https://doi.org/10.17221/222/2018-CJFS

Antioxidant properties and resveratrol content of Polish Regent wines from Podkarpacie region

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Citation: Bednarska S., Dabrowa A., Kisala J., Kasprzyk I. (2019): Antioxidant properties and resveratrol content of Polish Regent wines from Podkarpacie region. Czech J. Food Sci., 37: 252–259.

Abstract: The antioxidant properties of cool-climate Regent wines from Podkarpacie region, Poland were investigated. Total polyphenols, total flavonoids, total monomeric anthocyanins, *trans*-resveratrol concentration, radical scavenging ability using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and total antioxidant capacity with TEAC and FRAP methods were measured. The average contents of the compounds determined for Regent wines were comparable to the respective data available in literature found for warm-climate wines. Correspondence Analysis of the data revealed the relation of total polyphenols content and antioxidant capacity and radical scavenging ability of studied wines, and in lesser extent correlation with total flavonoids content. The total monomeric anthocyanins content and resveratrol concentration were poorly related to the total polyphenols content and antioxidant capacity.

Keywords: antioxidant capacity; Polish winemaking; polyphenols; Regent grapevine; resveratrol; wine

Wine is one of the most important source of polyphenolic antioxidants including anthocyanins, flavonols and stilbenes which mainly influence on wine quality and health effects (Flamini *et al.* 2013; Tangney & Rasmussen 2013; Haseeb *et al.* 2019). Exploring the content and composition profile of polyphenols in wine is the point of interest due to their antioxidant and immunomodulatory properties (Guilford & Pezzuto 2011; Haseeb *et al.* 2017). Polyphenols have been shown to regulate mechanisms responsible for the events leading to the initiation and development of metabolic syndrome predisposing to cardiovascular disease i.e. protection against oxidative and inflammatory damage or decrease in oxidized LDL cholesterol concentration (Fragopoulou *et al.* 2018; Finicelli *et al.* 2019).

Poland is now classified by European Union Council to zone A in wine production, *i.e.* the coldest region country which may produce wines. However wine production is still a small-scale operation, a dynamic increase in the number of vineyards and their area may be observed in recent years in Poland. Podkarpacie is a region located in south-east part of Poland, with a large number of vineyards, with characteristic influence of continental climate and harsh winters. Mainly hybrid varieties of grapes are thus grown in this region. One of the grapevines cultivated in Podkarpacie is Regent (Geilweilerhof 67-198-3), the hybrid of (Silvaner × Müller Thurgau) × Chambourcin, originally created in Germany (Vitis International Variety Catalogue, 2007). Regent grapevine combines the good wine quality and resistance

Supported by Department of Cell Biology and Biochemistry, University of Rzeszow.

https://doi.org/10.17221/222/2018-CJFS

to downy and powdery mildew (Figueiredo *et al.* 2012).

As the professional and commercial winemaking in Poland is in progress, some attempts of characteristic of Polish wines were undertaken (Tarko *et al.* 2010; Jeleń *et al.* 2011) but till this time no comparisons between single-cultivar wines of various vineyards were done. The aim of this study was to characterize and compare the antioxidant properties of several wines of Regent grapevine from Podkarpacie region.

MATERIAL AND METHODS

Samples preparation. Seven wines (W1–W7, numbers assigned randomly) produced from Regent grapevine (2011 vintage) were analysed, each one from another vineyard, all of them were single-cultivar wines except for W2 which was Regent + 8% Marechal Foch blend. The wine samples were kindly donated by the owners of vineyards located in Podkarpacie region, Poland (in alphabetical order): Winnica Łany, Winnica Maria Anna, Winnica Mieszko, Winnica Spotkaniówka, Winnica Sztukówka, Winnica Wiarus, Winnice Przeworskie. Wines were aliquoted after opening and frozen at –70°C until analysed.

Total polyphenols content. Total phenolic compounds content was estimated with Folin-Ciocalteu method (Singleton & Rossi 1965) using Tecan Infinite M200 microplate reader (Tecan, Switzerland). Polyphenols content was calculated using standard curve of gallic acid (0–200 mg/l) and expressed as mg/l GAE (gallic acid equivalents).

