



J. Serb. Chem. Soc. 85 (4) 517–529 (2020)
JSCS–5319

Serbian aromatized wine “Bermet”: Electrochemical, chemiluminescent and spectrophotometric determination of antioxidant activity

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(Received 4 April, revised 1 November, accepted 16 December 2019)

Abstract: Serbian aromatized wine “Bermet” from grapes grown on Fruška Gora Mountain has been in production since the 15th century. Ten commercial Bermets produced according to the traditional procedure by different manufacturers, and six prepared within the scope of this study were assessed for antioxidant (AO) activity using electrochemical, chemiluminescent and spectrophotometric AO assays. Direct current polarographic assay based on the decrease of anodic current of [hydrogen(peroxido)(1-)]hydroxidomercury(II) complex formation in alkaline H₂O₂ solution at potential of mercury oxidation, chemiluminescent H₂O₂ scavenging assay, as well as commonly used spectrophotometric assays (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) based Trolox equivalent antioxidant capacity (TEAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP)) were used. Total phenolic content (TPC) was determined by Folin–Ciocalteu assay. The results obtained were correlated using regression analysis, ANOVA and *F*-test. An integrated approach to AO capacity determination allowed a more comprehensive comparison between samples. The approach is based on the introduction of the relative antioxidant capacity index, calculated by assigning each AO assay equal weight, and by PCA analysis. In addition, the introduction of phenolic antioxidant coefficients, calculated as the ratio between individual AO capacity and TPC, enabled a better understanding of their relation.

Keywords: antiradical activity; hydrogen peroxide scavenge; Fruška Gora; phenolics; polarography.

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<https://doi.org/10.2298/JSC190404139G>

INTRODUCTION

Three categories of aromatized drinks can be distinguished by wine content, alcoholic strength, and presence of added alcohol: aromatized wines (vermouth, bitter aromatized wine, egg-based aromatized wine), aromatized wine-based drinks (sangria, bitter soda) and aromatized wine-product cocktails. The globally known aperitif, aromatized wine “Vermouth”, has been prepared since the 18th century, from base red or white wine, by adding a mixture of herbs and spices or their extracts, sugar and alcohol or distilled wine (brandy). Some herbs and spices impart an aromatic flavour, while others bring bitterness.¹ Flavouring substances from the hydroalcoholic extracts obtained from macerates give specific prevailing flavours and cause significant changes in physical and chemical quality of the base wine.^{2,3} Addition of hydroalcoholic plant macerates to the base wines improves the organoleptic, physical and chemical properties of the flavoured wines.⁴ Antioxidant properties of red and white Vermouth were investigated and compared with red and white wines used for their production.⁵

Aromatized wine, made exclusively from the grapes grown in vineyards located on the mountain Fruška gora (Serbia), is known under the name “Bermet”. The tradition of viticulture in this vineyard area started during the Roman period, while production of Bermet dates back to the 15th century. Production stopped during the communist era, but restarted a few decades ago. International registration of “Bermet” as a protected national drink of Serbia with geographic origin is currently in progress.

Bermet is prepared in a similar way to Vermouth, from white or red grapes, by maceration of herbs, fruits and spices. It is produced by mixing wine (min. 60 % vol of Bermet content), extracts of herbs, fruits and spices, and sugar (50–150 g L⁻¹). The obtained blend is fortified to the desired alcohol content (usually 16–18 vol. %) with alcohol or wine distillate. Final maturation of Bermet takes place in oak casks or inox tanks. Different dried parts of various plants (herbs, fruits and spices) such as seeds, twigs, leaves, bark or roots are used.⁶ Details of the extraction process differ between manufactures. Phenolic composition of Bermet is probably more complex than that of wine. Presence of bioactive compounds originating from herbs, fruits and spices could have a beneficial effect on health of moderate Bermet consumers, so there is a great interest to investigate the wine composition and activity. However, until now there have been no reports focused on quality characteristics or health-related parameters of Bermet.

