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THE INFLUENCE OF WINEMAKING ON THE CONTENT OF NATURAL ANTIOXIDANTS AND MINERAL ELEMENTS IN WINES MADE FROM BERRY FRUITS

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Abstract

Bioactive compounds are natural antioxidants essential for the normal functioning of a body. The main sources of antioxidants are fruit and vegetables. Bioactive compounds are essentially stable during the storage of raw material, but their processing leads to significant changes in the content of these compounds, which continue during the storage of end products. A substantial proportion of biologically active compounds is destroyed during technological processing as well as long-term storage of products in unsuitable conditions. Hence the aim of the study was to determine the effect of the winemaking process on the antioxidant potential and content of phenolic compounds (anthocyanins and tannins), vitamin C, minerals and catalase activity in wines obtained from black currant, red currant, strawberry, grape and raspberry. While grape wines are very popular because of their flavour and other health-promoting effects, wines produced from berry fruits are a source of numerous valuable natural antioxidant compounds that are beneficial for health. Wine made from black currants was found to contain the greatest quantity of antioxidants. Among the berry fruits analyzed, black currant proved to have the highest antioxidant properties, which was linked to its high content of polyphenolic compounds, vitamin C and mineral nutrients. Similarly, wine obtained from the fruit of black currant had the highest capacity to scavenge free radicals.

Keywords: wine, berry fruits, polyphenols, vitamin C.

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INTRODUCITION

Fruit and vegetables are a rich source of antioxidants, and according to much research are the most easily assimilated and most available sources of these compounds. These compounds are mainly represented by polyphenols (phenolic acids and a large group of flavonoids, including anthocyanins), vitamins – A, C, as well as organic acids and mineral nutrients (Jimenez-Garcia et al. 2013).

Among these substances, phenolic compounds play a particularly important role. Owing to their presence in berries and stone fruits, which are typical fruit plants grown in central Europe, the antioxidant activity of these fruits is two- or three-fold higher than that of citrus fruits (Rouseff, Nagy 1994). It has also been demonstrated that procyanidins have greater capacity to neutralize free radicals than quercetin derivatives or vitamins C, which determine the antioxidant potential of citrus fruits (Rice-Evans et al. 1997).

Bioactive compounds are essentially stable during the storage of raw material, but their processing leads to significant changes in the content of these compounds, which continue during the storage of end products. A substantial proportion of biologically active compounds is destroyed during technological processing as well as long-term storage of products in unsuitable conditions (Jimenez-Garcia et al. 2013). It should be emphasized, however, that depending on the type of raw material, all technological operations and properly conducted preliminary processing can have a beneficial effect and increase antioxidant activity. This may be associated with the extraction of polyphenolic compounds from inedible parts of fruit. Beneficial changes also include the transformation of antioxidant particles into a more active form, such as the conversion of the glycoside form to the aglycone form (Rice-Evans 2004). Antioxidant properties are also exhibited by products of chemical changes in fruit (e.g. non-enzymatic browning) generated during technological processes (Visioli et al. 2000).

Among fruit products, wines are characterized by high antioxidant potential (Howard et al. 2002). Currently, owing to the fact that wines have become an integral part of the culture of many countries, they are produced not only from grapes, but also from many other fruit, including mangos (Kumar, Prakasam 2009), bananas (Akubor et al. 2003), and cacao (Dias et al. 2007). In Poland, the most popular fruits for wine production are the antioxidant-rich berry fruits mentioned above, i.e. raspberries, strawberries and currants.

Despite numerous studies, knowledge about differences in the content of biologically active compounds in raw plant materials, and especially in processed food products, is still insufficient, so further research in this area is needed. It is assumed that antioxidants present in berries are not entirely extracted in the winemaking process. Hence the aim of the study was to determine the influence of the winemaking process on the antioxidant potential and content of phenolic compounds (anthocyanins and tannins), vitamin C, minerals and catalase activity in wines obtained from black currant, red currant, strawberry, grape and raspberry.

