015. The whole process is described in Fig. 1. Briefly, after the pomaces were collected and transported to Cluj-Napoca, in freezers, they were dried on polyethylene foils for 1 week in a room with 20-22 °C and 40% humidity. The material was manually turned multiple times a day in order to prevent any microbiological alterations. Then the pomaces were manually separated, bagged under vacuum and stored in a freezer at -18 °C. Prior to analysis, the seeds, the skins, and the whole pomace were ground into flours using a domestic coffee grinder (Heinner Coffee Grinder, 150P) and sieved to remove the debris.

Chemicals and reagents

All solvents were of HPLC quality, and all chemicals were of analytical grade (>99%). Methanol, ethanol (96.5%), deionised water, acetonitrile, formic acid, absolute ethanol and hydrochloric acid (37%) were purchased from Panreac (Barcelona, Spain). The commercial standards: trans-resveratrol, trans-piceid and (-)-epicatechin were bought from Phytolab (Vestenbergsgreuth, Germany) and Extrasynthese (Genay, France), respectively. The *trans* isomers of resveratrol and piceid (resveratrol-3-glucoside) were transformed into their respective cis isomers by UV irradiation (366 nm light for 5 min in quartz vials) of 25% MeOH solutions of the trans isomers. Wilkens *et al.* (2008) established that an irradiation time of 5 min was enough to convert almost the half of *trans* isomers of resveratrol and piceid into their corresponding *cis* isomers, without the

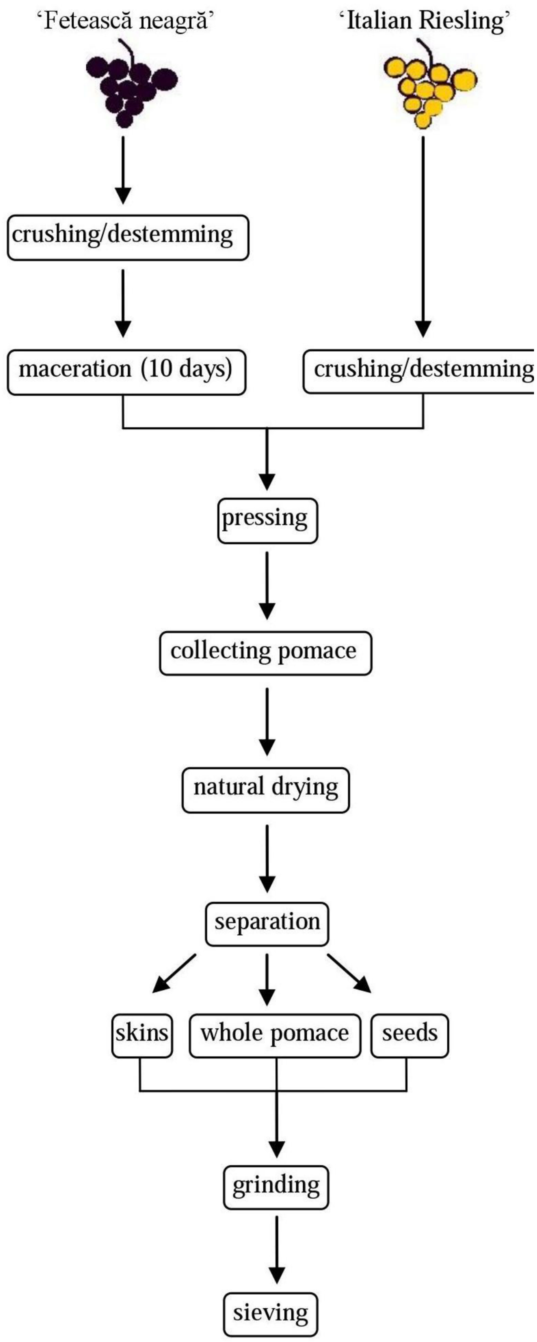

Fig. 1. Technological scheme for obtaining grape pomace flours

formation of degradation products. This process was necessary because the commercially available isomers for resveratrol and piceid have only *trans* configuration. However, in grape and wine is easy to find also their *cis* isomers. For that reason, this process was applied in order to do an accurate quantification of both geometrical isomers of resveratrol and piceid.