Cyclic voltammetry (CV)^{7,8} and differential pulse voltammetry (DPV)⁹ on glassy carbon working electrode (GCE) are most commonly used electrochemical techniques for antioxidant (AO) activity determination in wines. There are several papers where CV and DPV on GCE were used in parallel to determine the AO activity of wines^{10,11} and their anthocyanins.¹² CV also was used in

parallel to chronoamperometry for the evaluation of AO properties of red wines.¹³ Red and white wines¹⁴ were assessed for AO activity using a recently developed DC polarographic AO assay, based on the decrease of the current of [hydrogen(peroxido)(1-)]hydroxidomercury(II) complex (HPMC) formation in alkaline solutions of hydrogen peroxide at the potential of mercury oxidation, upon addition of antioxidants.¹⁵ Applicability to turbid and coloured samples is a general advantage of electrochemical assays over spectrophotometry, while the renewable surface of dropping mercury electrode provides fast and reproducible DC polarographic measurements compared to methods employing solid electrodes.

Chemiluminescent assays, known for their quick procedure as well as sensitivity, were also used with various alcoholic beverages. Enzyme-free peroxyoxalate chemiluminescence (POCL) assay using 9,10-diphenylanthracene as a fluorophore¹⁶ was used to assess H₂O₂ quenching activity of wines.¹⁷ On the other hand, martinis were assayed for their ability to quench luminescence, in a procedure in which hydrogen peroxide reacts with albumin-bound luminol.¹⁸

The aim of this study was to provide an insight into the AO activity of Bermets. Three pairs of white and red Bermets, prepared within the scope of this study from various types of grapes, as well as 5 pairs of white and red commercial Bermets, each pair from a different manufacturer, were assessed for AO activity using DC polarographic, chemiluminescent and three different spectrophotometric assays (DPPH, TAEC, FRAP). Phenolic content determined by FC assay is also considered as the measure of total reducing activity. The results obtained were correlated using regression analysis, ANOVA and *F*-test. Relative antioxidant capacity index (*RACI*), calculated by assigning equal weight to all applied assays and phenolic antioxidant coefficients (*PACs*), calculated as the ratio between particular AO capacity and TPC, were used for a more comprehensive comparison between analysed samples, as well as the assays used.

EXPERIMENTAL

Chemicals and details about experimental procedures applied are given in Supplementary material to this paper.

Bermets and wines samples. Commercial Bermets, red (cR) and white (cW) were obtained from five vineries: Vinarija Kiš, Sremski Karlovci (cR1 and cW1), Podrum Šukac, Sremska Kamenica (cR2 and cW2), Vinarija Aleks, Novi Sad (cR3 and cW3), Vinarija Kovačević, Irig (cR4 and cW4) and Vinarija Živanović, Sremski Karlovci (cR5 and cW5).

Three red and three white bermets (10 L of each) were prepared within the scope of this study at the Experimental field Radmilovac, Faculty of Agriculture, University of Belgrade, Serbia. Red (eR) and white (eW) bermets (16.5 vol. % of alcohol) were made from red and white wine (60 vol. %), varieties Cabernet sauvignon (2008, oak barrique cask – 1 year, 13.0 vol. % alc. (eR1) and 2010, oak cask – 1 year, 13.3 vol. % alc. (eR2)), Pinot noir (2010, 13.2 vol. % alc. (eR3), Riesling Rhine (2010, 12 vol. % alc. (eW1)), Chardonnay (2010, 11.8 vol. % alc. (eW2)) and Sauvignon blanc (2010, 13.1 vol. % alc. (eW3)). An extract containing 46

herbs (*Paris quadrifolia* L., *Polygonum aviculare* L., *Teucrium montanum* L., *Salvia officinalis* L., *Achillea millefolium* L., *Mentha piperita* L., *Thymus serpyllum* L., *Thymus vulgaris* L., *Matricaria chamomilla* L., *Teucrium chamaedrys* L., *Artemisia absinthium* L., *Melissa officinalis* L., Hawaiian hibiscus, *Eugenia caryophyllata* L., *Pimpinella anisum* L., *Cinnamomum div.*, *Vanilla planifolia*, *Rosa canina* L., *Juniperus communis* L., *Ceratonia siliqua* L., *Origanum vulgare* L., *Hypericum perforatum* L., *Plantago lanceolata*, *Arctostaphylos uva ursi*, *Morus alba* L., *Rosmarinus officinalis* L., *Alchemilla vulgaris* L., *Ocimum basilicum* L., *Sambucus nigra* L., *Equisetum arvense* L., *Capsella bursa-pastoris* L., *Cassia officinalis*, *Rubus fruticosus* L., *Betula* L., *Crataegus oxyacantha* L., *Viscum album* L., *Foeniculum vulgare* Mill., *Erythraea centaurium* Pers., *Viola tricolor* L., *Quercus*, *Calendula officinalis* L., *Utricle dioica* L., *Tussilago farfara* L., *Anagallis arvensis* L., *Taraxacum officinale* Web., *Euphorbia cyparissias* L., *Ficus carica* L.) and an extract of 8 fruits (*Vitis vinifera*, *Prunus domestica* L., *Pirus malus* L., *Rubus idaeus* L., *Citrus aurantium* L., *Citrus limonum* Risso, *Citrus paradise*) were added in quantity of 25 and 4 mL L⁻¹, respectively, to produce red and white Bermets.¹⁹ All Bermets obtained at laboratory level contained 70 g/L of sugar, 2 g/L citric acid and 86 ml/L purified wheat alcohol (96 vol. %).

