CHEMIJA. 2014. Vol. 25. No. 1. P. 51–55

Antioxidant activity of vine fruits depending on their colouring

Magdalena Kapłan[1],

Agnieszka Najda[2]

[1] *Chair of Seed Production and Nurseries, University of Life Science, 58 Leszczyński Street, 20-068 Lublin, Poland*

[2] *Department of Vegetable Crops and Medicinal Plants, University of Life Science, 58 Leszczyński Street, 20-068 Lublin, Poland*

Fruits of the vine and their products, including wine, are the raw material, which is a valuable source of biologically active substances for the human organism. The most important group of health-promoting compounds present in fruits are polyphenols, flavonoids, phenolic acids, flavones, flavonols, flavanones, flavanonols, catechins, and anthocyanin pigments. The aim of the present study was to determine the level of selected secondary metabolites and the antioxidant capacity of vine fruits depending on their colouring. The experimental material consisted of fruits derived from three vine cultivars characterized by different skin colouring: 'Regent' – dark blue fruits, 'Einset Seedless' – pink fruits, and 'Jutrzenka' – white fruits. A field experiment was conducted in 2010 and 2011 in Vineyard Faliszowice in Sandomierz Upland (50°39'N; 21°34'E). Shrubs of three cultivars were planted in spring 2003. The harvest was dependent on the results of sugar content measurements in grape extract using an Abbe refractometer. Fruits of the studied cultivars were subject to a comparative analysis of the content of secondary metabolites such as: total polyphenols, gallic acid, phenolic acids, flavonoids, tannins and anthocyanins. The results showed that the antioxidant activity of the studied grapes depended significantly on the colour of berries; fruits of 'Regent' and 'Einset Seedless' cv. had a significantly higher antioxidant capacity than the 'Jutrzenka' cv. It has been shown that contents of anthocyanins and flavonoids increased along with increasing colour intensity of grapes. Fruits of 'Einset Seedless' cv. having pink-coloured skin contained significantly more phenolic acids and tannins than the others.

Key words: vine, extract, polyphenols, anthocyanins, flavonoids, tannins

INTRODUCTION

Due to the taste and nutritional properties, vine fruits are valuable raw materials for the human organism and an abundant source of biologically active substances such as vitamins (A, B1, B2, C, PP), minerals (potassium, phosphorus, calcium, iron, boron, magnesium), pectins, pigments, tannins, oils, easily digestible carbohydrates, amino acids, fruit acids, and fiber. The most important group of health-promoting compounds present in fruits are polyphenols, flavonoids, phenolic acids, flavones, flavonols, flavanones, flavanonols, catechins, and anthocyanin pigments [1–6].

According to Yang et al. [5], 80% of all grapes are used for wine production, including 13% of dessert fruit, while the rest of the fruits are used for making raisins. In wine-producing countries, mainly cultivars derived from *Vitis vinifera* (Wine Grape), while in the colder regions, including Poland, the species belonging to the *Vitis labrusca*, *Vitis riparia*, *Vitis aestivalis*, *Vitis rupestris*, and *Vitis rotundifolia* are grown, which are used for wine manufacturing.

* Corresponding author. E-mail: magdalena.kaplan@up.lublin.pl

In Poland, vine is of little economic importance, but considerable interest in the cultivation of this species and wine production has been recently observed [7]. It is estimated that about 200 thousand vines are planted each year [8]. The grapevine has been present in home gardens because of its many decorative, taste, and health benefits.

The aim of the present study was to determine the level of selected secondary metabolites and the antioxidant capacity of vine fruits depending on their colouring.

EXPERIMENTAL

The experimental material consisted of fruits derived from three vine cultivars characterized by different skin colouring:

1. 'Regent' – ('Diana' ('Silvaner' × 'Mueller Thurgau') × 'Chambourcin'), origin: Germany, dark blue fruits;

2. 'Einset Seedless' – ('Fredonia' × 'Canner Seedless' ('Hunisia' × 'Sultania')), origin: USA, pink fruits;

3. 'Jutrzenka' – ('Seyve Villard' 12-375 × 'Pinot Blanc'), origin: Jasło, Poland, white fruits.

In 2010 and 2011 a field experiment was conducted in Vineyard Faliszowice in Sandomierz Upland (50°39'N; 21°34'E). Shrubs of three cultivars were planted in spring 2003. The harvest was dependent on the results of sugar content measurements in grape extract using an Abbe refractometer. Fruits of the studied cultivars were subject to a comparative analysis of the content of secondary metabolites presented below.

