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Phenolic compounds and vitamin C as sources of antioxidant activity in black currant fruit (*Ribes nigrum* L.)

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Abstract: The content of some important phenolic compounds (kaempferol, myricetin, quercetin, and ellagic acid), total phenolics (TPH), vitamin C, and total antioxidant capacity (TAC) in the fruit of three black currant cultivars (Ben Lomond, Ben Sarek and Malling Juel) were studied. The content of individual phenolic compounds showed variability among the studied cultivars. The main flavonol found in cv. Malling Juel was kaempferol (1.60 μg g^{-1} FW), whereas the lowest values of myricetin (5.46 μg g^{-1} FW) and quercetin (7.65 μg g^{-1} FW) were also observed in the fruit of this cultivar. Ellagic acid content was the highest in cv. Ben Lomond (12.90 μg g^{-1} FW), which is also characterized by lower values of individual flavonols compared to other cultivars tested. However, the highest TPH level was recorded in cv. Ben Lomond (4.71 mg g^{-1}FW).

Vitamin C content in fruit of cv. Malling Juel was at a very high level (141.4 mg $100g^{-1}$), whereas cv. Ben Lomond expressed the highest level of TAC (7.60 mg asc g^{-1} FW). Overall, the content and profile of phenolic compounds in the black currant cultivars showed significant contribution to the expressed antioxidant capacity which was confirmed by positive correlation between TPH and TAC obtained in this study. Besides total phenolics, vitamin C is also a significant contributor to the measured antioxidant capacity in black currant fruit indicating that some cultivars with lower phenolic content may express high TAC.

Key words: black currant, cultivar, phenolic compounds, vitamin C, antioxidant capacity.

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Introduction

In the past decades, pronounced attention has been paid to the antioxidant capacity of fruit as an eligible parameter of quality (Vangdal and Slimestad 2006). This parameter is strictly correlated to the presence of efficient oxygen radical scavengers, such as vitamin C and phenolic compounds, which have been shown to play an important role in controlling oxidative reactions in the human body and exhibit anticarcinogenic activities (Moyer *et al*. 2002, Sun *et al*. 2002, Henriquez *et al*. 2005, Šavikin *et al*. 2009).

Berry fruits, especially black currant (*Ribes nigrum* L.), represent rich sources of natural antioxidants. Furthermore, accumulating evidence exists, suggesting that genotype may have a profound influence on the content of bioactive compounds in berries (Benvenuti *et al*. 2004, Scalzo *et al*. 2005, Pantelidis *et al*. 2007).

Taking into account the importance of the antioxidant capacity exhibited by phenolics and vitamin C, the purpose of this study was to determine the phenolic profiles and vitamin C content in the fruit of different black currant cultivars.

Potential differences in expressed antioxidant capacity among cultivars tested will indicate the importance of improving the assortment in commercial plantings. It means that by the introduction of cultivars which, apart from high productivity and attractive fruit appearance, possess high nutritive and antioxidant values, it is possible to increase fruit consumption for beneficial health purposes without decreasing the commercial effect of production.

Material and methods

Plant material

Fruits of three black currant cultivars (Ben Lomond, Ben Sarek and Malling Juel) were harvested at the commercial plantation located in the Belgrade region and analysed during three-year period (2004 – 2006).

Samples were collected in triplicate at commercial maturity stage of each cultivar and extracted immediately after harvesting. Approximately 100 g of fruits were blended to a puree, and samples of 5 g were homogenised for 1 min in 20 ml of extraction solution containing methanol/water/hydrochloric acid at a ratio of 70:30:5 by volume. The homogenate was filtered through a filter paper and the filtrates were centrifuged for 15 min at 3000 x *g*. The methanol supernatant was divided into aliquots and frozen at -80^{o}C until analysis.

Analytical procedures

Determination of flavonols and ellagic acid content. Quantification of individual phenolic compounds was done by reversed phase HPLC analysis. Samples were injected in Waters HPLC system consisted of 1525 binary pumps, thermostat and 717+ autosampler connected to the Waters 2996 diode array detector (Waters, Milford, MA, USA). Chromatograms were gathered in 3D mode with extracted signals at specific wavelengths for different compounds (370, 326 and 254 nm, respectively). Separation of phenolics was performed on a Symmetry C-18 RP column 125 x 4 mm size with 5 µm particle diameter (Waters, Milford, MA, USA) connected to appropriate guard column. Two mobile phases, A (0.1% phosphoric acid) and B (acetonitrile) were used at flow of 1 ml min^{-1} with the following gradient profile: the first 20 minutes from 10 to 22 % B; next 20 minutes of linear rise up to 40 % B, followed by 5 minutes reverse to 10 % B and additional 7.5 minutes of equilibration time. The data acquisition and spectral evaluation for peak confirmation were carried out by the Waters Empower 2 Software (Waters, Milford, MA, USA).

