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CONTENT OF BIOACTIVE COMPOUNDS IN SEMI-DRY RED WINE

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Abstract

The aim of the present work was to compare the content of bioactive compounds (polyphenols and minerals) in semi-dry wines. The material for the study consisted of 6 types of semi-dry red wine originating from Bulgaria (Sofino Melnik), Hungary (Loci), France (Bongeronde) and Spain (Don Kichot). The wines were subjected to organoleptic scoring assessment, followed by analyses of crude ash, anthocyanins, the total content of phenol compounds, zinc, manganese, copper, iron and magnesium. Additionally, the browning index was determined. All the samples attained positive organoleptic assessment. The highest score for the color was given to the wines from France and Spain. The total acidity in the analyzed red wines ranged between 4.97 g dm^{-3} (Spanish wine) and 5.56 g dm^{-3} (Bulgarian wine), which fulfills the quality requirements for grape wine. Grape wine contains from 1.0 to 4.0 g dm^{-3} of polyphenols. The analyzed wine samples contained a slightly lower concentration of phenolic compounds: 1.28 g dm^{-3} on average. The average content of *p*-coumaric acid in the examined samples was 1.85 mmol dm^{-3}. The concentration of anthocyanins, compounds responsible for the color of wine, was significantly lower in Bulgarian and Hungarian wine samples (26.05±0.25 mg dm^{-3} and 23.60±0.80 mg dm^{-3} respectively), in comparison with French (59.90±0.20 mg dm^{-3}) and Spanish wine particularly (75.90±2.40 mg dm^{-3}). All the analyzed wine samples, independently of their place of manufacture, achieved high organoleptic assessment, especially for clarity and color. The highest rated wines from France were characterized a high level of bioactive compounds: total polyphenols and anthocyanins. They also had a high content of crude ash and minerals such as Mg, Mn, Zn. Relatively high concentrations of polyphenols, anthocyanins and crude ash were also determined in Spanish wines.

Key words: semi-dry red wine, mineral elements, phenolic compounds, anthocyanins.

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ZAWARTOŚĆ ZWIĄZKÓW BIOAKTYWNYCH W WINACH CZERWONYCH PÓŁWYTRAWNYCH

Abstrakt

Celem pracy było porównanie zawartości związków bioaktywnych (polifenole, antocyjany, taniny, składniki mineralne) w winach półwytrawnych. Materiał do badań stanowiły wina czerwone półwytrawne (po 6 prób w każdej grupie), pochodzące z Bułgarii (Sofino Melnik), Węgier (Loci), Francji (Bongeronde) i Hiszpanii (Don Kichot). Wina poddano punktowej ocenie organoleptycznej. Analizowano również zawartość popiołu surowego, garbników, kwasu szczawiowego, kwasowość ogólną, stężenie antocyjanów, zawartość związków fenolowych ogółem oraz zawartość cynku, manganu, miedzi, żelaza i magnezu. Oznaczono indeks zbrązowienia. Wszystkie poddane badaniom próby win uzyskały pozytywną ocenę organoleptyczną, a najkorzystniejszą ocenę pod względem barwy – wina pochodzące z Francji i Hiszpanii. Kwasowość ogólna analizowanych win czerwonych półwytrawnych wynosiła od 4,97 g dm^{-3} (wina hiszpańskie) do 5,56 g dm^{-3} (wina bułgarskie) i spełniała wymagania jakościowe dla win gronowych. Wina gronowe zawierają od 1,0 do 4,0 g dm^{-3} polifenoli. Badane próby win zawierały nieco mniej związków fenolowych, średnio 1,28 g dm^{-3}. Zawartość kwasu *p*-kumarowego wynosiła średnio 1,85 mmol dm^{-3}. Antocyjany to związki, które odpowiedzialne są za barwę win. Ich stężenie było istotnie niższe w winach bułgarskich oraz węgierskich (odpowiednio 26,05±0.25 mg dm^{-3} i 23,60±0.80 mg dm^{-3}), w porównaniu z winami francuskimi (59,90±0.20 mg dm^{-3}), a przede wszystkim hiszpańskimi (75,90±2,40 mg dm^{-3}). Wszystkie poddane badaniom wina, niezależnie od miejsca ich wytworzenia, uzyskały wysoką ocenę organoleptyczną, szczególnie w zakresie oceny klarowności i barwy. Najwyżej oceniono wina pochodzące z Francji, w których stwierdzono także wysoki poziom związków bioaktywnych – polifenoli ogółem i antocyjanów oraz wysoką zawartość popiołu surowego i składników mineralnych, takich jak Mg, Mn, Zn. Podobnie dość wysokie stężenie polifenoli, antocyjanów, a także popiołu surowego oznaczono w winach hiszpańskich.