MATERIAL AND METHODS

Chemicals and reagents

All reagents were purchased from Sigma-Aldrich (Germany). All chemicals and solvents were analytical grade.

Samples - plant material and wine

All berries (*Ribes nigrum*, *Ribes rubrum*, *Fragaria ananassa*, *Vitis californica* and *Rubus idaeus*) were purchased from 15 local farms near Lublin, Poland, during the 2013 season (three samples of each berry of 11 kg). For laboratory assays of the content of antioxidant compounds and minerals, 0.5 kg was taken from each sample and frozen until analysis. Wine was made from the remaining quantity of fruit. About 10 L of each wine was prepared. Berries were crushed before fermentation and sugar was added to 23°Bx. Fermentation was carried out in 30 L glass carboys, adding a yeast inoculum of 0.25 g L^{-1} Madeira (Biowin Polska). The first fermentations were carried out at a controlled temperature of 23°C for 8 weeks. Then, the wine was decanted into clean carboys and the second stage of alcohol fermentation was carried out. After 6 months, the wine was bottled in 750 ml dark glass bottles and the wines were stored at 16°C until analysis. Each wine was prepared three times.

Determination of anthocyanins, polyphenols, tannins and vitamin C

Compounds were extracted from fruit samples (1 g) in aqueous ethanol (10 mL, 50% v/v) and sonicated for 20 min. Then, the samples were centrifuged (10 min at 4,000 *g*) and the supernatant was removed for analysis. Anthocyanins were determined by the pH differential method (Lee et al. 2005). The total polyphenol content was determined using the Folin-Ciocalteu method (Tsao, Yang 2003) and calculated as amount of gallic acid equivalent (GAE) in g L^{-1} or g kg^{-1} of sample. Tannins were determined by the method of Ribereau-Gayon et al. (2006). The amount of vitamin C was determined using the Folin-Ciocalteu method according to norm PN-A-04019/98.

Determination of microelements

Content of copper, zinc, iron and manganese were determined using atomic absorption spectrometry with flame atomization (FAAS). All experiments were carried out in a UNICAM SOLAR 939 spectrometer.

Determination of antioxidant activity based on the DPPH reagent

The extraction procedure was based on methods reported by Musa et al. (2011). A DPPH radical scavenging assay was performed according to the method reported by Brand-Williams et al. (1995) with some modifications. A 50 μL volume of sample or methanol (control) was added to 1.95 mL of a methanolic solution of DPPH (20 mg L^{-1}). The mixtures were shaken vigorously and left to stand in the dark at room temperature for 12 min, and then absorbance was read at 515 nm. Results are presented as mmol Trolox L^{-1}.

Catalase activity assay

The berry material (2 g) was homogenized with 0.1 mol L^{-1} potassium phosphate buffer, pH 7.0. Extracts were centrifuged at 12,000 *g* for 30 min and the supernatant was removed for analysis. Catalase activity was measured according to Luhova et al. (2003).

Statistical analysis

At least three analyses were run for each wine and berry for polyphenols, anthocyanins, ascorbic acid, tannins, catalase activity, iron, copper, zinc, manganese and antioxidant activity. Each analysis consisted of triplicate measurements of each sample and data were averaged over three measurements. Parameters were expressed as mean and standard deviation. Significant differences between raw berry fruits and wines were calculated by Independent *t*-test. Significance of differences between means was determined by the Duncan's multiple range test for significance levels of $P < 0.05$. Single-metabolite analysis of variance (Anova) was performed using Statistica software.

RESULTS AND DISCUSION

Berry fruits and the wines made from them are characterized by a high content of bioactive compounds with antioxidant activity. The results obtained during the determination of polyphenolic compounds and vitamin C in the berries and the wines are shown in Table 1. The highest content of bioactive compounds was noted in the fruit of black currant. Similar observations were made by Benvenuti et al. (2004), who found the highest total concentration of polyphenolic compounds (8.885 g kg^{-1}) in black currants, and the lowest one in raspberries (1.406 g kg^{-1}).