Total flavonoids content. Total flavonoids content was determined with the method of Zhishen *et al.* (1999), using Varian Cary50 spectrophotometer (Varian, USA). Flavonoids content was calculated using standard curve of catechin (0–100 mg/l) and expressed as mg/l CAE (catechin equivalents).

Anthocyanins content. The total monomeric anthocyanins content was estimated by the pH differential method (Lee *et al.* 2005) using Varian Cary50 spectrophotometer (Varian, USA). The concentration (mg/l) of total monomeric anthocyanins was calculated from the difference in absorbance at 510 and 700 nm at pH 1.0 subtracted with the difference in absorbance at pH 4.5 for each wine sample, the molar absorbance coefficient 26900 M/cm for cyanidin 3-glucoside and molecular weight 449.2 g/mol.

Antioxidant capacity assays. Total antioxidant capacity of wine samples estimated with FRAP assay was performed according to (Benzie & Strain 1996) and with TEAC method by a modification of the assay of reduction of 2,2'-azinobis(3-ethylbenzthiazoline sulfonate) cation radical ($ABTS^+$) (Re *et al.* 1999) using Varian Cary50 spectrophotometer and the results were expressed as mmol/l Trolox equivalents (*TE*).

The ability to react with free radicals was estimated with DPPH scavenging assay (Brand-Williams *et al.* 1995) using Tecan Infinite M200 microplate reader (Tecan, Switzerland). DPPH (%) radical scavenging by wine antioxidants was calculated as follows:

$$\text{DPPH scavenging (\%)} = (A_c - A_w) / A_c \times 100 \quad (1)$$

where: A_c – absorbance of control sample; A_w – absorbance of wine sample

Estimation of resveratrol content. Resveratrol concentration in the wines analysed was assessed by HPLC. The HPLC system consisted of a Shimadzu LC-10AS pump (Shimadzu, Japan), SIL-10AD VP autoinjector (Shimadzu, Japan) and SPD-10A UV spectrometric detector (Shimadzu, Japan). The column Kinetex XB-C18 (100 × 4.6 mm) (Phenomenex, USA) packed with 2.6 µm particle size has been used. Chromatographic separations were monitored at 310 nm.

Statistical analysis. Two techniques of the comparison of average values of biochemical features were used. Depending of the homogeneity of variance (Brown-Forsyth test), ANOVA or Kruscal-Wallis test, as well as appropriate post-hoc tests were applied. To fit the biochemical parameters to the studied wines Correspondence Analysis (*CA*) was undertaken. These variables (parameters and wines samples) were described with coordinates in multidimensional analysis. The choice of the number of dimension was based on the analysis of own values and the screen test. The first dimension explained the largest percentage of the variability. Another method for grouping data is cluster analysis, which allows to formation groups of variables (type of wine) characterized by some type of 'similarity'. The analyses were performed using Statistica ver. 10 software (StatSoft, Inc., USA).

RESULTS AND DISCUSSION

We analysed seven Regent wines from Podkarpacie region in Poland from the point of view their anti-

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oxidant properties. Thus we evaluated the content of total polyphenols in these wines, total flavonoids, anthocyanins, and total antioxidant capacity with various methods and the content of resveratrol as the health properties of wine drinking are attributed in large part to this compound.

Total polyphenols in the wines analysed was found from about 1700 mg/l GAE for W6 and W4 to over 3000 mg/l GAE for W3 and W5 (Figure 1A). The polyphenols content of wines depends on the grape variety, wine production technology and environmental factors as the climate, the vineyard location, soil or atmospheric conditions during ripening. In contrast to Mediterranean regions characterized by long growing seasons with moderate to warm temperatures ensuring the grapevine long warm periods during the crucial phenological stages, in Poland, 65% days of a year is determined by transformed polar-maritime air, mean yearly temperatures oscillates around 8°C and mean vegetation period lasts 220 days. Maximal mean temperature is about 18°C for July (average from 1997–2010 years) and 2 or 3 months yearly have the mean temperature below 0°C (Figure 2). The diurnal minus temperatures may hold in March but the ground frosts may occur even in May during grapevine bud bursting.

The average content of polyphenols in the wines tested is 2373 ± 627 mg/l GAE indicating that cool-climate Regent wines do not diverge from warm climate wines with respect to this parameter (López-Vélez *et al.* 2003; Minussi *et al.* 2003; Granato *et al.* 2011).