Spectrophotometric methods applied. Total phenol content (TPC) was determined according to a modified Singleton *et al.*²⁰ method. DPPH radical scavenging assay was performed according to Brand–Williams, Cuvelier & Berset.²¹ The Trolox equivalent AO capacity (TEAC) was measured using ABTS radical cation decolorization assay.²² The ferric reducing/antioxidant power (FRAP) assay was carried out as reported by Benzie & Strain.²³

Determination of AO capacity by DC polarographic HPMC assay. DC polarographic assay was used according to a previously reported procedure.¹⁵

Determination of hydroxyl free radical-scavenging activity (SA_{HFR}). Chemiluminescence (CL) was measured according to Parejo *et al.*²⁴

Determination of relative antioxidant activity index (RACI). Central tendency is most often used to compare the AO activity of complex food samples determined using multiple assays,²⁵ where samples are ranked based on the mean value and standard deviation of the assays used. Since the units and the scale of the data from various chemical methods are different, the data in each dataset should be transformed into standard scores, dimensionless quantities derived by subtracting the mean from the raw data, then divided by the standard deviation, according to the following equation:

$$\text{Standard score} = \frac{(x - \mu)}{\sigma} \quad (1)$$

where x represents the raw data, μ the mean, and σ the standard deviation. The standard scores of a given sample for different assays, when averaged, give a single unitless value named RACI, which is a specific combination of data from different chemical methods, regardless of the units they are expressed in and with no variance between them.

Statistical analysis. Descriptive statistical analyses were performed using Microsoft Excel software (Microsoft Office 2007). Results were expressed as the mean±standard deviation (SD). Principal component analysis (PCA), used as a pattern recognition technique, was applied within assay descriptors to characterize and differentiate various analysed wine samples. Furthermore, the evaluation of correlation matrix, ANOVA and F -test, as well as PCA of obtained results were performed using StatSoft Statistica 10.

RESULTS AND DISCUSSION

A multilateral approach was used to determine a reliable AO capacity of 10 commercial Bermets and 6 Bermets prepared within the scope of this study at laboratory scale. A direct current (DC) polarographic assay, chemiluminescent assay and 4 spectrophotometric assays were used for each of the samples. A rapid, simple and reliable AO assay based on the decrease of anodic current from HPMC formation in alkaline hydrogen peroxide solution, at the potential of mercury oxidation, upon addition of AOs, was developed and optimized by Sužnjević *et al.*¹⁵ DC polarographic assay HPMC has previously been used on various individual phenolics, food samples^{26,27} and alcoholic beverages, including beer and spirits, as well as red and white wines^{7,28,29} The chemiluminescence assay, based on the generation of hydroxyl free radicals, which oxidise luminol, leading to a sequence of reactions that end in light emission, has been used to evaluate the hydroxyl free radical scavenging activity (SA_{HFR}).¹⁷ The peroxyoxalate chemiluminescence-based assay for the evaluation of H_2O_2 scavenging activity, employing 9,10-diphenylanthracene as fluorophore, has been used on various wines.¹⁸ We have also used three spectrophotometric assays (ABTS, DPPH and FRAP), the most widely used in the analysis of complex food samples. Since it determines the total reducing activity, FC could be considered a measure of AO activity.³⁰

The antioxidant activity of Bermets determined by the different AO assays. Polarographic anodic current decrease upon addition of Bermets was followed. Polarograms of the initial solution of hydrogen peroxide before and after addition of the tested samples are provided in Fig. 1. The anodic current decreased upon gradual addition of analysed samples in a dose-dependent manner, as can be seen from Fig. 1 inserts.