Although raspberries are the least abundant in bioactive compounds, their extraction by wine is the highest, which may be related to the thickness of the skin (Paredes-López et al. 2010). This is also implicated by the lowest percentage of extraction of bioactive compounds from currants, whose skin is the thickest among all the tested berries.

Table 1

The experimental results of the determination of bioactive compounds in raw berries and wines ($\bar{x}$ ± SD)

Berries	Phenolic compounds			Tannins			Anthocyanins			Vitamin C		
	raw (g GAE kg^{-1})	wine (g GAE L^{-1})	(%) extraction*	raw (g kg^{-1})	wine (g L^{-1})	(%) extraction*	raw (g kg^{-1})	wine (g L^{-1})	(%) extraction*	raw (g kg^{-1})	wine (g L^{-1})	(%) extraction*
Black currant	6.242^{d} ± 0.202	1.845^{c} ± 0.099	28.98^{ab} ± 0.63	3.323^{c} ± 0.111	1.522^{c} ± 0.131	44.90^{b} ± 2.41	1.903^{d} ± 0.189	0.221^{b} ± 0.038	11.38^{a} ± 0.85	1.009^{b} ± 0.037	0.351^{b} ± 0.027	34.10^{c} ± 1.40
Red currant	4.754^{b} ± 0.133	1.062^{a} ± 0.133	22.38^{a} ± 2.17	2.825^{b} ± 0.086	0.936^{a} ± 0.056	33.20^{a} ± 0.97	0.936^{c} ± 0.028	0.107^{a} ± 0.010	11.45^{a} ± 0.73	0.594^{a} ± 0.058	0.111^{a} ± 0.015	18.72^{a} ± 0.71
Strawberry	5.157^{c} ± 0.151	1.585^{b} ± 0.046	31.08^{b} ± 0.08	3.411^{c} ± 0.104	1.181^{b} ± 0.050	35.00^{a} ± 0.41	0.687^{b} ± 0.047	0.204^{b} ± 0.021	30.02^{c} ± 1.03	0.547^{a} ± 0.047	0.076^{a} ± 0.019	14.05^{a} ± 2.29
Grape	5.057^{c} ± 0.213	1.396^{b} ± 0.112	27.41^{ab} ± 1.05	3.501^{c} ± 0.150	1.132^{b} ± 0.062	32.11^{a} ± 0.38	0.550^{a} ± 0.079	0.122^{a} ± 0.011	22.03^{b} ± 1.21	0.519^{a} ± 0.015	0.143^{a} ± 0.044	27.36^{b} ± 2.75
Raspberry	3.179^{a} ± 0.091	1.073^{a} ± 0.064	33.92^{b} ± 1.04	2.250^{a} ± 0.091	0.886^{a} ± 0.069	39.57^{ab} ± 1.47	0.726^{b} ± 0.066	0.151^{a} ± 0.021	20.90^{b} ± 1.01	0.498^{a} ± 0.047	0.132^{a} ± 0.024	26.64^{b} ± 2.34

a,b,c,d – means within columns with different superscripts differ significantly at $p \leq 0.05$,
* % extraction was calculated as (content of compound in wine/content of compound in berry) · 100%. Density of wines was respectively for black currant 1.020 g mL^{-1}, red currant 0.998 g mL^{-1}, strawberry 0.989 g mL^{-1}, grape 1.007 g mL^{-1} and raspberry 0.995 g mL^{-1};

Differences in the content of bioactive compounds, mainly polyphenols, are caused by numerous factors, including the variety of fruit and environmental factors such as insolation, temperature, degree of ripeness and storage after harvest. These factors also affect the colour of fruit (Wang, Lin 2000), which is significantly associated with the total content of anthocyanins and the proportions of individual compounds of this group. Among the fruits tested, grapes, with the lightest skin, had the lowest content of anthocyanins. The literature data give a very wide range of concentrations of these compounds in grapes, from 0.3 to 7.50 g kg^{-1} (Wang et al. 1997). According to Wang et al. (1997), the content of anthocyanins in raspberries ranges from 0.2 to 0.6 g kg^{-1}, which was confirmed in the present study. A high anthocyanin content is also linked to a high content of tannins. In fruit with a large quantity of anthocyanins they are stabilized by the formation of copolymers with tannins, which give the fruit, and in particular the wine, its characteristic flavor (Cheynier et al. 2006).