Słowa kluczowe: wina czerwone półwytrawne, składniki mineralne, związki fenolowe, antocyjany.

INTRODUCTION

Red wine is a rich source of healthy components, such as phenolic acids, anthocyanins and (+)catechins (tannins). Polyphenols are believed to have an antioxidant effect, which contributes to protecting low-density lipoproteins (LDL) from oxidation (NIGDIKAR et al. 1998, ZIMA et al. 2005, AMAROWICZ et al. 2008). Wine contains hydroxybenzoic (mostly gallic and protocatechic) and hydroxycinnamic (mostly caffeic and *p*-coumaric) acids and high concentrations of some of their derivatives (HERNANDEZ-ORTE et al. 2006). Grapes and wine contain many mineral elements. The most important minerals include potassium, magnesium, calcium and sodium, whereas iron, boron, silicon and zinc occur only in insignificant amounts (ppm range), and there are merely trace quantities of aluminum, lead, cadmium, fluorine, copper or selenium (<1 mg dm^{-3}). The content of mineral elements is affected by numerous factors, such as the type of grapevine, climate and soil as well as the method of wine production (COOK et al. 1995, RUPASINGHE, CLEGG 2007).

With the growing awareness of the positive impact on health (the French paradox) and the increasing consumption of red wines, it is interesting to compare their chemical composition and organoleptic characteristics. As mentioned earlier, wine quality is significantly affected by the region in which grapevine grows, which is why wines from different parts of Europe were analyzed. The objective of the present work was to compare the content of bio-active substances (polyphenols, minerals) in semi-dry red wine originating from different vineyards in Bulgaria, Hungary, France and Spain.

MATERIAL AND METHODS

The material consisted of semi-dry red wine originating from Bulgaria (Sofino Melnik, 11% vol.), Hungary (Loci, 10.5% vol.), France (Bongeronde, 10% vol.) and Spain (Don Kichot, 11% vol.) produced in 2009.

Bulgarian wine Sofino Melnik is a multistrain wine from the south-western region of this country (the Mediterranean climate), more specifically from the Struma Valle. Hungary Loci is a multistrain red wine from Ěszak-Alföld. French semi-dry red wine Bongeronde is a multistrain wine made from grapes growing in Languedoc. Spanish wine Don Kichot is a multistrain wine from La Mancha. Each of the examined groups consisted of 6 wine types (6 wines x 4 countries) in two replications. In total, 48 samples were examined. The wine was subjected to organoleptic assessment. A panel of tasters consisted of 20 people (10 males and 10 females), aged from 35 to 45 years. The assessment was made on a 5-point scale in accordance with the standards PN-90 A-79120/02. Laboratory analyses were performed in order to determine the content of crude ash according to PN-90A-79120/09, the share of tannins, based on formation of insoluble tannins with heavy metal salts (AOAC, 1995). Total acidity was determined according to PN-90A-79120/07 and expressed in tartaric acid. Total anthocyanins were determined using the pH differential method described by Lee et al. (2005). The methods described by Somers and Evans (1974) were used to determine the browning index of the wines. The measurements were carried out with a Unicam 5625 UV/VIS spectrophotometer. All samples were analyzed by HPLC-DAD (Waters) for phenolic acids. The analytical HPLC system (Gilson) employed consisted of two pumps 306, a detector UV-VIS DAD 170 and an injector 20 µL. Chromatographic separations were performed on a reversed phase column Symmetry C_{18} (5.0 µm, 250_4.6 mm i.d., Waters). Phenolic acids were separated using eluents, A: 1% acetic, B: 50% acetonitryle, both in water (v:v). Elution gradient was following: 0-10 min 92% A, 11-40 min 70% A, 41-55 min 60% A, 56-72 min 92% A. The monitoring wavelength was 320 nm. The results were calculated from the calibration curve.

The total content of phenolic compounds was determined by the spectrophotometric method of Folin-Ciocalteau (Goristein et al. 2000) and expressed as gallic acid equivalent (GAE). For assays of minerals, the samples were pre-dried in a dryer at a temperature of 60°C for 24 hours, and then dried at 105°C for another 24 hours. Afterwards, the samples were weighed, incinerated in a muffle furnace at a temperature of 450°C and solubilized in 6N of spectrally-pure hydrochloric acid. The content of zinc, manganese, copper, iron, and magnesium was determined in the mineralizates by the ASA method (PN-EN 14082:2004) using a UNICAM 939 spectrometer. Analysis of the content of oxalic acid was performed following the method presented by Brzozowska et al. (1999). The determination of the browning index was based on the procedure recommended by Wrolstad et al. (1982).