Antioxidant activity, expressed as HPMC (%) vs. volume (V) curve slope, was compared with results obtained from chemiluminescent and spectrophotometric assays (Table I). AO activity, determined polarographically, ranged from 48.3 to 201 % mL^{-1} . Red Bermets contained from 523 to 1835 mg GAE L^{-1} phenolics. TPC range in red wines, reported previously by Gorjanović¹⁴ was 1700–2314 mg GAE L^{-1} . According to Arnous¹⁶ and Kefalas¹⁷, aged red Greek wines showed higher variations, from 1217 to 3772 and from 620 to 4735 mg GAE L^{-1} . TPC of white wines ranged from 164 to 346.¹⁴

The total phenolic content in base wines used to produce the small-scale Bermets was found to be higher than in commercial Bermets. Phenolic contents in white base wines were 209.8, 199.5 and 193.7, and in red base wines 1903.6, 1537.0 and 1430.3 mg GAE L^{-1} . According to the HPMC assay, the AO activity of the base white and red wines used for Bermets preparation was found to be higher than in the Bermets (145, 159, 196 and 39.9, 56.1, 84.2 % mL^{-1} , respectively). According to FRAP (2.5, 1.94, 1.91 and 28.4, 23.65, 23 mM Fe(II) L^{-1})

and *TEAC* (2.46, 2.39, 2.38 and 20.27, 17.1, 16.7 mM TE L⁻¹), AO activities of base wines also exceeded AO activities of the Bermets.

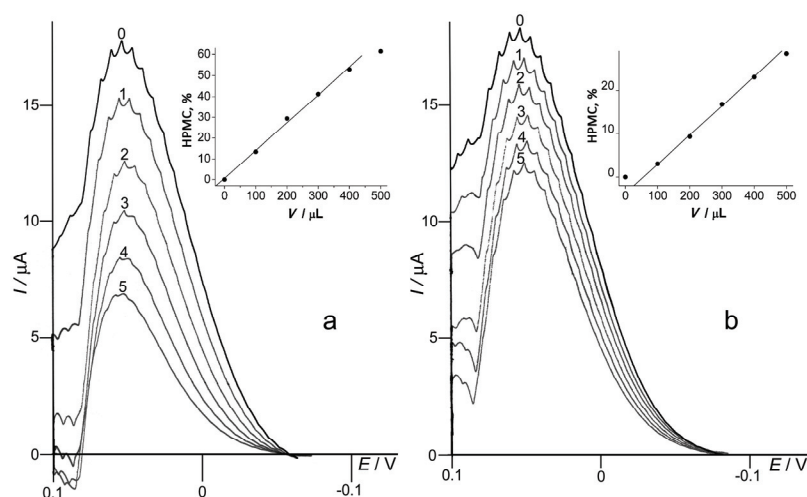


Fig. 1. Anodic DC polarographic curves of 5 mmol L⁻¹ solution of H₂O₂ in CL buffer (pH 9.8) before (0) and after addition of five equal aliquots of red (a) and white (b) Bermets (Šukac): (1–5) 100–500 μL. Inserts: red and white Bermets effects on anodic limiting current in 5 mmol L⁻¹ H₂O₂ in CL buffer (pH 9.8) (*I* decreases vs. volume of added samples).

TABLE I. Bermets total phenolic content (*FC-GAE*) and AO capacity established by DC polarographic (*HPMC*), chemiluminescent (*SA_{HFR}*) and spectrophotometric assays (*DPPH*, *TAEC*, *FRAP*). Results are given with standard deviations (*SD*)