Determination of total phenolics. The amount of total phenolics (TPH) in extracts was determined according to the Folin-Ciocalteu's spectrophotometric (2501 PC Shimadzu, Kyoto, Japan) procedure (Singleton and Rossi, 1965) using gallic acid (GA) as a standard for the calibration curve. Samples were mixed with 0.25 N Folin-Ciocalteu reagents and after 3 min 0.2 M sodium carbonate solution were added and incubated for 60 min. Results were read at 724 nm and expressed as milligrams of GA equivalent per gram of fresh weight (mg GAE g^{-1} FW).

Determination of vitamin C. Vitamin C was quantified using the reflectometer set of Merck Co (Merck RQflex) according to their protocol for the juice of red fruit (Ascorbic Acid in Red Coloured Fruit Juices, Merck). Fruit sample (5 g) and 20 ml oxalic acid (1%) were mixed, homogenised for 1 min, and filtered. PVPP (polyvinylpolypyrrolidone) (500 g) was added to 10 ml of the filtered sample, to remove phenols, and 6-7 drops of H_2SO_4 (25 %) were added, to reduce pH to below 1. Results were expressed as mg ascorbic acid 100 g^{-1} fresh weight (FW).

Determination of the total antioxidant capacity. Determination of total antioxidant capacity (TAC) was done following the ABTS method of Arnao et al. (1999). The reaction mixture contained 2 mM ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), 15 µM hydrogen peroxide and 0.25 µM horse radish peroxidase (HRP) in 50 mM phosphate buffer pH 7.5. The reactions were monitored at 730 nm (2501 PC Shimadzu, Kyoto, Japan) at 25^{o}C until a stable absorbance was obtained due to ABTS radical formation. Afterwards, different concentrations (0.1-0.8 mM) of ascorbic acid were added for a standard curve set-up. Adding of methanolic extracts of raspberry in reaction mixture resulted in absorbance decreasing as a consequence of ABTS radical depletion. Absorbance alterations were read from standard curve and results were expressed as milligrams of ascorbic acid equivalent per gram of fresh weight (mg asc g^{-1} FW).

Statistical analysis

Statistical analysis was performed using software Statistica 6.0 for Windows (StatSoft Inc., Tulsa, OK, USA). Data from the three-year investigation were calculated by ANOVA for mean comparison, and intergenotype significance of differences were calculated according to LSD test. Data are reported as means. Correlations between TAC and TPH were calculated according to Pearson's test. Differences at $P \leq 0.05$ were considered to be statistically significant.

Results and Discussion

Great variability existed among the examined cultivars, regarding their content of individual flavonols (Table 1). The main flavonol found in cv. Malling Juel was kaempferol (1.60 µg g^{-1} FW), whereas the lowest values of kaempferol was observed in the fruit of cv. Ben Lomond (1.06 µg g^{-1} FW). Cv. Ben Sarek had the highest average content of myricetin and quercetin (7.24 µg g^{-1} FW and 8.73 µg g^{-1} FW, respectively) which was affected by weather conditions, and therefore varied widely from year to year.

Tab. 1. Flavonols and ellagic acid content in the fruit of black currant cultivars

		Flavonols (µg g^{-1}FW)			Ellagic acid
		Kaempferol	Myricetin	Quercetin	(µg g^{-1}FW)
Cultivar	Ben Lomond	1.06 b	5.56 b	7.97 b	12.90 a
	Ben Sarek	1.29 ab	7.24 a	8.73 a	5.70 c
	Malling Juel	1.60 a	5.46 b	7.65 b	9.95 b
Year	2004	1.20 b	6.14 a	12.85 a	15.05 a
	2005	0.93 b	6.61 a	6.42 b	7.40 b
	2006	1.83 a	5.50 b	5.07 b	6.10 c
Cv x Year	Ben Lomond 2004	0.98 bcd	4.87 cd	12.54 a	24.99 a
	2005	0.72 cd	8.31 a	7.47 b	8.15 cd
	2006	1.49 ab	3.49 e	3.90 c	5.56 ef
	Ben Sarek 2004	1.25 bcd	7.37 ab	13.87 a	6.16 def
	2005	0.61 d	5.92 cd	8.17 b	6.72 cde
	2006	2.02 a	8.42 a	4.15 c	4.21 f
	Malling Juel 2004	1.36 abc	6.17 bc	12.15 a	14.01 b
	2005	1.46 ab	5.61 cd	3.63 c	7.33 cde
	2006	1.98 a	4.59 de	7.16 b	8.52 c
ANOVA (F test)	Cv	4,055*	15,583**	3,958*	63,276**
	Year	11,914**	4,848*	15,267**	112,989**
	Cv x Year	$0{,}378^{ns}$	19,409**	4,932**	44,591**