Two major groups are distinguished within polyphenols – flavonoid compounds (anthocyanins, flavonols, and flavanols) and non-flavonoid compounds (hydroxycinnamic acids, benzoic acids, and stilbenes) (Waterhouse 2002; Mushtaq & Wani 2013).

Mainly resveratrol and flavonoids, and among them several compounds (flavanols catechin and epicatechin, flavonols quercetin and rutin) attracted detailed concern for their antioxidant properties and their impact on biological effects of moderate wine drinking as modulation HDL/LDL ratio, prevention platelet aggregation, modulating immune response (Guilford & Pezzuto 2011).

Total flavonoids and total monomeric anthocyanins were quantified for Regent wines (Figure 1B and C). The concentration of flavonoids ranged from ~500 mg/l CAE to ~1500 mg/l CAE (Figure 1B), the average concentration was 957 ± 442 mg/l CAE. The content of flavonoids in Regent wines seems relatively low with respect to the average content of polyphenols (957 ± 442 mg/l CAE versus 2373 ± 627 mg/l GAE). However, even lower concentration of total flavonoids was shown for Montenegrin Merlot wines (~215 mg/l versus ~3000 mg/l of total phenolic content) (Dordević *et al.* 2018). Moreover, in case of several wines (W2, W6, and W7) the content of total monomeric anthocyanins was approximately the same as the concentration of flavonoids (Figure 1B and C) indicating that mean content of non-anthocyanin flavonoids (flavonols and flavanols) may vary from surprisingly low to the concentrations observed for other wines like Brazilian red wines (Granato *et al.* 2010).

The concentration of monomeric anthocyanins in Regent wines ranged from ~50 mg/l (W4) to ~900 mg/l (W5 and W7) (Figure 1C). The main impact of anthocyanins on red wine quality is principal contribution in wine colour which is an important attribute of red wines (He *et al.* 2012). Anthocyanins content in wines depends not only on their content in grapes but also on the winemaking procedures (Gonzalez-San Jose *et al.* 1990). The typical concentrations of monomeric anthocyanins in young red wines was reported about 500 mg/l (He *et al.* 2012). We estimated similar average content for Regent wines, the average content of anthocyanins for all wines studied was found 612 ± 293 mg/l. Chemically grape anthocyanins are 3-mono- and 3,5-di-glucosides of anthocyanidins. The grapes anthocyanin profiles may be used as a chemotaxonomic criterion to distinguish grape varieties (González-Neves *et al.* 2007; Versari *et al.* 2014). Acylated monoglucosylated anthocyanins (mainly malvidin 3-glucoside) are typical for *Vitis vinifera L.* varieties while diglucosylated anthocyanins are more typical for other *Vitis* varieties and the interspecific hybrid grapevines obtained from hybridisation of American grapevine species with European *V. vinifera* grapevines.

The anthocyanins profile of Regent grapevine was estimated recently for grapes grown in Czech Republic and south-west region of Poland revealing malvidin-3,5-*O*-diglucoside as the main compound (Balík *et al.* 2013; Wojdyło *et al.* 2018). The anthocyanins profile has been found as relatively stable for the grapevine variety while their concentrations could vary between vintages and different cultivated zones depending on climate factors (Gil-Muñoz *et al.* 2010). Our study indicates the diversities in anthocyanins content in Polish Regent wines from different vineyards (Figure 1C).

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The antioxidant activity of wine may depend also on others factors than phenolic composition, like methodological aspects of the assay employed and interactions of phenolic and non-phenolic compounds contained in wine (Granato *et al.* 2010). Figure 1D shows the radical scavenging ability of the wines tested, the highest for W1, W3 and W5 (over 70% scavenging of DPPH radical). The highest antioxidant capacity of W1, W3 and W5 Regent wines was confirmed with TEAC and FRAP methods although some discrepancies between the values obtained with different methods may be observed as well as highest reactivity