| Sample | <i>HPMC</i> % mL ⁻¹ | <i>FC-GAE</i> mg GAE L ⁻¹ | <i>DPPH EC₅₀</i> mL mL ⁻¹ | <i>FRAP</i> mM Fe(II) L ⁻¹ | <i>TEAC</i> mM TE L ⁻¹ | <i>SA_{HFR}</i> mM QE L ⁻¹ |
|--------------------------------------|-----------------------------------|---|--|--|--------------------------------------|--|
| Commercial Bermets | | | | | | |
| cR1 | 199±6 | 1717±28 | 0.330±0.003 | 22.7±0.5 | 16.4±0.4 | 53.84±0.03 |
| cR2 | 134±5 | 1367±21 | 0.218±0.006 | 19.9±0.3 | 14.0±0.1 | 49.81±0.02 |
| cR3 | 80±1 | 1005±23 | 0.178±0.003 | 18.0±0.4 | 13.6±0.1 | 46.37±0.06 |
| cR4 | 202±7 | 1836±12 | 0.400±0.002 | 26.5±0.4 | 20.8±0.4 | 128.31±0.03 |
| cR5 | 126±5 | 523±2 | 0.088±0.004 | 7.4±0.3 | 6.1±0.1 | 48.91±0.04 |
| cW1 | 61±2 | 387±9 | 0.046±0.002 | 5.4±0.3 | 3.5±0.2 | 23.09±0.03 |
| cW2 | 86±3 | 275±15 | 0.025±0.001 | 3.1±0.1 | 2.5±0.1 | 18.02±0.05 |
| cW3 | 52±1 | 376±14 | 0.048±0.003 | 5.7±0.3 | 3.5±0.1 | 24.88±0.01 |
| cW4 | 120±4 | 450±12 | 0.046±0.002 | 6.0±0.2 | 4.8±0.2 | 10.55±0.01 |
| cW5 | 48±2 | 261±4 | 0.025±0.003 | 3.4±0.1 | 2.6±0.1 | 18.46±0.01 |
| Bermets obtained at laboratory scale | | | | | | |
| eR1 | 120±3 | 1375±4 | 0.288±0.004 | 22.5±0.3 | 14.6±0.1 | 75.48±0.03 |
| eR2 | 143±4 | 1176±18 | 0.254±0.008 | 18.8±0.2 | 12.8±0.4 | 70.10±0.07 |
| eR3 | 161±9 | 1126±21 | 0.183±0.004 | 18.1±0.3 | 12.1±0.2 | 65.78±0.04 |
| eW4 | 40±2 | 142±5 | 0.016±0.002 | 1.4±0.1 | 1.8±0.1 | 16.52±0.02 |
| eW5 | 50±1 | 133±3 | 0.013±0.002 | 1.2±0.1 | 1.6±0.1 | 15.92±0.01 |
| eW6 | 51±2 | 124±5 | 0.010±0.001 | 1.1±0.1 | 1.6±0.1 | 13.24±0.02 |

The correlation between AO activities determined by different assays. Regression analysis at the significance level of ($p < 0.05$) revealed that Bermet AO capacity determined using *HPMC* assay correlated with *TPC* (0.885) determined using a *FC* assay (Table II). A better correlation between *TPC* and AO capacity of red and white wines determined by *HPMC* (0.997) was reported by Gorjanović¹⁴. SA_{HFR} that ranged from 10.55 to 128.31 μM quercetin equivalents L^{-1} correlated well with *TPC* and *HPMC* (0.87 and 0.79, respectively). Correlation between SA_{HFR} and *TPC* was in agreement with a previous study focused on wine (0.8363).³¹ Correlations of AO activity determined polarographically with antiradical activities against DPPH and *TAEC* were significant (0.865 and 0.855), while the correlation with *FRAP* was slightly lower (0.841). A high correlation was found between DPPH scavenging and the AO activity of red and white wines determined by DC polarography (0.986).

TABLE II. Correlation coefficients between *FC*-GAE and *HPMC*, *FRAP*, *TAEC* and DPPH for commercially available Bermets and those obtained at laboratory scale ($p < 0.05$)

| | <i>FC</i> -GAE | DPPH | <i>FRAP</i> | <i>TEAC</i> | SA_{HFR} | <i>RACI</i> |
|----------------|----------------|-------|-------------|-------------|------------|-------------|
| <i>HPMC</i> | 0.885 | 0.865 | 0.841 | 0.855 | 0.794 | 0.906 |
| <i>FC</i> -GAE | | 0.986 | 0.989 | 0.988 | 0.872 | 0.989 |
| DPPH | | | 0.976 | 0.981 | 0.914 | 0.990 |
| <i>FRAP</i> | | | | 0.992 | 0.881 | 0.982 |
| <i>TEAC</i> | | | | | 0.899 | 0.988 |
| SA_{HFR} | | | | | | 0.927 |

Lower correlations between Bermet AO activity obtained using DC polarography and *TPC*, as well as DPPH scavenging, in comparison with previously reported¹⁴ correlations for various red and white wines can be explained by the more uniform phenolic profile of wines. A wide variety of phenolics originating from various herbs and spices, added, contribute to Bermet AO activity to different extents.