Wines produced from black currants had significantly higher concentrations of bioactive compounds than the other wines. Only the content of anthocyanins was similar in the wines made from black currants and from strawberries (Table 1). The content of bioactive compounds in wine is influenced by winemaking technology (Villano et al. 2006). The processes of fermentation and maceration that the fruit undergoes during wine production, as well as the storage of wine decrease the content of polyphenolic compounds. Polyphenols are oxidized by reactive oxygen species, first to semiquiones and then to quinones. The rate of this reaction largely depends on pH – an increase in wine pH of one unit causes an increase in the concentration of phenolate ions, i.e. acceleration of the oxidation of polyphenols (Danilewicz 2003). According to Di Majo et al. (2008), the presence and arrangement of OH and OCH_3 groups also influence the antioxidant activity of polyphenols. The vitamin C present in fruits and passed on to wine prevents oxidation of polyphenols by free radicals, thereby preventing the browning of wine.

Apart from bioactive compounds, enzymes, including catalase, are also involved in the antioxidant defense of an organism. The highest catalase activity was found in the fruit of grapevine (Table 2), but the results were considerably lower than those presented by Rani et al. (2004), probably because the grapes were of a different variety. The production process of wine from blackcurrant or raspberry led to an over 30% inhibition of catalase activity.

Besides catalase, other enzymes also participate in antioxidant defense. These are built from protein units bound to ions of transition metals such as zinc, copper, manganese and iron (CuZn-SOD, Mn-SOD, Fe-SOD). Mineral elements are natural antioxidants but, on the other hand, Fe(II) and Cu(I) ions catalyze the Fenton reaction generating hydroxyl free radicals (Zago, Oteiza 2001). Table 3 presents the content of selected mineral elements in the berry fruits and wines produced from them. As in the case of polyphenolic compounds, the black and red currant fruit had a higher content of min-

Table 2

Catalase activity (U g^{-1}) and DPPH (mmol Trolox L^{-1}) radical scavenging activity of berries and wines ($\bar{x}$ ± SD)

Berries	Catalase			DPPH		
	raw	wine	(%) inhibition*	raw	wine	(%) inhibition
Black currant	32.35^{a} ± 3.85	20.51^{a} ± 2.62	36.60^{c} ± 0.56	15.82^{b} ± 1.02	11.21^{b} ± 0.78	29.14^{d} ± 0.36
Red currant	50.02^{b} ± 5.12	47.06^{c} ± 1.89	5.92^{a} ± 0.53	9.43^{a} ± 0.69	9.08^{a} ± 0.55	3.71^{b} ± 0.39
Strawberry	58.78^{c} ± 3.25	47.06^{c} ± 2.92	19.94^{b} ± 0.54	13.63^{b} ± 0.86	9.24^{a} ± 0.59	32.21^{d} ± 0.05
Grape	64.71^{d} ± 4.34	52.94^{d} ± 1.99	18.19^{b} ± 2.42	11.24^{ab} ± 0.77	9.26^{a} ± 0.63	17.62^{c} ± 0.04
Raspberry	47.05^{b} ± 1.97	32.35^{b} ± 3.74	31.23^{c} ± 5.08	9.09^{a} ± 0.60	9.01^{a} ± 0.71	0.88^{a} ± 0.06

a,b,c,d – means within columns with different superscripts differ significantly at $p \le 0.05$,
* % inhibition was calculated as 100% (activity/content of compound in wine/activity/content of compound in berry) · 100%);

eral elements, including copper, zinc and iron, than the other berry fruits. The values obtained were consistent with the research by Gąsioł, Domagała-Świątkiewicz (2012). Based on our results, an interaction between the mineral content (except zinc) and the efficiency of their extraction was observed.