Determination of total phenolic content

The amount of total phenolic was determined using Folin–Ciocalteu reagent, as described by Singleton and Rossi [9]. About 1g of raw homogenised samples was extracted with 80% aqueous methanol (4.5 ml) on a mechanical shaker for 2 h. The mixture was centrifuged at 10,000 rpm for 15 min and the supernatant decanted into polypropylene tubes. The pellets were extracted under identical conditions. Supernatants were combined and filtered through Whatman No. 1 filter paper. The clear extracts were analysed both for determination of phenolic content and antioxidant activity. Results were expressed as milligram gallic acid equivalents (GAE)/100 g fresh mass.

Total flavonoids estimation

The studied material was investigated for the total content of flavonoids, using the modified Christ and Müller method, calculated for quercetin QE [10]. Absorbance was measured at 425 nm on a HITACHI U-2900 spectrophotometer.

The content of flavonoids was calculated from the equation:

$$X = \frac{8.75 \times A}{m},$$

where m (g) was the amount of fresh mass.

Total phenolic acids estimation

It was carried out according to the Arnov method [11]. One milliliter of the sample was mixed with 5 ml of distilled water, 1 ml 0.5 M HCl, 1 ml of the Arnov reagent and 1 ml 1 M NaOH, and subsequently adjusted to 10 ml with distilled water. The absorbance was measured at 490 nm. The total phenolic acid content was expressed as the caffeic acid equivalent (CAE).

Tannin estimation

The amount of tannin estimation was determined using the Pharmacopoeia procedure [10]. The content of tannins was expressed as fresh and dry weight percentage.

Anthocyanins estimation by means of colorimetry

Samples of raw material (1.0 g) were extracted with 50 ml HCl (1 mol · dm^3) and heated in a water bath for 1 hour. The obtained extract was hydrolyzed with 20 ml n-buthanol, and then two 10 ml n-buthanol portions were added as a solution. Anthocyanin extracts were rinsed in a 50 ml flask with n-buthanol. The absorbance was measured immediately at 533 nm [12].

The percentage of anthocyanins, as delphynidyn chloride, was calculated from the expression:

$$P = \frac{A \times V \times F}{m},$$

where P is total anthocyanins (mg · 100 g^{-1}), A is the absorbance at 533 nm, V is the value of buthanol phase (50 ml), F is the coefficient for delphinidyn chloride (2.6), m is the mass of the sample to be examined (mg).

Determination of antiradical activity (AA)

A 0.1 ml aliquot of the methanol extract prepared above was mixed with 3.9 ml of an 80% ethanolic 0.6 mM DPPH solution. The tubes were vortexed for 15 s and allowed to stand for 180 min, as described by Cai et al. [13]. After this, the absorbance of the mixture was measured at λ = 517 nm wavelength using the HITACHI UV-Vis spectrophotometer (UV-Vis model U-2900, Shimadzu, Kyoto, Japan). Most tested compounds react completely within 180 min in this condition. Reaction time for vitamin C is less than 1 min due to its fast oxidation. Ethanol (80%) was used as a blank solution, and the DPPH solution without test samples (3.9 ml of DPPH + 0.1 ml of 80% ethanol) served as the control. All tests were performed in triplicate. The antiradical activity of the test samples was expressed as the median effective concentration for radical scavenging activity (EC_{50}): TP (mg) of the antioxidant (test sample) required for a 50% decrease in the absorbance of DPPH radicals, and inhibition (%) of the DPPH absorbance = $(A_{control} - A_{test}) \times 100/A_{control}$. A plot of the absorbance of DPPH vs concentration of the antioxidant was made to establish the standard curves (dose-response curves) and to calculate that EC_{50}. $A_{control}$ is the absorbance of the control (DPPH solution without the test sample), and A_{test} is the absorbance of the test sample (DPPH solution plus

0.1 ml of 5 μM test compound). Ascorbic acid served as a standard. The results of the assay were expressed relative to an ascorbic acid equivalent.

Chemicals

All reagents and solvents were analytical grade chemicals from Merck (Darmstadt, Germany) or Sigma Chemical Co. (St. Louis, MO, USA).

Results achieved from laboratory experiments were statistically processed by means of the variance analysis method and Tukey confidence intervals at 5% confidence level.