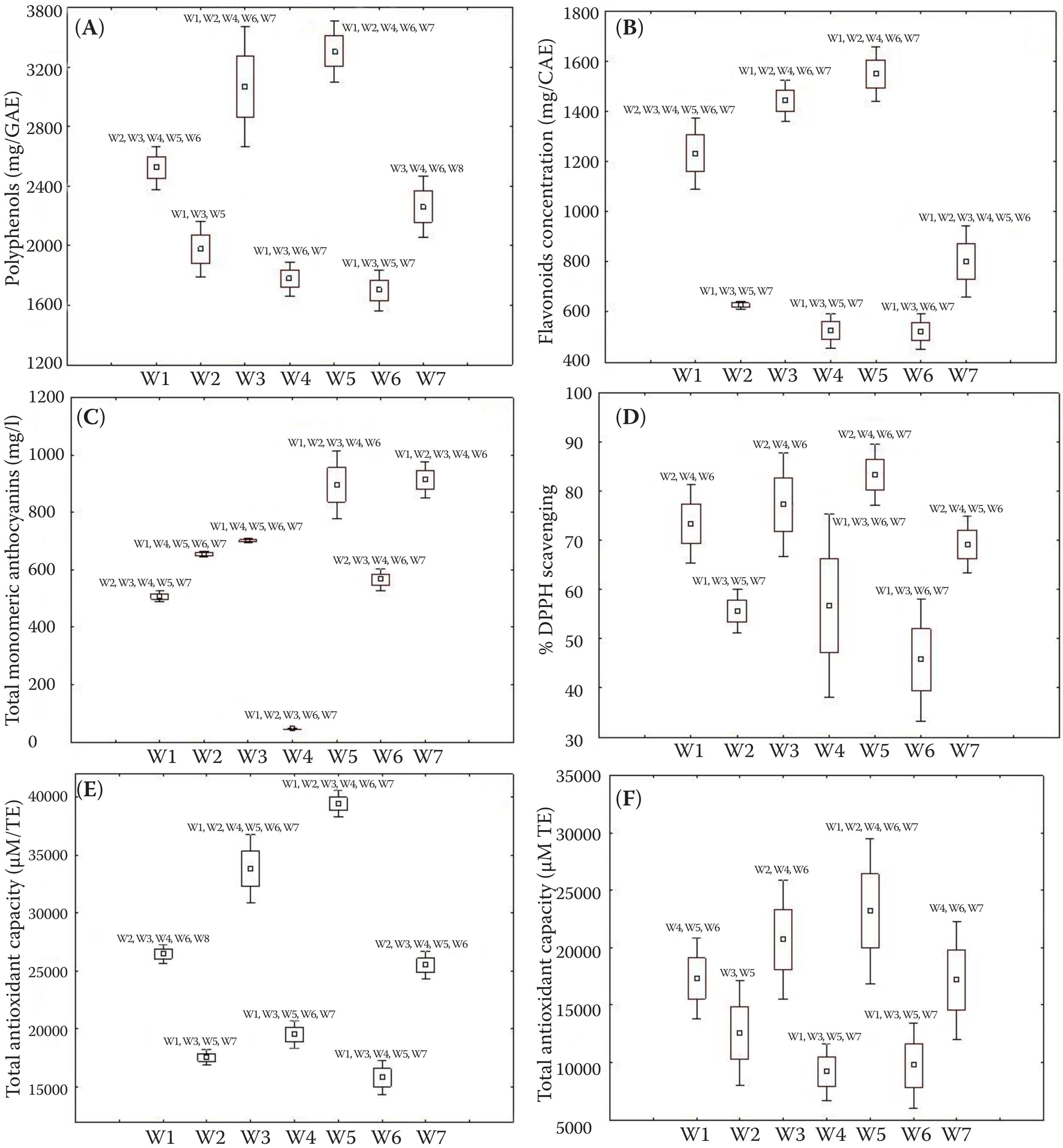

Figure 1. Antioxidant properties of Regent wines, estimated with FRAP method: (**A**) total polyphenols content, (**B**) total flavonoids content, (**C**) total monomeric anthocyanins content (**D**) radical scavenging activity (% of DPPH); (**E**) antioxidant capacity estimated with TEAC method; (**F**) antioxidant capacity

Boxes on the plots represents mean ± s.d.; whiskers – mean ± 1.96 × s.d.; W1–W7 – wines statistically differ at $P < 0.05$

