

Bioactive compounds and fatty acid profile of grape pomace

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The aims of experiment were to determinate the values of bioactive compounds and fatty acid profile in white dried grape pomace *Vitis vinifera* "Pinot Gris". Grape pomace originated from winery of the University farm Kolíňany, centre Oponice. The dry matter and crude fat content was determined after the preparation of samples. The dried grape pomace contained 94.2% of dry matter and 8.40% of crude fat. This research was conducted on antiradical activity (DPPH), total polyphenols, total phenolic acids, total flavanoids and fatty acid profile. The results confirmed that the grape pomace is considerable source of bioactive compounds, with high antioxidant activity, value of total phenolic acids and total polyphenols. From fatty acids profile are grape pomace significant source of polyunsaturated fatty acids, mainly essential linoleic acid (68.62 g 100 g⁻¹ of fatty acids). They are characterized by wide ratio of n6/n3 fatty acids.

Keywords: grape by-product, fatty acid, bioactive compound, nutrition

1 Introduction

The majority types of natural antioxidant compounds are flavonoid and phenolic acids in complexed of free forms. These compounds have been identified in some vegetables and they have high of correlation antioxidant activity (Einbond et al., 2014; Soares, 2002). Grape berries are consumed as a table fruit, juice, wine and others. Grapes and products from grape are important components in human and animal nutrition (Lavee, 2000; Juráček et al., 2019). Grape pomace is a winemaking by-product. They are rich in bioactive compounds which have a lot of health benefits. Isolation and further utilization of these compounds is an important issue in pomace grape waste management. Different industries have different requirements for specific types of compounds and selective extraction methods for isolation of these types should be developed (Pintač et al., 2018). Grape is a rich source of natural substances – carbohydrates, vitamins and minerals, organic acids and polyphenols (Vauzour et al., 2010). These substances are known for their differently positive impact on health of humans and animals (Capcarová et al., 2017; Habánová et al., 2019; Juríková

et al., 2019; Michalcová et al., 2019). Some antioxidants have antimicrobial, cardioprotective and anticancer effects (Manca et al., 2016; Rodríguez-Morgado et al., 2015). Except polyphenols and triterpenoid compounds can be present. These substances have high potential in medicine since they since their results antidiabetic, anti-inflammatory, antioxidant, immunomodulatory, hepatoprotective and gastroprotective properties, were found. Saturated fatty acids do not contain double bonds, they occur mainly in coconut and palm oil, the most common being myristic acid, palmitic acid and stearic acid. Monounsaturated fatty acids contain one double bond, reducing the LDL (low-density lipoprotein) cholesterol fraction. In nature, oleic acid is the most widespread from monounsaturated fatty acids. Polyunsaturated fatty acids contain two or more double bonds. Polyunsaturated fatty acids have irreplaceable role in the structures of lipoprotein membranes, in the brain, nervous system, inflammatory processes and cardiovascular processes. The polyunsaturated fatty acids group includes essential fatty acids such as linoleic acid (n-6) and α -linolenic acid (n-3) (Ivanko, 2012; FAO,

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2010). The linoleic acid reduces risk of coronary heart disease, adiposity, mortality, improves glycaemic control (Wu et al., 2014; Farvid, 2015; Belury et al., 2018; Li et al., 2018) and α -linolenic acid improves inflammation, hepatic steatosis, insulin sensitivity, and risk of coronary heart disease (Lenighan et al., 2019). The aim of the study was to determinate the values of bioactive compounds and fatty acid profile in white dried grape pomace *Vitis vinifera* "Pinot Gris".

2 Material and methods

The samples of grape pomace Pinot Gris variety ($n = 4$) used in this experiment were from the Winery of the University farm Koliňany, centre Oponice. The samples were predried at 60 °C (dryer of the type: HS 402 PA Chirana) and following mechanically prepared on a laboratory shredder (mesh size 1 mm sieve). After adjustment the samples were analysed on dry matter content (drying on dryer of the type: Pol-Eco Aparatura at $103 \pm 2^\circ\text{C}$) and crude fat content (Soxhlet extraction with light petroleum on Soxtec System HT). The dried grape pomace contained 94.2% of dry matter and 8.40% of crude fat. After fat extraction, the fatty acids profile was determined in the Laboratory on Department of Animal Nutrition (Faculty of Agrobiolgy and Food Resources, SUA in Nitra). The analyses of bioactive compounds were performed in cooperation with the Department of Technology and Quality of Plant Products (Faculty of Biotechnology and Food Science, SUA in Nitra). Statistical evaluation of results was processed using one-way ANOVA (IBM SPSS v. 20.0).