Samples preparation for the polyphenols extraction

The extracts of grape pomace flour were prepared by weighing 5 g of each type of flour and adding 50 mL of alcoholic solution 50% and 1 mL of potassium metabisulfite 5.25%. All the extracts were then placed in a water bath at 50 °C for one week and manually stirred each day. Prior to each analysis, the extracts were centrifuged and the supernatants were further used.

Spectrophotometric determination of total polyphenolic index (TPI)

The TPI was determined by measuring the 280 nm absorbance of a 1:200 dilution of grape flour extracts (Ribéreau-Gayon *et al.*, 2006). Aqueous (-)-epicatechin solutions (0, 20, 40, 60, 80, 100 mg/L epicatechin) were used to establish a calibration chart for reporting TPI.

$TPI = ABS_{280} \times dilution$

Spectrophotometric determination of total anthocyanins content (TAC)

A 250 μL of supernatant, 250 μL ethanol 96.5% and 5 mL HCl 0.9 M were placed in a 10 mL tube and stirred. A 1 mL was then mixed with 0.4 mL of distilled water (A) and 1 mL was bleached with 0.4 mL potassium metabisulfite 15% (B). The mixtures were agitated then left into repaus for 10 minutes in order for the reaction to take place. The absorbance was read at 520 nm using a Helios Alpha UV-vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). All results were expressed as mg malvidin-3-*O*-glucoside/g DW and concentrations were calculated according to the above formula. The value 875 is a mathematical constant which was established from data obtained from various red grape cultivars (Ribéreau-Gayon *et al.*, 2006).

$TAC = (A-B) \times 875$

Samples preparation for the HPLC analysis

A 9 ml of the initial extracts were taken and placed under a low-pressure vacuum for 24 hours for the complete evaporation of alcohol (Univapo 100 ECH, Uni Equip, Germany). The samples were then frozen and immediately freeze-dried using a lyophilizer (Christ Alpha 1-4; Martin Christ, Osterode am Harz, Germany), redissolved in 4 mL of alcoholic solution 50% and used for further analysis.

Stilbenes from 'Fetească neagră' grape pomace flours were isolated from diluted samples using ECX SPE cartridges (40 μm, 500 mg, 6 mL; Scharlab, Sentmenat, Barcelona, Spain) according to the procedure described by Lago-Vanzela *et al.* (2011). The eluates were then dried in a rotary evaporator (37 °C), redissolved in 3 mL of 20% methanol in water, and directly injected into the HPLC equipment. Extracts from 'Italian Riesling' were directly injected for the determination of stilbenes. Prior to injections, samples were filtered with a 0.20 μm, polyester membrane, Chromafil PET 20/25, Machery-Nagel, Deuren, Germany.

HPLC-DAD-ESI-MS/MS identification and quantification of stilbenes

HPLC identification of stilbenes from grape pomace flours with the method described by Lago-Vanzela *et al.* (2011) was performed using an Agilent 1200 series system equipped with DAD (Agilent, Germany) and coupled to an AB Sciex 3200 Q TRAP (Applied Biosystems) electrospray ionization mass spectrometry system (ESI-MS/MS). The chromatographic system was managed by an AgilentChemStation (version B.01.03) data-processing station. The mass spectral data were processed with Analyst MDS software (Applied Biosystems, version 1.5). The samples were injected into a Zorbax Eclipse XDB-C18 reversed-phase column (4.6 × 250 mm; 5 μm particle; Agilent).

The flow rate was 0.63 mL/min, the column was thermostated at 40 °C, and the injection volume was 50 μL. The gradient solvents program used for HPLC separation was presented in Table 1. For identification, the ESI-MS/MS was used in negative ionization mode using a combination of −EMS (enhanced mass spectrum; MS conditions) and −EPI (enhanced product ion; MS/MS conditions) experiments, with the following parameters: scan, 100−650 Da (1000 Da/s); declustering potential, −45V; entrance potential,−12 V; collision energy, −20 (arbitrary units); curtain gas, 15 psi; collision gas, high; ion spray voltage, −4000 V; temperature, 425 °C; ion source gas 1, 70 (arbitrary units); ion source gas 2, 50 (arbitrary units); and Q3 barrier, 12 V. For quantification, DAD chromatograms were extracted at 320 nm.