Relative antioxidant capacity index (RACI). *RACI* was calculated by assigning equal weight to each of the used assays. As a relative index, *RACI* provides an accurate AO capacity ranking of foods. *RACI* ranking for the investigated Bermets is presented in Fig. 2.

Positive values of *RACI* ascribed to red Bermets decrease from 1.99, obtained for the red Bermet produced by Kiš vinery, to 0.33 for the red Bermet produced by Aleks. The only exception is the red Bermet produced by vinery Živanović with a negative *RACI* value (−0.16). All white Bermets showed negative *RACI* values (0.51–1.99), with the highest value obtained for the sample from vinery Kiš, and the lowest values observed in Bermets obtained at laboratory scale.

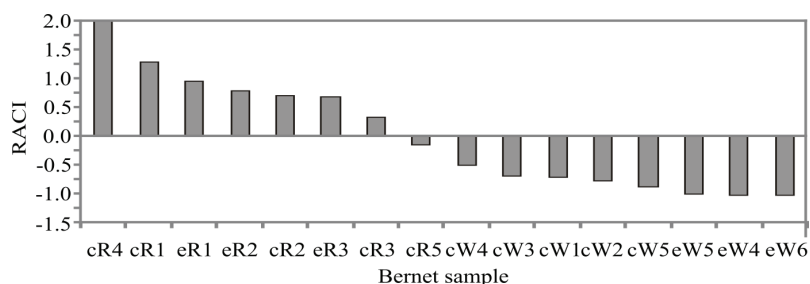


Fig. 2. Relative antioxidant capacity index (*RACI*) of 5 commercial red (cR1-5) and 5 white (cW1-5) Bermets as well as 3 red (eR1-3) and 3 white Bermets (eW1-3) obtained at laboratory scale.

Phenolic antioxidant coefficients (PAC): Introduction of *PAC* allows for a more comprehensive understanding of AO properties of complex samples containing numerous individual AOs. In contrast to high correlations between AO activities determined by various assays obtained by regression analysis, introduction of *PAC* values stresses the discrepancies between them. For example, the highest AO activity, according to all assays used, including *FC*, and consequently the highest *RACI*, was found in Kiš red wine; however, its *PAC* values varied from highest *PAC_{SA}*, *PAC_{TEAC}*, very high (second to last) *PAC_{HPMC}* to medium *PAC_{DPPH}* and low *PAC_{FRAP}*.

The red wine with the lowest *RACI* had the highest *PAC_{HPMC}*, medium *PAC_{FRAP}* and *PAC_{TEAC}*, but the lowest *PAC_{SA}* and *PAC_{DPPH}*. Differences between white wines were even more pronounced. For example, C2 white wine, with medium *RACI* values among white wines, had the lowest *PAC_{HPMC}* and *PAC_{SA}* and the highest *PAC_{TEAC}*, *PAC_{FRAP}* and *PAC_{DPPH}*.

As seen in Fig. 3, there was a visible agreement between *PAC_{HPMC}* and *PAC_{SA}* particularly for white wines. Inverse order of *PAC_{HPMC}* and *PAC_{SA}* on the one hand and *PAC_{FRAP}* and *PAC_{DPPH}* on the other was observed for white wines.

Generally, red wines AO capacity correlated with *TPC*, rather than individual polyphenol content. This showed that both *TPC* and total flavonoids may provide a significant contribution to the overall AO status of wines, while total anthocyanins appear to be a less important factor in this respect.³¹ The antioxidant potency of white wines was also correlated with *TPC* and with two major classes of white wine polyphenols, total hydroxycinnamates (*THC*) and total non-hydroxycinnamates (catechin, epicatechin, gallic acid) (*TNHC*).³² Reducing effects are likely to be exerted by the flavanol fraction, whereas antiradical efficiency is primarily due to the sum of total hydroxycinnamates and total flavonoid contents. This suggests that the overall AO status of white wines³³ is the result of a synergy between these two factors.