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of wine components in TEAC method (Figure 1D–F). TEAC assay revealed the largest distinction between the wines W1, W3 and W5. The average antioxidant capacity of all wines tested was 25440 ± 8721 µM *TE* estimated with TEAC assay and 15688 ± 5382 µM *TE* with FRAP assay. The values again do not diverge from the antioxidant activities reported for warm climate wines (Fernández-Pachón *et al.* 2004; Staško *et al.* 2008; Granato *et al.* 2010; Šeruga *et al.* 2011). Figure 5 shows the close correlation of antioxidant activity estimated with TEAC and DPPH methods with the polyphenols concentrations in Regent wines (but smaller with FRAP assay) what is in agreement with the data from literature (Fernández-Pachón *et al.* 2004; Staško *et al.* 2008).

Resveratrol is present in various structural forms in grapes, the main is *trans*-resveratrol. Its content in grapes and wines is largely dependent on grape varieties and the conditions of grapevine growth *i.e.* the degree of exposure to stress (Fernández-Mar *et al.* 2012). Resveratrol is produced under physiological stress, as a response to *Botrytis cinerea* or *Plasmopara viticola* infections and to abiotic stress factors like UV radiation, heavy metal exposure, preharvest chemical treatment (Lachman *et al.* 2009; Fernández-Mar *et al.* 2012; Garrido & Borges 2013). The highest *trans*-resveratrol levels were found in the wines made from Pinot Noir and St. Laurent and the average value varied widely between regions (Lachman *et al.* 2009). The *trans*-resveratrol concentrations of 2.8 ± 2.6 mg/l for Merlot wines (average for many samples from different regions), 1.8 ± 0.9 mg/l for Syrah, 1.7 ± 1.7 mg/l for Cabernet Sauvignon were reported (Fernández-Mar *et al.* 2012).

For Polish Regent wines we found the *trans*-resveratrol content between 0.82 mg/l for W6 to 11.13 mg/l for W7 (Figure 3), the average content for all wines

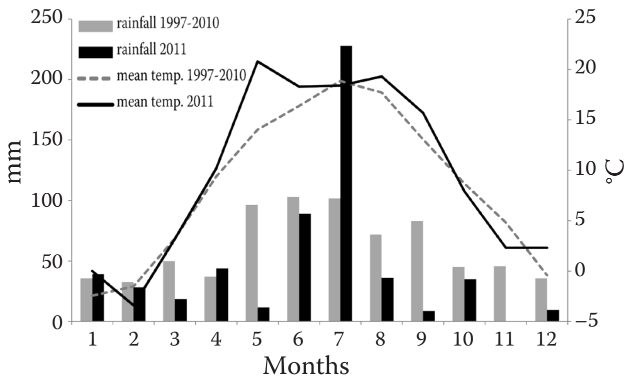

Figure 2. Climatic diagram for Rzeszow, Podkarpacie region and voivodeship for years 1997–2010, and 2011

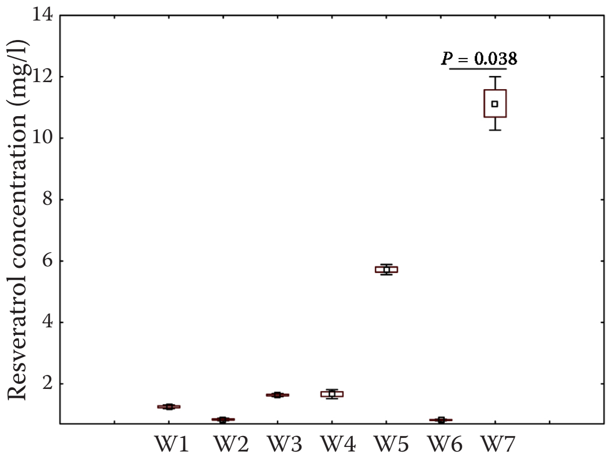

Figure 3. *Trans*-resveratrol concentration in Regent wines, assessed by HPLC

The boxes on the plot represent mean ± s.d.; whiskers – mean ± 1.96 × s.d.; statistical significance of differences between means was estimated using Kruscal-Wallis non-parametric ANOVA

tested is 3.30 ± 3.85 mg/l and does not differ from values shown for wines of typical wine regions or is even higher. This confirms the general tendency noted by Lachman *et al.* (2009) that wines from relatively warm and dry climatic conditions tended towards lesser resveratrol content.