Owing to its high content of polyphenols and vitamin C, black currant exhibits high antioxidant activity expressed as the capacity to scavenge DPPH free radicals (Table 2). With respect to the other fruits, their antioxidant activity increased with the content of polyphenols. The antioxidant activity of black currant has been confirmed by the results of studies conducted in both *in vitro* and *in vivo* conditions. Anthocyanins isolated from black currants have been shown to inhibit the production of ROS in HL-60 and SH-SY5Y cells (Gosh et al. 2006). A consequence of the antioxidant activity of the components of black currant is their ability to counteract oxidative modifications of biologically important cell components, such as DNA, lipids and proteins (Gosh et al. 2006, Farombi et al. 2004).

Among the berry fruits analyzed, black currant proved to have the highest antioxidant properties, which was linked to its high content of polyphenolic compounds, vitamin C and mineral nutrients. Similarly, the wine obtained from the fruit of black currant had the highest capacity to scavenge free radicals. Although raspberry fruit are the least abundant in polyphenols and vitamin C, their extraction by wine is highest. While grape wines are very popular owing to their flavor and their other health-promoting effects, wines made from berry fruits are a source of numerous valuable natural antioxidant compounds that are beneficial for health.

Table 3

Content of microelements in raw berries (mg kg^{-1} fresh weight) and wines (mg L^{-1})

Berries	Copper			Zinc			Iron			Manganese		
	raw	wine	% ex-traction*	raw	wine	% ex-traction*	raw	wine	% ex-traction*	raw	wine	% ex-traction*
Black currant	0.901^{c} ± 0.040	0.211^{a} ± 0.020	22.96^{a} ± 1.18	2.671^{b} ± 0.171	2.003^{b} ± 0.192	73.52^{a} ± 2.39	8.492^{c} ± 0.281	5.370^{c} ± 0.581	61.99^{d} ± 4.75	2.993^{b} ±0.211	0.401^{b} ± 0.031	13.13^{b} ± 0.09
Red currant	1.105^{c} ± 0.006	0.210^{a} ± 0.041	20.04^{a} ± 3.06	2.732^{b} ± 0.150	1.927^{b} ± 0.131	70.67^{a} ± 0.92	9.261^{d} ± 0.391	3.052^{b} ± 0.130	33.02^{b} ± 0.03	2.054^{a} ±0.175	0.322^{a} ± 0.043	15.71^{c} ± 0.76
Strawberry	0.681^{b} ± 0.011	0.203^{a} ± 0.031	30.14^{b} ± 4.07	2.120^{a} ± 0.221	2.037^{b} ± 0.142	97.15^{c} ± 3.36	3.920^{a} ± 0.121	1.852^{a} ± 0.132	47.77^{c} ± 1.91	5.515^{c} ±0.210	0.640^{c} ± 0.020	11.73^{b} ± 0.08
Grape	0.382^{a} ± 0.012	0.261^{ab} ± 0.032	67.85^{d} ± 3.64	1.891^{a} ± 0.181	1.742^{a} ± 0.120	91.48^{c} ± 2.49	9.221^{d} ± 0.191	1.733^{a} ± 0.203	18.66^{a} ± 1.81	5.051^{c} ±0.341	0.232^{a} ± 0.091	4.56^{a} ± 0.49
Raspberry	0.673^{b} ± 0.010	0.302^{b} ± 0.041	45.10^{c} ± 4.57	2.612^{b} ± 0.183	2.141^{b} ± 0.184	82.38^{b} ± 1.31	6.133^{b} ± 0.261	2.027^{a} ± 0.141	33.22^{b} ± 0.89	2.931^{b} ±0.120	0.453^{b} ± 0.081	15.53^{c} ± 2.13

a,b,c,d – means within columns with different superscripts differ significantly at $p \leq 0.05$,
* % extraction was calculated as (content of mineral in wine/content of mineral in berry) · 100%. Density of wines was respectively for black currant 1.020 g mL^{-1}, red currant 0.998 g mL^{-1}, strawberry 0.989 g mL^{-1}, grape 1.007 g mL^{-1} and raspberry 0.995 g mL^{-1};

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