Results and Discussion

Total polyphenols index (TPI)

When grape pomace flours were compared from the point of view of TPI, no statistical differences could be observed between the same flour forms of the two varieties (Fig. 2). The highest TPI was determined in 'Italian Riesling' seed flour (103.62 mg epicatechin/g DW), followed closely by 'Fetească neagră' seed flour (94.40 mg

Table 1. Gradient solvents program used for HPLC separation

Solvent type	Time							
	0	7	38	52	52.5	57	58	65
Solvent A (%)	96	96	70	50	30	-	-	96
Solvent B (%)	4	4	17	30	40	50	50	4
Solvent C (%)	-	-	13	20	30	50	50	-

Solvent A: acetonitrile/water/formic acid in a ratio of 3/88.5/8.5
Solvent B: acetonitrile/water/formic acid in a ratio of 50/41.5/8.5
Solvent C: methanol/water/formic acid in a ratio of 90/1.5/8.5

epicatechin/g DW). As expected, TPI decreased in skin flours for both varieties (68.01 mg epicatechin/g DW in 'Fetească neagră' and 56.29 mg epicatechin/g DW in 'Italian Riesling') and values were statistically significant ($p < 0.05$) when compared to all other forms of flour, except when compared to 'Fetească neagră' mixed flour (81.84 mg epicatechin/g DW). The TPI found in grape seed flours of 'Fetească neagră' and 'Italian Riesling' was quite similar with those reported by Ross *et al.* (2011) for 'Cabernet Sauvignon' grape seed flour (95.2-134.2 mg tannic acid/g DW). According to the present study, grape seed flours contained much more polyphenols than grape skin flours, finding which is consistent with previous research on grape marc seeds and skins resulted from Brazilian winemaking (Rockenbach *et al.*, 2011). Concerning TPI in grape skin flours, independent on the variety, our results are two to threefold higher than those reported by Deng *et al.* (2011), which ranged from 11.6 to 15.8 mg GAE/g dry matter for white skin varieties and 21.4 to 26.7 mg GAE/g dry matter for red skin varieties, respectively. In whole pomace flours, other studies report a concentration in TPI of 236.6 g/kg GAE for 'Öküzgözü' grape pomace, 65.93 g/kg for 'Narince' (Gül *et al.*, 2013), 4.826 mg GAE/100g dry weight basis in white whole pomaces and 5.402 mg GAE/100 g dry weight basis in red grape pomaces (Makris *et al.*, 2007). In general, the literature reported high differences between TPI in skin and whole pomace of white and red varieties (Makris *et al.*, 2007; Deng *et al.*, 2011; Gül *et al.*, 2013), or between defatted seed flours obtained from both red or white grape pomaces (Lutterodt *et al.*, 2011). In this study, this kind of differences was reported only between 'Italian Riesling' skin and mixed flour, but the phenomenon was not observed between 'Fetească neagră' flours. Since it was observed that the proportion of seeds and skins, in the whole pomaces of the two analyzed varieties, and the winemaking technique were different it can be assumed that so was their polyphenolic contribution to the final product. Differences between 'Fetească neagră' and 'Italian Riesling' grape seeds flours were not significant. This may be due to the fact that phenolic compounds are only extracted from the grape seeds after ethanol formation during red wine fermentation (Kammerer *et al.*, 2004). Any other discrepancies between our results and those obtained in other studies could be due to the hypothesis formulated by Iacopini *et al.* (2008), stating that genotypes affect the polyphenol content in grape skin and seeds. The fact that during winemaking the extraction of substances from seeds require a longer contact time and a higher temperature might explain the statistical differences between 'Fetească neagră' skin and seed flours (Cheynier *et al.*, 2006; Gil *et al.*, 2012).