Taking into account all the parameters tested, the similarity of the Regent wines was analysed by hierarchical cluster analysis (Stanimirović *et al.* 2018). Cluster analysis (Figure 4) grouped on the left the wines W4, W6, W2 with the lowest polyphenols and flavonoids content and on the right the cluster with wines of high content of polyphenols. Wine 7 with the highest content of resveratrol, seems distinguished in the dendrogram.

In Correspondence Analysis method only two dimensions were taken into account because the percentage of explained variability was high – over 90%. Some of parameters did not fit to any samples. There were resveratrol and anthocyanins content and in the smaller degree, flavonoids. Except of W4, the rest of parameters very well described the wines tested. The Correspondence Analysis also indicated that the wines formed two groups which slightly differed from each other (Figure 5). The composition of these groups is in accordance with results of cluster analysis (Figure 4).

The concentration of resveratrol was noted as a very variable factor, significantly different values were estimated for the same grapevine varieties grown in the same vineyard and from different vintage

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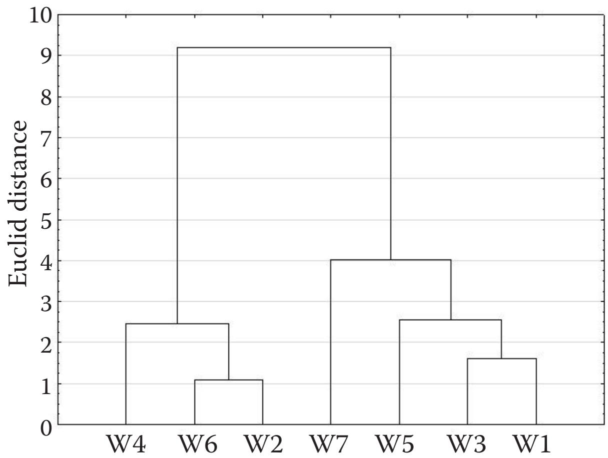

Figure 4. Dendrogram of Regent wines based on their composition and antioxidant properties

(Gómez Gallego *et al.* 2012). The very high precipitation (over twice fold comparing to the average from 1997–2010) in July 2011 (Figure 2) and thus increased susceptibility to fungal infections during grape ripening may also contribute to the diversities in resveratrol content in Regent wines vintage 2011. The presence of resveratrol was not reported in Regent grapes grown in 2016 year in south-west region of Poland (Wojdyło *et al.* 2018).

Similar relationship with respect to the impact of resveratrol and anthocyanins on total antioxidant capacity of red wines indicating that mainly flavonoids contribute to wine total antioxidant capacity estimated with DPPH scavenging and TEAC methods was noted recently for other wines (Granato *et al.* 2010, 2011; Xiang *et al.* 2014).

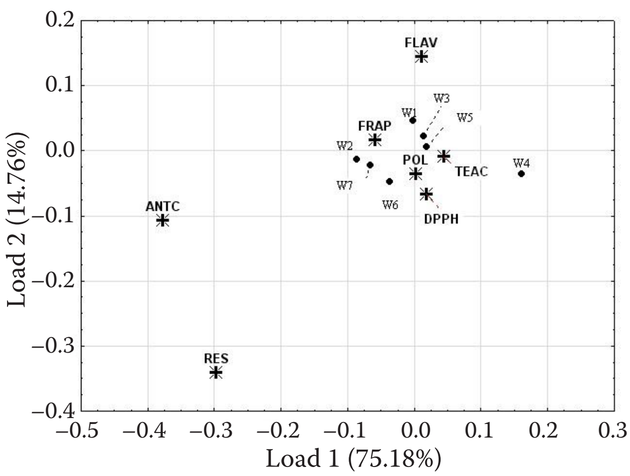

Figure 5. Correspondence analysis of the composition and antioxidants properties of Regent wines
POL – polyphenols; FLAV – flavonoids; ANTC – anthocyanins; RES – resveratrol

CONCLUSIONS

Our report shows that cool-climate wines may be comparable with those from traditional and well-established winemaking regions with respect to the composition and antioxidant properties, and corroborates the opportunity to produce good quality single cultivar red wines in cold climate.

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Received: 2018–08–03
Accepted after corrections: 2019–07–08