RESULTS AND DISCUSSION

It was shown that extract contents for the studied fruits significantly differed between cultivars and amounted to: 'Einset Seedless' – 17.5%, 'Jutrzenka' – 18.3%, and 'Regent' – 19.3% (Figure). Studies performed by Krośniak et al. [4] revealed that extract content in vine fruits ripening under Polish conditions was very divergent depending on the cultivar. In the case of light-fruit cultivars, values of the parameter were at the levels: 16.4% for 'Muscat Odeski', 21.1% for 'Jutrzenka', and 22.8% for 'Seyval Blanc', whereas for dark-berry cultivars: 'Mareachal Foch' and 'Rondo' – 19.2%.

The results unveiled that the antioxidant activity of tested vine fruits significantly depended on their colouration (Table 1). It has been shown that fruits of 'Einset Seedless' cv. and 'Regent' cv. contained remarkably more polyphenols than the 'Jutrzenka' cv. It has been observed that pink-colored fruits of 'Einset Seedles' cv. had slightly more polyphenols than the 'Regent' cv. with dark skin of the fruit. These results confirm the study by Krośniak et al. [4], which showed that the polyphenol content significantly depended on the colour of the skins and the cultivar of grapes. The authors proved that red-colored fruits ('Rondo' and 'Marechal Foch' cv.) contained considerably more polyphenols than white cultivars ('Jutrzenka', 'Seyval Blanc', and 'Muscat Odeski'). The influence of a cultivar on the studied parameter among cultivars with the same colour of berries appeared to be significant.

The content of phenolic acids in the studied fruits depended remarkably on the cultivar and ranged from 5.10 up to 26.7 mg 100 g FM (Table 1). It has been shown that fruits of 'Regent' cv. with dark-coloured skin contained significantly less phenolic acids than 'Einset Seedless' cv. (red, purple) and 'Jutrzenka' cv. (white). Similar results were obtained by Bunea et al. [15], who studied ferrulic acid, quercetin, and resveratrol in 9 cultivars of vine grapes grown by means of the conventional and organic method. They found that fruits of 'Muscat de Hamburg' cv. with dark-coloured skin contained less ferrulic acid as compared to those with white-coloured skin 'Timpuriu de Cluj' cv. Yang et al. [5] also reported that 'Baco Noir' contained less total phenolic acids and flavonoids than white cultivars such as 'Riesling' cv. and 'Vidal Blanc' cv. According to Yang et al. [5], Anastasiadi et al. [16], and Bunea et al. [15], the quantitative and qualitative composition of phenolic acids is significantly affected by genetic features, environmental conditions, as well as by agrotechnical management.

The analysis showed that the level of tannins in vine fruits substantially depended on their colouring (Table 1). Among

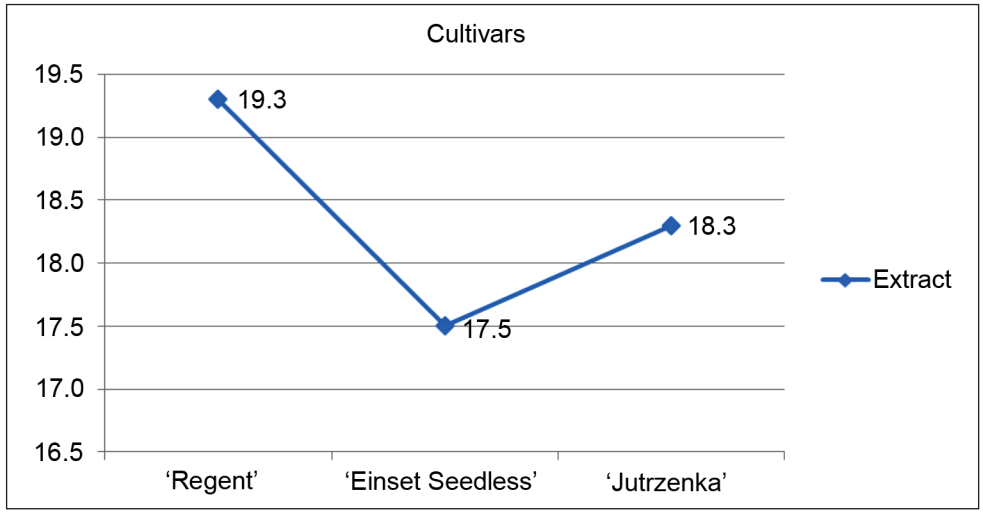

Figure. Soluble solids content of investigated grape cultivars (g 100 g^{-1})