2.1 Determination of bioactive compounds

The antiradical activity, total polyphenols using Folin-Ciocalteu reagent, total flavonoids and total phenolic acids in grape pomace was determined. An amount of 0.20 g of samples was analyzed. Preparation of the extract: the homogenized sample was extracted in 80% ethanol on a shaker (GFL 3005, Germany) for 2 h at room temperature. Subsequently, the extract was centrifuged (Rotofix 32 A, Hettich, Germany; 4,000 RPM, 10 minutes) and the supernatant was used for measurement (DPPH, total polyphenols, phenolic acids and flavonoids).

Antioxidant activity – DPPH method

In the presence of antioxidants, the purple color of the stable DPPH radical changes to yellow. At 515 nm, there were a decrease in absorbance, which was recorded at regular intervals. The decrease in absorbance was recorded until the reaction equilibrium was reached on a spectrophotometer (Sánchez-Moreno et al., 1998). Trolox ($R_2 = 0.99$) was used as a standard and the results were expressed in mg TEAC g^{-1} of sample.

Total polyphenols

The Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965) to determine the total amount of polyphenols was used. After reacting the phenolic compounds with Folin-Ciocalteu, a blue color develops. Gallic acid ($R_2 = 0.998$) was used as the standard and the results were expressed in mg GAE g^{-1} of sample.

Total content of phenolic acids

The spectrophotometric method according to Farmakopea Polska (1999) to determine the total content of phenolic acids was used. The method is based on increasing the absorbance- phenolic acids form a purple complex with the Arnov's reagent, the intensity of which is directly proportional to the absorbance. Caffeic acid ($R_2 = 0.999$) was used as a standard and the results were expressed in mg CAE g^{-1} of sample.

Total flavonoid content

The spectrophotometric method according to Lamaison and Carnat (Willett, 2002) based on the formation of a colored flavonoid-aluminum complex to determine the content of flavonoids was used. Quercetin ($R_2 = 0.9977$) was used as the standard, and the results were expressed in mg QE g^{-1} of sample.

2.2 Determination of fatty acid profile

The fat from samples was received by extraction with organic solvent. Triglycerides (fat) were hydrolysed (saponified) into glycerol and free fatty acids. Fatty acids were derivatized to the methyl esters. For the derivatisation, including hydrolysis and methylation, 2 N KOH in methanol was used. FAME's were then separated and identified by GC/FID (Instrumentation chromatographic system: Agilent 6890 GC). FAME's were separated according to the carbon number and the degree of saturation. Moreover, the position of the double bond(s) and the geometric configuration */cis/trans/* was also important parameters for their determination. For FAME identification a 37 component C4-C24 mixture Supelco 47885-U was used. The amount of each FAME was expressed as the percentage from sum of all present FAME's (100%).

3 Results and discussion

3.1 Bioactive compounds

Antioxidant activity

The average value of antioxidant activity 9.17 ± 0.15 mg TEAC g^{-1} of grape pomace was detected (Table 1). Pastrana-Bonilla et al. (2003) recorded the average antioxidant activity of red grape skins 3.20 mg of TEAC g^{-1} . Ruberto et

Table 1 Bioactive compounds of dried grape pomace

Compounds	Unit	Average \pm SD	Min.	Max.
Antiradical activity DPPH	mg TEAC g ⁻¹	9.17 \pm 0.15	9.03	9.33
Total polyphenols	mg GAE g ⁻¹	27.38 \pm 1.38	26.16	28.87
Total phenolic acids	mg CAE g ⁻¹	13.27 \pm 0.62	12.65	13.69
Total flavonoids	mg QE g ⁻¹	0.12 \pm 0.01	0.11	0.13