The results presented in the work are the arithmetic means obtained from 12 measurements (6 types of wine, two replications) analyzed statistically with Statistica ver. 5 software, and p=0.05 and p=0.01 regarded as a statistically significant value.

RESULTS AND DISCUSSION

All types of the analyzed wine obtained a positive organoleptic scoring (Table 1). The highest score for the color was given to the wines from France and Spain, while the Bulgarian and Spanish wine types had the best clarity. The aroma and flavor of the French wine obtained the highest score and this type of wine had the highest total score among all the analyzed types.

Table 1

Organoleptic assessment of semi-dry red wines

Wine origin	Number of points in a 5-point scale				General opinion
	C	K	A	T	
Bulgaria	3.8 ± 0.91	4.9 ± 0.38	3.4 ± 0.46	2.9 ± 0.35	satisfactory
Hungary	3.4 ± 0.54	4.6 ± 0.22	3.1 ± 0.26	3.2 ± 0.17	satisfactory
France	4.1 ± 0.55	4.6 ± 0.19	3.6 ± 0.30	3.6 ± 0.21	good
Spain	4.5 ± 0.47	4.8 ± 0.31	3.1 ± 0.14	2.6 ± 0.22	satisfactory

C – colour, K – clarity, A – aroma, T – taste

The key criterion while purchasing groceries is their sensory attractiveness and one of its elements is color. The color of wine red depends mainly of the anthocynins. These compounds are found predominantly in the grape skin and are released in the process of maceration. The longer the maceration period before decanting young wine, the richer the wine in dyes and tannins (Balík, Kumšta 2008).

The acidity of wine depends on its content of organic acids, such as tartaric, malic, citric, succinic or lactic acid (MAĆKIW 2003). This is a significant trait which gives wine its valuable properties enhancing digestion and ideally wine should contain 2.0-5.0 g dm^{-3} of organic acids (GAWLIK, BIALIK 1998). According to PN-A-79122:1996, the total acidity of grape wine should range from 3.5 to 9.0 g dm^{-3}, as converted into tartaric acid. The total acidity of the analyzed semi-dry red wine was from 4.97 g dm^{-3} in Spanish wine to 5.56 g dm^{-3} in Bulgarian wine, which met the requirements set for the quality of grape wine (Table 2). The total value of acidity in the analyzed wine was not affected by the amount of oxalic acid, which was significantly higher in Bulgarian and Hungarian wine than in the wine from France and Spain (Table 2).

Table 2

Content of oxalic acid and total acidity and browning in semi-dry red wines

Item	Wine origin				Mean
	Bulgaria	Hungary	France	Spain	
Oxalic acid (mg %)	$1.80^a \pm 0.61$	$1.71^a \pm 0.21$	$5.67^b \pm 0.95$	$5.49^b \pm 1.56$	3.66 ± 2.07
Total acidity (g dm^{-3})	5.56 ± 0.42	5.39 ± 0.54	5.06 ± 0.38	4.97 ± 0.27	5.24 ± 0.88
Browning index	$2.97^a \pm 0.16$	$3.37^{ab} \pm 0.06$	$4.29^b \pm 0.19$	$3.75^{ab} \pm 0.11$	3.59 ± 1.11

a, b – values in the same rows marked with different letters differ significantly at $p \leq 0.05$

Oxalic acid is fairly high in the concord grapes and ooccasionally, crystals of calcium oxalate form in wine. The development occurs late, commonly after bottling. The redox potential of most young wines stabilizes the complex formed between oxalic acid and metal ions, such as iron. However, as the redox potential of wine rises during aging, ferrous oxalate changes into the unstable ferric form. After dissociation, oxalic acid may bond with calcium, forming calcium oxalate crystals (JACKSON 2000).

The health-promoting value and the quality of wine also depend on its content of phenolic compounds (IWANOW et al. 2001). Grapes are a rich source of phenolic compounds and red grape wine contains from 1.0 to 4.0 g dm^{-3} of polyphenols (LOPEZ-VELEZ et al. 2003). The analyzed semi-dry red wine originating from France and Spain was relatively low in these compounds (1.45 g dm^{-3} in both replications). However, this value was significantly higher (p=0.05) compared with the wine from Bulgaria (1.25±0.03 g dm^{-3}) or Hungary (0.97±0.02 g dm^{-3}) – Table 3. Both the content of polyphenols and the share of anthocyanins and tannins, and hence the wine's color and its intensity, depend on the type of grapes, the degree of their maturity and the climate in which the fruit is cultivated (the warmer the climate, the darker the wine) (BALÍK et al. 2008), which can justify a higher concentration of the above compounds in French and Spanish wine varieties.