Table 1. **The total phenolic, phenolic acid and tannins contents in 3 grape cultivars (mean for 2010–2011)**

Cultivar	Colour	Total phenolic, mg 100 g FM	Phenolic acid, mg 100 g FM	Tannins, % FM
'Regent'	Dark purple	337.2 ± 3.71 a	5.10 ± 0.01 c	0.12 ± 0.006 b
'Einset Seedless'	Red, purple	365.8 ± 1.03 a	26.7 ± 0.11 a	0.30 ± 0.011 a
'Jutrzenka'	White	258.8 ± 1.44 b	13.2 ± 0.04 b	0.07 ± 0.00 c

Explanation: Means followed by the same letter are not significantly different at α = 0.05.

Table 2. **The total flavonoid, total anthocyanin contents and scavenging ability of 3 grape cultivars (mean for 2010–2011)**

Cultivar	Total flavonoids, mg 100 g^{-1} FM	Total anthocyanin, mg 100 g^{-1} FM	DPPH, μM TE/g^{-1} FM
'Regent'	396.4 ± 21.3 a	55.1 ± 4.8 a	81.4 ± 2.5 a
'Einset Seedless'	104.1 ± 13.1 b	6.4 ± 1.2 b	68.1 ± 4.2 a
'Jutrzenka'	84.8 ± 7.3 c	2.5 ± 0.7 b	41.1 ± 1.7 b

Explanation: Means followed by the same letter are not significantly different at α = 0.05.

studied cultivars, significantly higher level of tannins was found in fruits of 'Einset Seedless' cv., while remarkably lower level was in 'Jutrzenka' cv. According to Matthews and Nuzzo [17], tannins are present in the skin, seeds, and peduncles. Their content in fruit juice (a must) and wine depends on the crop technique, shrub loading, and climatic conditions, methods of maceration and fermentation circumstances. These compounds have a spectrum of important properties that affect the colour, colour stability, astringency, and wine depth [18].

The content of flavonoids in the studied fruits ranged from 84.8 to 396.4 mg of cyanidin 3-glucoside equivalents / 100 g of grapes, which differed significantly between the assessed cultivars (Table 2). It was observed that the flavonoid content significantly increased with an increase in the colour intensity of fruit. However, these facts do not confirm findings of Yang et al. [5], who did not show any univocal effect of fruit colour on the tested parameter. Among cultivars from *Vitis vinifera* species, significantly higher flavonoid content was found for 'Pinot Noir' cv. (dark purple) rather than for others: 'Cabernet Franc' cv. (dark purple), 'Chardonnay' cv. (green), and 'Riesling' cv. (green), while for hybrids, significant differences were observed between 'Catawba' cv. (pink) and 'Cayuga White' cv. (green) vs 'Baco Noir' cv. (dark purple), 'Chancellor' cv. (dark purple), 'De Chaunac' cv. (dark blue), 'Marechal Foch' cv. (dark purple), and 'Vidal Blanc' cv. (green).

The anthocyanin content in the studied vine fruits substantially depended on their colouration and ranged from 2.5 mg 100 g^{-1} FM to 55.1 mg 100 g^{-1} FM (Table 2). Fruits of 'Regent' cv. had significantly more anthocyanins than 'Einset Seedless' cv. and 'Jutrzenka' cv. The content of these compounds in 'Regent' cv. was almost 150 times greater than that in 'Jutrzenka' cv. and 62 times higher than in 'Einset Seedless' cv. Studies carried out by Yang et al. [5] involving cultivars with green-coloured skin did not reveal any presence of anthocyanins, whereas for pink-coloured fruits of 'Catawa' cv. this parameter reached 8.1 mg · 100 mL of fresh must. In the case of grapes with dark coloured skin, those authors demonstrated a significant impact of a cultivar on the evaluated parameter; the anthocyanin content ranged from 49.8 to 239.6 mg 100 mL and it differed significantly. Similarly, in the study performed by Mazza [1], the total content of anthocyanins in the fresh weight of ripe vine fruits ranged from 30 to 750 mg g^{-1} depending on the cultivar. Numerous studies have demonstrated that the anthocyanin and tannin contents largely depend on the cultivar, species, ripeness degree of the fruits, production localization, and climate [1, 5, 19, 20].