TEAC – trolox equivalent antioxidant capacity, GAE – equivalent galic acid, CAE – equivalent caffeic acid, QE – equivalent quercetin

al. (2007) found significantly lower values in grape pomace, probably due to a different variety and different treatment. The values of antioxidant activity in the given experiment were as follows: Nero d'Avola 0.40 mg, Nerello Mascalese 0.58 mg Nerello Cappucci 0.42 mg, Frappato 0.40 mg and Cabernet Sauvignon 0.55 mg TEAC g⁻¹. In another study with dried grape pomace, which was extracted in 80% ethanol, the antioxidant activity 8.21 mg TEAC g⁻¹, which is a comparable value with the results of the our experiment was recorded. The red grape pomace originated from an unspecified variety grown in Romania (Chedea et al., 2017). Chamorro et al. (2012) carried out an experiment in which they analyzed unspecified grape pomace from Spain. They found values of antioxidant activity of up to 18.15 mg TEAC g⁻¹. The antioxidant activity of grape pomace is many times higher than in grape berries, which was also confirmed by the results of Ivanišová et al. (2019), who found values of antioxidant activity from 0.02 to 0.41 mg TEAC g⁻¹ in berries of white grape varieties.

Total polyphenols

The value of total polyphenols in dried pomace from the white grape variety of the Pinot Gris variety 27.38 \pm 1.38 mg GAE g⁻¹ was determined. Antonioli et al. (2015) analyzed in their research the total polyphenols in dried Malbec grape pomace had an average value of polyphenols 31.6 mg GAE g⁻¹. Spigno et al. (2007) examined Barbera grape pomace and measured a average value of 42.50 mg GAE g⁻¹, Vatai et al. (2009) carried out an experiment with lower values of 17.3 mg GAE g⁻¹ in dried pomace of the red grape variety Refošk. Other research (Rockenbach et al. 2011) carried out with dried pomace of several red varieties of Cabernet Sauvignon 74.7 mg, Bordeaux 63.3 mg, Merlot 46.2 mg, Isabel 32.62 mg GAE g⁻¹. Total content of polyphenols in grape pomace is many times higher than in grape berries, which was also confirmed by the results of Ivanišová et al. (2019), value determination polyphenols in berries of white grape varieties from 0.26 to 1.06 mg GAE g⁻¹.

Total phenolic acids

In carried experiment, the value of total phenolic acids was determined to 13.27 \pm 0.62 mg CAE g⁻¹. Librán et al.

(2013) found a lower value of this parameter in the range 0.39–5.02 mg CAE g⁻¹. Baydar et al. (2007) performed an experiment with grapes of white grapes of the Turkish variety Narince, which were dried at room temperature. In this case, they found higher values of phenolic acid content of 24.00 \pm 4.3 mg CAE g⁻¹. Further research by Xu et al. (2014), in which they examined skins from the Muscadine variety, which originates in the southeastern United States and Mexico, and found a phenolic acid content 23.15 mg CAE g⁻¹. The total content of polyphenols from Cabernet Sauvignon red grape pomace from the Maipo region of Chile was 10 mg CAE g⁻¹ (Ishida et al., 2015), which was the value that most closely matched our results. Ivanišová et al. (2019) recorded lower values of total phenolic acids (0.11–0.50 mg CAE g⁻¹) in white grape varieties (berries), which are lower values than in the analyzed grape pomace samples.

Total flavonoids

The average value of total flavonoids 0.12 \pm 0.01 mg QE g⁻¹ was found. In an experiment by Librán et al. (2013) a range of values of the content of total flavonoids (0.03–4.98 mg QE g⁻¹) was determined on Tempranillo red grape compacts analyzes of several samples. Poudel et al. (2008) performed an experiment with 5 varieties of wild grapes and 2 varieties of cultivated grapes. The grape skins were examined and the following results were found in wild varieties: Ebizuru 2.1 \pm 0.1 mg, Ryukyuganebu 1.2 \pm 0.1 mg, Shiohitashibudou 3.4 \pm 0.3 mg, Shiragabudou 1.3 \pm 0.1 mg, Yamabudou 1.2 \pm 0.1 mg QE g⁻¹; in breeding varieties: Bailey Alicante A 2.5 \pm 0.1 mg, Muscat of Alexandria 0.3 \pm 0.1 mg QE g⁻¹. In another experiment, Braga et al. (2016) carried out the experiment again with compacts of red grapes of the Pinot Noir variety, which were frozen and subsequently lyophilized. Subsequent analyzes gave total flavonoid values of 1.76 \pm 0.05 mg QE g⁻¹. Ozdemir et al. (2018) examined the skins and pulp of the Turkish red grape variety Okugozu and found total flavonoid values: skins 0.01 mg QE g⁻¹ and pulps 0.12 mg QE g⁻¹. The content of total flavonoids in grape pomace is lower than in grape berries, which was also confirmed by the results of Ivanišová et al. (2019), they found values of total