Data are the means of three replications. Values within column followed by the same letter are not significantly different at $P > 0.05$. *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$

Ellagic acid content was the highest in cv. Ben Lomond (12.90 µg g^{-1} FW), which is also characterized by lower values of individual flavonols compared to other cultivars tested. The two-fold lower amount of ellagic acid was recorded in cv. Ben Sarek (5.70 µg g^{-1} FW). Interest for ellagic acid has particularly increased during the past decade due to its possible antimutagenic, anticarcinogenic and antioxidative effects (Häkkinen *et al*., 2000).

The average content of TPH in black currant cultivars ranged from 2.46 mg GA g^{-1} FW (Ben Sarek) to 4.71 mg GA g^{-1} FW (Ben Lomond) (Table 2). Šavikin et al. (2009) reported for total phenolics in black currant fruit an average of 3.80 mg

GA g^{-1} FW. Environmental factors may have occasioned the variability in TPH content as seen with individual flavonols content.

Tab. 2. Total phenolic content (TPH), Total antioxidant capacity (TAC) and Pearson's correlation coefficients between TPH and TAC in the fruit of black currant cultivars

		Total phenolic (mg GA g^{-1}FW)	Total antioxidant capacity (mg asc g^{-1}FW)	Pearson's correlation coefficients
Cultivar	Ben Lomond	4.71 a	7.60 a	0.952**
	Ben Sarek	2.46 c	3.83 c	0.630^{NS}
	Malling Juel	3.85 b	5.68 b	0.685*
Year	2004	3.69 b	5.30 b	
	2005	4.28 a	7.15 a	
	2006	3.05 c	4.67 c	
Cv x Year	Ben Lomond 2004	4.34 b	6.97 b	
	2005	6.26 a	10.42 a	
	2006	3.54 cde	5.41 de	
	Ben Sarek 2004	2.45 f	2.49 f	
	2005	3.10 e	5.96 cd	
	2006	1.83 f	3.04 f	
	Malling Juel 2004	4.28 b	6.44 bc	
	2005	3.48 de	5.06 e	
	2006	3.79 bcd	5.55 de	
ANOVA (F test)	Cv	84.169**	150.731**	
	Year	24.577**	70.568**	
	Cv x Year	15.066**	39.328**	

Data are the means of three replications. Values within column followed by the same letter are not significantly different at $P > 0.05$. NS, non significant; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$.

Black currant fruits are the richest source of vitamin C among all berries (Hägg *et al*. 1995, Szajdek and Borowska 2008). Our results confirmed this finding by achieving the highest value in cv. Malling Juel (141.4 mg 100 g^{-1} FW), followed by cv. Ben Sarek (132.6 mg 100 g^{-1} FW) (Figure 1). These results are in accordance with the previous published data (Benvenuti *et al.* 2004). In addition, its content were significantly higher than those in other fruits well known for their high vitamin C content, such as strawberries (Roberts and Gordon 2003) and kiwi fruits (Nishiyama *et al*. 2004).

The highest antioxidant activity of black currant fruit has been proved (Milivojević 2008; Benvenuti *et al*. 2004; Moyer *et al*. 2002; Lister *et al*. 2002). However, this study pointed out that the high antioxidant capacity of black currants is strongly influenced by cultivar. Cv. Ben Lomond had the highest average value of TAC (7.60 mg asc g^{-1} FW), which was 2-fold higher than that obtained by cv. Ben Sarek (3.83 mg asc g^{-1} FW) (Table 2).