The antioxidant activity of fruit extracts made from the studied fruits as determined by the DPPH method ranged from 41.1 to 81.4 μM TE g^{-1} and it significantly depended on the cultivar (Table 2). It has been shown that fruits of 'Regent' cv. and 'Einset Seedless' cv. had considerably higher antioxidant activity than 'Jutrzenka' cv. Research by Katalinić et al. [6], which involved assessing the antioxidant activity of 14 vine cultivars, showed no significant effect of the vine fruit colour and cultivar on the tested parameter; considering white cultivars, the DPPH value ranged from 52.8 to 291.0 μM TE g^{-1}, while for red ones it was from 58.0 to 239.0 μM TE g^{-1}.

CONCLUSIONS

The antioxidant activity of the studied vine grapes determined as the total polyphenols and the DPPH parameter depended significantly on the colour of berries. Fruits of 'Regent' cv. and 'Einset Seedless' cv. had significantly higher antioxidant capacity than 'Jutrzenka' cv. Fruits of 'Einset Seedless' cv. characterized by pink-coloured skin contained more phenolic acids and tannins than others. It has been shown that the flavonoid content remarkably increased along with the increase of grape colour intensity. The anthocyanin level in fruits of the studied vine cultivars significantly depended on their colouration.

Received 20 June 2013
Accepted 20 November 2013

References

1. G. Mazza, *Crit. Rev. Food Sci*., **35**, 341 (1995).
2. R. R. Montealegre, R. Peces, J. L. Vozmediano, et al., *J. Food Compos. Anal.*, **19**, 687 (2006).
3. J. M. Pezzuto, *J. Agric. Food Chem.*, **56**, 6777 (2008).
4. M. Krośniak, M. Gąstoł, P. Banach, et al., *Żywność. Nauka. Technologia. Jakość,* **4(65)**, 116 (2009).
5. J. Yang, T. E. Martinson, R. H. Liu, *Food Chem*., **116**, 332 (2009).
6. V. Katalinić, S. S. Mozina, D. Skroza, et al., *Food Chem.*, **119**, 715 (2010).
7. M. Kapłan, *Acta Agrobot.*, **64(4)**, 189 (2011).
8. J. Lisek, *The Amateur Viticulture,* Wydawnictwo Działkowiec, Warszawa (2002).
9. V. Singleton, J. Rossi, *Am. J. Enol. Viticult.*, **16**, 144 (1965).
10. *Polish Pharmacopoeia VII,* Wyd. PTFarm, Warszawa (2005).
11. *Polish Pharmacopoeia VI*, Wyd. PTFarm, Warszawa (2002).
12. K. Miłkowska, H. Strzelecka, *Herba Pol.*, **41(1)**, 11 (1995).

13. Y. Z. Cai, M. Sun, H. Corke, *J. Agric. Food Chem.*, **51(8)**, 2288 (2003).
14. J. H. Chen, C. T. Ho, *J. Agric. Food Chem.*, **45**, 2374 (1997).
15. C. I. Bunea, N. Pop, A. Babes, et al., *Bulletin UASVM Horticulture*, **69(1)**, 104 (2012).
16. M. Anastasiadi, H. Pratsinis, D. Kletsas, et al., *Food Res. Int*., **43**, 805 (2010).
17. M. A. Matthew, V. Nuzzo, *Acta Hortic.*, **754**, 423 (2007).
18. A. Weston, *Proceedings of the 29th Annual New York Wine Industry Workshop*, 6 (2005).
19. F. Mattivi, C. Zulian, G. Nicolini, et al., *Ann. N. Y. Acad. Sci.*, **957**, 37 (2002).
20. A. C. Munoz-Espada, K. V. Wood, B. Bordelon, et al., *J. Agric. Food Chem*., **52**, 6779 (2004).

Magdalena Kaplan, Agnieszka Najda

VYNUOGIŲ ANTIOKSIDACINIO AKTYVUMO RYŠYS SU JŲ SPALVA

S a n t r a u k a

Trijose skirtingose vynuogių rūšyse buvo nustatyti ir tarpusavyje palyginti antrinių metabolitų – polifenolių, galo rūgšties, fenolių rūgščių, flavonoidų, taninų ir antocianinų – kiekiai. Nustatyta, kad tirtųjų vynuogių antioksidacinės savybės yra glaudžiai susiję su jų spalva.