Table 2 Fatty acid profile of dried grape pomace

Fatty acid (g 100 g ⁻¹ FA)	Average ± SD	Min.	Max.
C16:0	8.43 ±0.30	8.00	8.70
C16:1	0.22 ±0.004	0.22	0.23
C18:0	3.98 ±0.13	3.80	4.09
C18:1 <i>cis</i> n9	16.27 ±0.23	15.95	16.47
C18:2 <i>cis</i> n6	68.62 ±0.80	67.93	69.75
C18:3 n3	0.50 ±0.02	0.48	0.54
C20:0	0.20 ±0.009	0.19	0.21
C20:1 n9	0.20 ±0.001	0.20	0.21
C22:0	not detected	/	/
Polyunsaturated fatty acids	69.13 ±0.82	68.42	70.29
Monounsaturated fatty acids	16.69 ±0.23	16.36	16.90
Saturated fatty acids	12.61 ±0.43	12.00	13.00
n6/n3	136.16 ±4.12	130.09	138.83

FA – fatty acids, C16:0 – palmitic acid, C16:1 – palmitoleic acid, C18:0 – stearic acid, C18 – 1*cis* n9-oleic acid, C18:2 – *cis* n6-linolenic acid, C18:3 n3 – α -linolenic acid, C20:0-arachidic acid, C20:1 n9-*cis*-11-eicosenoic acid, C22:0 – behenic acid

flavonoids in white grape varieties (berries) from 0.18 up to 0.35 mg GAE g⁻¹.

86.60/1 (Pinot Blanc) and 89.59/1 (Zweigelt) in grape pomace from Slovakia.

3.2 Fatty acid profile

Essential linoleic acid had the highest proportion of polyunsaturated fatty acids in dried grape pomace (Table 2). Consistent with our results, Parry et al. (2011) recorded linoleic acid values of 68.70 g (Chardonnay) and 72.30 g 100 g⁻¹ FA (Tinta Cao) in grape pomace. Similar values of linoleic acid in grape pomace are reported by Ribeiro et al. (2015) 54.58 g (Cabernet Sauvignon) and 65.49 g 100 g⁻¹ FA (Merlot). Dried grape pomace had proportion of total unsaturated fatty acids 85.82 g 100 g⁻¹ FA. The high proportion of unsaturated fatty acids in grape pomace was also confirmed by the results of Parry et al. (2011) (90.50 g and 88.50 g 100 g⁻¹ FA from Chardonnay and Tinta Cao varieties). In the performed experiment, the proportion of monounsaturated fatty acids was 16.69 g 100 g⁻¹ FA, which is consistent with the results of Yi et al. (2009), who report a value of 16.50 g 100 g⁻¹ FA (Royal Rouge). From the monounsaturated fatty acids, oleic acid (16.27 g 100 g⁻¹ FA) had the largest share, which was also confirmed by the results of Iora et al. (2015) with values of 11.82 to 14.15 g 100 g⁻¹ FA in grape pomace of three varieties (Merlot, Cabernet and Tanat). Palmitic acid accounted for the highest proportion of saturated fatty acids in grape pomace. Ratio of n6/n3 in grape pomace was higher in comparison with results of Yi et al. (2009) where n6/n3 ratio was 36.90/1 and 20.80/1 (Caberen Sauvignon and Royal Rouge) in milk of cows fed with TMR than in milk of grazing cows. Kolláthová et al. (2020) reported lower ratio of n6/n3 41.72/1 (Green Veltliner),

4 Conclusions

The results confirmed that grape pomace are considerable source of bioactive compounds, they are characterized high antioxidant activity, value of total phenolic acids and total polyphenols. The grape pomace are valued by high proportion of polyunsaturated fatty acids, in particular linoleic acid. The grape pomace are noted for a low of saturated fatty acids content. Summarily, the grape pomace have responsible position in nutrition following a values of bioactive compounds and essential linoleic acid concentration.

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