Table 3

Content of biological activities compounds in semi-dry red wines

Item	Wine origin				Mean
	Bulgaria	Hungary	France	Spain	
Total phenolic ($g\ dm^{-3}$)	$1.25^a \pm 0.03$	$0.97^a \pm 0.02$	$1.45^b \pm 0.03$	$1.45^b \pm 0.01$	1.28 ± 0.33
Anthocyanins ($mg\ dm^{-3}$)	$26.05^a \pm 0.25$	$23.60^a \pm 0.80$	$59.90^b \pm 0.20$	$75.90^c \pm 2.40$	46.36 ± 20.11
p-coumaric acid ($mmol\ dm^{-3}$)	$1.95^a \pm 0.44$	$2.84^b \pm 0.08$	$1.45^a \pm 0.01$	$1.17^a \pm 0.33$	1.85 ± 0.25
Tannins (%)	$2.57^a \pm 0.79$	$5.35^b \pm 0.98$	$4.68^b \pm 0.81$	$3.24^a \pm 0.59$	3.96 ± 1.23

a, b, c, d – values in the same rows marked with different letters differ significantly at $p \leq 0.05$

Numerous studies have proven very beneficial influence of polyphenols on human health (the French paradox) and have enabled us to determine which are most effective. The group of compounds with documented bioactive effects includes, among others, hydroxybenzoate and hydroxysuccinic acids.

A wide discrepancy in the number and concentrations of phenolic acids was found among the examined wines, which contained from five (French wines) to eighteen phenolic acids (Spanish wines). The concentration of *p*-coumaric acid was used for a comparative study of all the semi dry wines because it occurred in a high content in all the samples. According to TUSZYŃSKI and SROKA (1999), the content of *p*-coumaric acid in red wine is around 1.56 mmol dm^{-3}. The same content is quoted by MAĆKIW (2003). RASTIJA et al. (2009) reported significant differences in the concentration of *p*-coumaric acid in Croatian red wines, where it ranged from 1.7 to 7.4 mg dm^{-3}. As regards *p*-coumaric acid in the analyzed wines (Table 3), its average content was 1.85 mmol dm^{-3}, being the highest in the Hungarian wine (2.84±0.08 mmol dm^{-3}).

The results concerning the total content of anthocyanins were confirmed by the total content of polyphenol compounds in the analyzed wine. Their concentration was significantly lower in the Bulgarian and Hungarian wine, (26.05±0.25 mg dm^{-3} and 23.60±0.80 mg dm^{-3}, respectively) compared to the wine from France (59.90±0.20 mg dm^{-3}) and Spain (75.90±2.40 mg dm^{-3}) – Table 3. Similar correlations were noted for the semi-sweet red wine analyzed by CZECH (2007), where the highest anthocyanin content was recorded in Spanish wine and the lowest in the Bulgarian varieties. According to TSANOVA-SAVOVA et al. (2002), the content of anthocyanin in Bulgarian red wines varied from 22 to 274 mg dm^{-3}.

Anthocyanins are responsible for the color of wine. However, they are exposed to different factors during the manufacturing process and storage, such as the presence of oxygen, metal ions or light, which lead to condensation processes and consequently brown polymer formation (PENG et al. 1998). An increased share of brown polymers deteriorates the color of wine, making it less vivid (CZAPSKI, WALKOWIAK-TOMCZAK 2008). Bulgarian wine was characterized by the lowest degree of browning (2.97±0.16), while this value was the highest in French wine (p=0.05) – Table 2.

The concentration of anthocyanins in red wine gives it a purple shade, whereas tannins are responsible for its lighter shade of orange or yellow. In time, the color of wine changes towards lighter shades as anthocyanins are less durable than tannins (MANIAK, KUNA-BRONIOWSKA 2007). The content of tannins in the analyzed wine samples ranged from 2.57±0.79% (Bulgarian wine) to 5.35±0.98% (Hungarian wine) – Table 3. The results revealed statistically significant differences at the level of p=0.05.