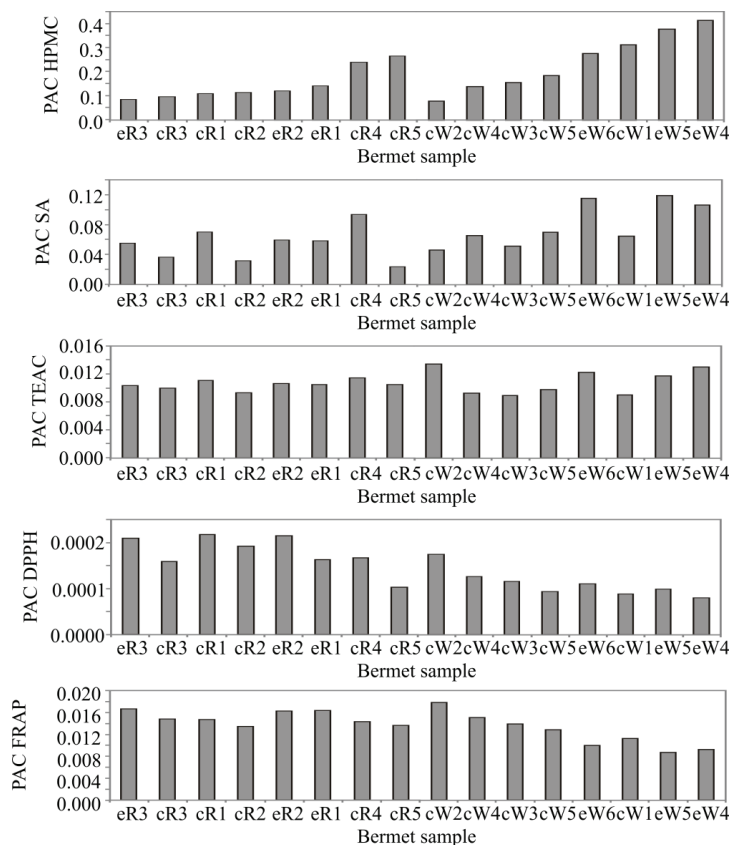


Fig. 3. Phenolic antioxidant coefficients (*PAC*) of 5 commercial red (cR1-5) and 5 white (cW1-5) Bermets as well as 3 red (eR1-3) and 3 white Bermets (eW1-3) obtained at laboratory scale.

The different ratios of major phenolics present in red and white wines, as well as large differences in their individual AO activities determined by the AO assays used in this study, may be responsible for the *PAC* variations. The AO activity of flavonoids measured previously by HPMC was found to be remarkably higher than the activity of both cinnamic and benzoic acids³⁴.

In contrast, according to spectrophotometric assays, higher activity was observed for cinnamates and gallic acids. For example, the catechin/CGA activity ratio was 3 when determined by HPMC, 1 when determined by *FRAP* and *DPPH* and 0.7 by *TAEC*. In addition, some physiologically active substances such as methylxanthines, hop bitter acids and methylpyridinium were found to be active, according to HPMC assays but not according to spectrophotometric assays.³⁴

PCA analysis of red and white Bermets. The PCA allows for a considerable reduction in the number of variables and the detection of structure in the relation-

ship between the measurement parameters and the different samples of red and white Bermets that provide complementary information. The full auto scaled data matrix, consisting of the obtained results, was submitted to PCA. A scatter plot was obtained for samples using the first two principal components (PCs) from PCA of the data matrix (Fig. 4), to visualize the data trends and the discriminating efficiency of the used descriptors.

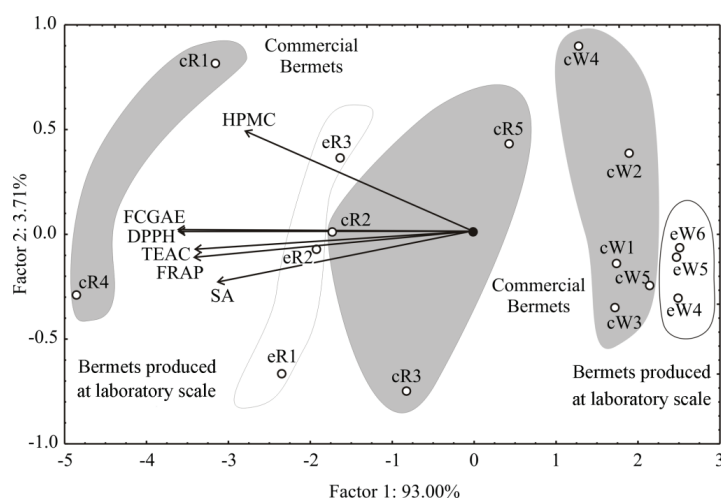


Fig. 4. Biplot diagram of red and white Bermets AO characteristics.