Total anthocyanins content (TAC)

The differences in TAC of grape pomace flours is presented in Table 2. As expected, no anthocyanins were determined in the flours obtained from 'Italian Riesling', because white cultivars lack the UFGT expression, a gene involved in anthocyanins synthesis (Boss *et al.*, 1996). Also, as support to these results, other studies reported the lack of anthocyanins in white grapes (Kammerer *et al.*, 2004; Ivanova *et al.*, 2011). On the other hand, this analytical was performed by taking into account the early discovery of the occurrence of anthocyanins in white berries of Muscat Gordo Blanco cultivar (Gholami and Coombe, 1995). Regarding the results of this study, all forms of flour from 'Fetească neagră' contained anthocyanins. The highest TAC could be found in 'Fetească neagră' skin flour (35.98 mg malvidin-3-glucoside/g DW), followed by mixed flour obtained from the same variety (26.52 mg malvidin-3-glucoside/g DW), a difference that was statistically significant at $p < 0.05$. The lowest quantity of TAC was determined in 'Fetească neagră' seed flours (5.25 mg malvidin-3-glucoside/g DW). Though anthocyanins do not occur naturally in seeds, their presence can be attributed to the diffusion from skins during grape maceration (Kammerer *et al.*, 2004; Gül *et al.*, 2013). The TAC in sample flours of 'Fetească neagră' skins are a lot higher than those found in the same type of flour produced from a red Turkish grape (Gül *et al.*, 2013) or in the skin from red grape marc of 'Lemberger', 'Spätburgunder', 'Swarzriesling' and 'Trollinger', but lower than the TAC in 'Cabernet Mitos' (Kammerer *et al.*, 2004). Grape pomaces skins of native French varieties also contain a very low TAC (Ky *et al.*, 2014). These studies clearly show that the variety is really important in terms of TAC, but the difference between our result and those reported by previous research could also be related to the analytical methods involved (Bunea *et al.*, 2012a). Higher values of TAC, compared to whole grape pomaces analyzed in other studies, were also found in mixed flours from 'Fetească neagră' (Corrales *et al.*, 2008).

Table 2. Total Anthocyanins Content (TAC) in different flour forms from 'Fetească neagră' and 'Italian Riesling'

Variety	Flour form	TAC (mg malvidin-3-glucoside/g DW)
'Fetească neagră'	Skin flour	35.98 ± 0.94 c
	Seed flour	5.25 ± 0.30 a
	Mixed flour	26.52 ± 1.98 b
'Italian Riesling'	Skin flour	n.d.
	Seed flour	n.d.
	Mixed flour	n.d.

n.d. – not detected
Values are expressed as the mean ± standard deviation (n = 3). Different letters within the same column indicate significant differences (Tukey HSD test; $p < 0.05$) among the different flours.

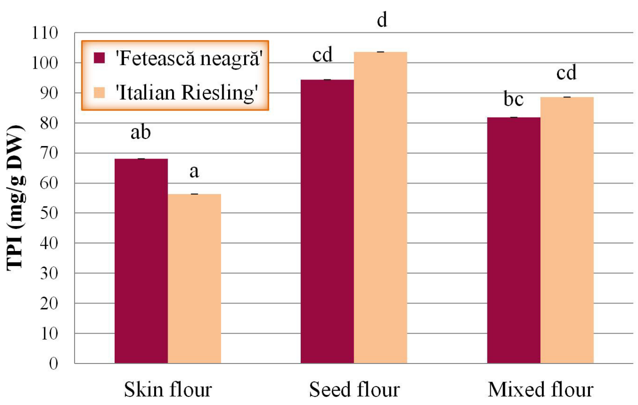

Fig. 2. Total polyphenols index (TPI) in grape pomace flours

Stilbenes

Stilbenes in grape pomace flours were also quantified through an HPLC-DAD-ESI-MS/MS analysis (Tabel 3). Big differences in the content of *trans*-piceid between the seed flours of 'Fetească neagră' and 'Italian Riesling' were reported. Thus, the seed flour from the black variety presented a *trans*-piceid concentration (29.01 μg/g DW) around twofold higher than the same flour form of the white grapes (13.10 μg/g DW), statistically significant at $p < 0.001$. The same trend was followed by the mixed flours when compared to each other ($p < 0.01$). No differences were observed between the skin flours from the two varieties. On the other hand, differences ($p < 0.05$) were found among 'Fetească neagră' grape pomace flours from the point of the *trans*-piceid amount. The lowest concentration was determined in skin flour (13.48 μg/g DW), followed by mixed flour (20.07 μg/g DW) and by seed flour at the highest rate (29.01 μg/g DW). The amount of *trans*-piceid among 'Italian Riesling' flours was constant.