Tannins form colorful complexes with ion metals. They also affect the bitterness of wine and, under the effect of oxygen and high temperature, are oxidized into dark-colored compounds. The content of mineral elements in the analyzed semi-dry red wine samples is presented in Table 4. Analyzing the content of mineral elements, it should be noted that the number

Table 4

Content of dry ash (g dm^{-3}) and selected mineral elements (mg dm^{-3}) in semi-dry red wines

Item	Wine origin				Mean
	Bulgaria	Hungary	France	Spain	
Dry ash	$0.89^a \pm 0.03$	$1.19^a \pm 0.08$	$1.63^b \pm 0.03$	$2.56^c \pm 0.11$	1.57 ± 0.80
Copper	$0.40^b \pm 0.04$	$0.24^{ab} \pm 0.06$	$0.13^a \pm 0.04$	$0.09^a \pm 0.03$	0.21 ± 0.10
Magnesium	$50.3^a \pm 6.10$	$117.2^b \pm 6.50$	$90.2^b \pm 10.40$	$102.4^b \pm 7.60$	90.0 ± 29.45
Iron	$6.41^b \pm 0.96$	$9.47^c \pm 0.86$	$5.98^b \pm 0.73$	$4.67^a \pm 0.95$	6.63 ± 2.14
Zinc	$1.26^a \pm 0.09$	$2.08^b \pm 0.16$	$3.06^c \pm 0.17$	$1.61^a \pm 0.12$	2.00 ± 0.75
Manganese	$0.67^a \pm 0.11$	$1.13^b \pm 0.06$	$0.94^b \pm 0.05$	$0.32^a \pm 0.04$	0.76 ± 0.33

a, b, c – values in the same rows marked with different letters differ significantly at $p \leq 0.05$

of some microelements must be limited to maintain good health-promoting and organoleptic traits (COOK et al. 1995). An increase in the content of minerals may lead to turbidity and precipitation of lees in the wine. According to RĘCZAJSKA and JĘDRZEJCZAK (1998), the maximum limits in red wines are 5.0 mg dm^{-3} of zinc, 3.5 mg dm^{-3} of copper and 18.0 mg dm^{-3} of iron. Following the regulation by the Ministry of Health and Social Welfare, the acceptable content of zinc in wine is 5.0 mg dm^{-3}, and the content of copper in alcohol must not be higher than 1.0-4.0 mg dm^{-3}. Neither of these values was exceeded in any of the analyzed wine samples. The French wine was characterized by a significantly higher content of most analyzed mineral elements (magnesium, iron, zinc and manganese). The wine originating from Hungary also proved to be a rich source of minerals, particularly magnesium, iron and manganese. The Bulgarian wine had a significantly higher content of copper and iron, while the Spanish wine was richer in magnesium. The content of copper, manganese and iron in the analyzed wine samples was close to the results noted by BULIŃSKI et al. (1995) for red grape wine and MALIK and CZECH (2005). The concentration of zinc in the examined wines was significantly higher than in the wine studied by BULIŃSKI et al. (1995). Our results suggest some regularity related to the fact that certain metals may indirectly affect the degradation of anthocyanins by catalyzing the oxidation of ascorbic acid with the formation of H_2O_2 (PENG et al. 1998). Two elements participate in the processes of forming hydrogen peroxide, namely copper and iron. A higher content of these minerals in the Bulgarian wine may have led to the lowering of the content of anthocyanins in those wine samples.

The content of ash in wine, that is the components left after evaporation and roasting, depends mainly on the presence of minerals. The concentration of minerals in wine is slightly lower than in the fruit, which are used to proliferate yeast in the fermentation process (POGORZELSKI et al. 2005). The content of crude ash in the analyzed wine samples was significantly lower in the Bulgarian and Hungarian wines (0.89±0.03 g dm^{-3}; 1.19±0.08 g dm^{-3}, respectively). The highest share of crude ash was recorded in the Spanish wine (2.56 g dm^{-3}) – Table 4. According to WZOREK et al. (2005), the content of ash in red wine should not exceed 1.7 g dm^{-3}, whereas POGORZELSKI et al. (2005) suggest that the mean ash content in wine ranges from 1.8 to 2.5 g dm^{-3}. The analyzed semi-dry wine samples revealed a slightly lower crude ash concentration: 1.57 g dm^{-3} on average.

CONCLUSIONS

All the analyzed wine samples, regardless of their origin, achieved a high organoleptic assessment score, especially for clarity and color. The highest rated wine was made in France. It was characterized by a high level of bioactive compounds: total polyphenols and anthocyanins. It also had a high content of crude ash and minerals such as Mg^{+2}, Mn^{+2}, Zn^{+2}. Similarly, relatively high concentrations of polyphenols, anthocyanins and crude ash were determined in the Spanish wines. The results of our analyses showed a relationship between the content of bioactive compounds and the place of manufacture of wine. Consumption of wines, especially French ones, may contribute to increasing the supply of compounds producing beneficial effects in the human body.

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