The angles between corresponding variables indicate the degree of their correlation (narrow angles corresponding to high correlations). As can be seen, there is a clear separation of the sixteen samples of red and white Bermets, according to the used AO assays and FC. A distinct discrimination between the commercial Bermets and those obtained at the laboratory scale is also evident. The orientation of the vector describing the variable in factor space indicates an increasing trend for these variables. The samples on the left side of the graph showed better AO and FC scores, with increased *HPMC*, *DPPH*, *TEAC*, *FRAP* and *SA* values, as well as FC. The superior AO activity, for observed red and white Bermets, determined by the assays used, including FC, was observed at the left side of the graph. Red Bermets showed better AO results, according to PCA. The points shown in the first factor plane, which are geometrically close to each other, indicate a similarity of patterns. The geometrical location of different samples observed in the factor space was also indicative of the *RACI* value – the maximum *RACI* is observed at the left side of the graph. As can be seen from Fig. 4, the different groups of Bermets (red or white, commercially available or obtained at laboratory scale) were positioned along the first factor coordinate, in which the spectrophotometric assays were the most dominant. However, the differentiation

of the samples within the same group could be observed along the second factor coordinate, in which the most dominant variable was HPMC polarographic assay.

CONCLUSION

Results presented indicate that the analysed Bermets, both produced commercially and obtained at laboratory scale, have AO capacities comparable to the AO capacities of wines, but generally slightly lower. We have found supporting evidence for the claim that parallel use of various AO assays is the prerequisite for reliable determination of AO activity. According to the results, the different groups of Bermets could be identified by commonly used spectrophotometric assays (TAEC, DPPH and FRAP), while the differentiation of the Bernet samples within the same group could be performed by HPMC polarographic assay. This paper has shown that it is possible to compare different types of Bermets by their AO activity and principal components analysis. This multivariate analysis allowed for a better differentiation among Bernet samples which could be necessary for authenticity control.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: <http://www.shd.org.rs/JSCS/>, or from the corresponding author on request.

Acknowledgements. This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grants No. 43010 and No.31093.

ИЗВОД

СРПСКО АРОМАТИЗОВАНО ВИНО „БЕРМЕТ“: ЕЛЕКТРОХЕМИЈСКО, ХЕМИЛУМИНИСЦЕНТНО И СПЕКТРОФОТОМЕТРИЈСКО ОДРЕЂИВАЊЕ ЊЕГОВЕ АНТИОКСИДАТИВНЕ АКТИВНОСТИ

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Српско ароматизовано вино Бермет се производи од грожђа из фрушкогорских винограда још од петнаестог века. Антиоксидативна активност десет бермета произведених на традиционалан начин у различитим винаријама и шест бермета произведених у пилот погону, одређена је електрохемијским, хемилуминисцентним и спектрофотометријским методама. Примењене су: поларографска метода једносмерном струјом базирана на смањењу анодне струје грађења хидроксопрехидроксожива(II) комплекса у алкалној средини у присуству водоник-пероксида на потенцијалу оксидације живе; хемилуминисцентна метода базирана на елиминисању водоник-пероксида; уобичајене спектрофотометријске методе (ABTS, DPPH и FRAP). Садржај укупних фенола одређен је Folin–Ciocalteu методом. Добијени резултати су упоређени методом регресионе анализе, ANOVA и F-тест. Интегрални начин поређења антиоксидативних капацитета базиран на увођењу индекса релативног антиоксидативног капацитета, који је примењен уз доделу једнаког значаја свим примењеним антиоксидативним методама и применом

анализе главних компонената пружио је свестраније поређење испитиваних бермета. Поред тога, увођење фенолних антиоксидативних коефицијената (количника антиоксидативног капацитета и садржаја укупних фенола) пружио је бољи увид у њихов међусобни однос.

(Примљено 4. априла, ревидирано 1. новембра, прихваћено 16. децембра 2019)

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