In terms of the *cis*-piceid, all concentrations in 'Italian Riesling' flours (including the one in skin flours) were lower compared to the same flours forms of 'Fetească neagră' and statistically different at $p < 0.001$, ranging from 0.58 to 0.74 μg/g DW. Compared to its *trans*- isomer, this compound was found, with no exception, in a much higher rather. The richest sample in *cis*-piceid was represented by 'Fetească neagră' seed flour (9.40 μg/g DW), followed by mixed flour (5.63 μg/g DW) and the lowest concentration in skin flour (3.01 μg/g DW). When compared to each other, the flours from the black variety were statistically significant at $p < 0.05$.

Trans-resveratrol was the only compound, which was not determined in all flour samples. Thus, it was not identified in seed and mixed flours of 'Italian Riesling'. In skin flour of the same variety it was determined in a concentration that was around threefold lower (0.98 μg/g DW) than the one in the same flour form of 'Fetească neagră' (2.92 μg/g DW), which also represented the richest sample in *trans*-resveratrol. The lowest concentration of *trans*-resveratrol among 'Fetească neagră' grape pomace flours was identified in seed flour (0.57 μg/g DW), statistically significant at $p < 0.05$ when compared to all other samples of flour. This stilbene was the only one that was found in the highest concentration in skin and not in seed flours.

Cis-resveratrol concentrations ranged from 0.90 μg/g DW ('Italian Riesling' skin flour) to 10.14 μg/g DW ('Fetească neagră' seed flour). Significant differences ($p < 0.05$) were found between 'Italian Riesling' skin and seed flour in terms of *cis*-resveratrol concentration. All amounts of *cis*-resveratrol were a lot lower in 'Italian Riesling' grape pomace flours when compared to the ones in the same flour forms of 'Fetească neagră'.

Previous studies stated that in grape berries, stilbenes are considered to be located essentially in the skin (Gatto *et al.*, 2008; Wang *et al.*, 2010). According to Sun *et al.* (2006) and Wang *et al.* (2010) some stilbenes were also reported in the seeds in lower concentrations. With grape skins being considered major contributes to wine stilbene content, compared to seeds and stems (Sun *et al.*, 2006), might explain the higher concentrations of some compounds in 'Fetească neagră' seed flour and not in 'Fetească neagră' skin flour. The higher temperature and alcohol concentration needed to extract polyphenols from grape seeds is also an important factor (Cheynier *et al.*, 2006; Gil *et al.*, 2012). The differences in winemaking technique and certain technological steps, like pressing, could be responsible for the constant concentration of the majority of analyzed stilbenes, among 'Italian Riesling' grape pomace flours (Paixão *et al.*, 2007). Resveratrol was shown to be synthesized in grapes as a response to stress or microbial infection. It can also be produced after the application of some chemical treatments with herbicide or fungicide or by UV-light exposure (Gerogiannaki-Christopoulou *et al.*, 2006). Thus, differences in microclimate, phytosanitary and technological conditions of the grapes may account for the observed stilbenes variability. Furthermore, some studies have shown that *trans*-resveratrol is present in wines in a higher concentration and suggested that cis-resveratrol might be present in grapes in combined form, which could be liberated by hydrolysis during fermentation or maceration processes (Vrhovsek *et al.*, 1997). *Cis*-resveratrol can also increase through the photochemical isomerization of partial trans-resveratrol during winemaking processes (Sun *et al.*, 2006). This may account for the higher *cis*-resveratrol concentration in 'Fetească neagră' flours compared to its *trans*-isomer. Previous studies have shown that not all seeds contain stilbenes, supporting the lack of these compounds in 'Italian Riesling' seed flours (Di Lecce *et al.*, 2014). Because seed flour did not contain *trans*-resveratrol and the concentration in skin flour from 'Italian Riesling' is low, it might be possible that the compound did not rich the detection limit in the mixed flour of the same variety. Lachman *et al.* (2009), also observed that *trans*-resveratrol in the must was mainly below the detectable limit.