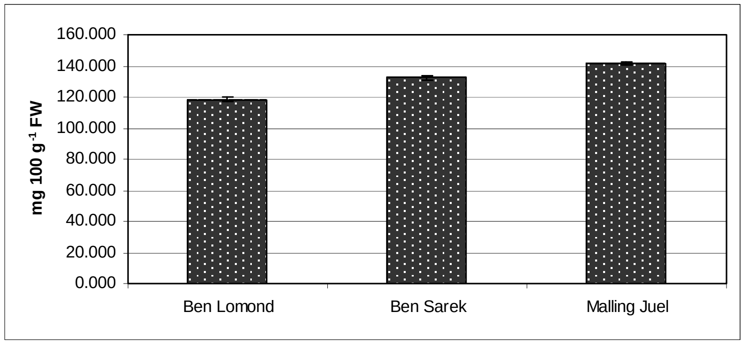

Fig. 1. Vitamin C content in the fruit of black currant cultivars (mean values for period of 2004-2006 ± standard error)

A very significant correlation between TAC and TPH was only found in cv. Ben Lomond (r = 0.952) confirms the possibility of using the parameter "total phenolics" as an indicator of antioxidant capacity. Lower correlation coefficients between TPH and TAC found in cvs. Ben Sarek and Malling Juel (r = 0.630 and r = 0.685, respectively) could be due to the very high vitamin C content recorded in their fruit. Similar results have been reported by other researchers (Kalt 2001, Lister *et al*. 2002, Scalzo *et al*. 2005, Milivojević 2008) who also found a linear correlation between TPH and TAC in different berries.

Conclusions

Black currants are a significant source of phenolic compounds and vitamin C. Many phenolic compounds (kaempferol, quercetin, myricetin, and ellagic acid) were detected and differences in the phenolic profiles were also observed among the cultivars used in this study. Antioxidant capacity, as an important fruit quality parameter, varied greatly among the cultivars and was highly correlated with total phenolics in cv. Ben Lomond. The present study indicates that cv. Malling Juel is an extremely rich source of vitamin C, demonstrating its contribution to the measured overall antioxidant capacity.

Overall, the data obtained suggest that cvs. Ben Lomond and Malling Juel clearly hold high position among the studied black currant cultivars and could be considered as a good source of natural antioxidants.

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FENOLNE KOMPONENTE I VITAMIN C KAO IZVORI ANTIOKSIDATIVNE AKTIVNOSTI PLODA CRNE RIBIZLE (*Ribes nigrum* L.)

- originalni naučni rad -

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Rezime

Rad prikazuje rezultate trogodišnjih ispitivanja sadržaja važnih fenolnih jedinjenja (flavonola – kampferola, miricetina i kvercetina, elaginske kiseline i ukupnih fenola), vitamina C, kao i vrednosti antioksidativnog kapaciteta registrovane u plodu tri sorte crne ribizle Ben lomond, Ben sarek i Moling džuel. Istraživanja su realizovana u periodu od 2004. do 2006. godine u laboratorijama Katedre za voćarstvo Poljoprivrednog fakulteta Univerziteta u Beogradu i Instituta za multidisciplinarna istraživanja iz Beograda.

Ustanovljeno je variranje u sadržaju pojedinih individualnih fenolnih komponenti između ispitivanih sorti crne ribizle, pri čemu je kod sorte Moling džuel registrovan najveći prosečni sadržaj kampferola (1,60 $\mu g\ g^{-1}$), ali i najniže prosečne vrednosti sadržaja miricetina (5,46 $\mu g\ g^{-1}$) i kvercetina (7,65 $\mu g\ g^{-1}$).

Među ispitivanim sortama crne ribizle najveći sadržaj elaginske kiseline imala je sorta Ben lomond (12,90 $\mu g\ g^{-1}$), koja se odlikuje nešto nižim vrednostima sadržaja flavonola u plodu, ali i najvećim sadržajem ukupnih fenola za ispitivani period (4,71 $mg\ g^{-1}$).

U pogledu sadržaja vitamina C, sorta Moling džuel je pokazala superiornost (141,4 mg $100g^{-1}$), dok se najvećim antioksidativnim kapacitetom ploda za trogodišnji period ispitivanja odlikuje sorta Ben lomond (7,60 mg ask g^{-1}).

Na osnovu većine analiziranih parametara može se konstatovati da sadržaj i profil fenolnih komponenata prisutnih u plodovima ispitivanih sorti crne ribizle, ima značajan doprinos u ispoljenoj antioksidativnoj aktivnosti, što potvrđuje i pozitivna korelativna zavisnost između sadržaja ukupnih fenola i antioksidativnog kapaciteta ploda. Pored ukupnih fenola, značajan doprinos antioksidativnom kapacitetu ploda daje i vitamin C. To objašnjava pojavu da su neke sorte posedovale visok antioksidativni kapacitet ploda uz, istovremeno registrovan, umeren sadržaj fenolnih jedinjenja.