Grape pomace flours from 'Fetească neagră' and 'Italian Riesling', in general, showed lower concentrations in stilbenes, compared to other by-products from different varieties. However, sometimes, the analyzed samples contained compounds that other varieties were lacking. For example, seeds from 'Albariño' a white variety grown in Vilafranca del Penedès totally lacked stilbenes (Di Lecce *et al.*, 2014). The same situation was encountered in the case of *trans*-piceid, *trans*-resveratrol and *cis*-piceid in the skin of 'Cabernet Franc' and 'Cabernet Sauvignon' coming from Brazilian winemaking process in São Paulo (Barcia *et al.*, 2014a). *Trans*-resveratrol was also absent in skin by-products of 'Pinot noir', 'Isabel', 'Sangiovese', 'Negro Amaro' and 'Primitivo'. In the same study, this compound was determined in the seeds of the same varieties, except for 'Pinot noir', ranging from 1.11 to 3.75 mg/100 g DW (Rockenbach *et al.*, 2011). Reported to our results these varieties have a *trans*-resveratrol concentration that is higher than the one found in our samples. Furthermore, Dang *et al.* (2014) found significant amounts of *trans*-resveratrol in seeds from the winemaking of 'Riesling', while in our study it was determined in a low concentration only in skin flour. The concentration of piceid compounds in 'Fetească neagră' skin flour is also lower than the one found in fermented skins of 'BRS Lorena' from 2012 vintage. On the other hand, the skins from the same variety did not contain any stilbenes in the climatic year of 2011 (Barcia *et al.*, 2014b).

Table 3. Stilbenes in grape pomace flours of 'Fetească neagră' and 'Italian Riesling'

Parameter	'Fetească neagră' skin flour	'Fetească neagră' seed flour	'Fetească neagră' mixed flour	'Italian Riesling' skin flour	'Italian Riesling' seed flour	'Italian Riesling' mixed flour	Sign. A	Sign. B	Sign. C
Trans-piceid (μg/g DW)	13.48 ± 1.01 a	29.01 ± 2.79 c	20.07 ± 1.91 b	14.69 ± 0.28 a	13.10 ± 1.28 a	12.35 ± 0.94 a	n.s.	***	**
Cis-piceid (μg/g DW)	3.01 ± 0.55 b	9.40 ± 0.51 d	5.63 ± 0.65 c	0.74 ± 0.00 a	0.67 ± 0.14 a	0.58 ± 0.14 a	***	***	***
Trans-resveratrol (μg/g DW)	2.92 ± 0.00 d	0.57 ± 0.14 ab	1.72 ± 0.49 c	0.98 ± 0.25 b	n.d.	n.d.	***	-	-
Cis-resveratrol (μg/g DW)	3.65 ± 0.42 cd	10.14 ± 1.02 e	5.38 ± 0.43 c	0.90 ± 0.14 a	2.67 ± 0.63 bc	1.41 ± 0.76 ab	**	***	***

Values are expressed as the mean ± standard deviation (n = 3). Different letters within the same column indicate significant differences (Tukey HSD test; $p < 0.05$) among the different flours. Sign. A between skin flours of 'Fetească neagră' and 'Italian Riesling'; Sign. B between seed flours of 'Fetească neagră' and 'Italian Riesling'; Sign. C between mixed flour of 'Fetească neagră' and 'Italian Riesling': *, **, *** and ns mean significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively. DW - dry weight; n.d. – not detected

Conclusions

In conclusion, flours from both varieties were proved to be rich in polyphenols. 'Fetească neagră' flours, especially skin flours, are rich in anthocyanins. The flours from the red variety, mainly seed flour, are a better source of stilbenes than 'Italian Riesling' grape pomace flours. In 'Fetească neagră' flours, besides *trans*-resveratrol, all other stilbenes were detected in a higher concentration in the seed and mixed flour. In 'Italian Riesling ' flours, the *cis*- and *trans*-piceid had a constant concentration among the different flours, while *trans*-resveratrol was detected only in skin flour. *Cis*-resveratrol was more concentrated in the seed flour of the white variety. Thus, these grape pomace flours could be considered good sources of antioxidants for alternative industries.

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