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# CONTENTS OF MAIN PHENOLICS AND ANTIOXIDATIVE CAPACITY IN FROZEN RASPBERRY FRUITS (*RUBUS IDAEUS* L.) FROM ARILJE GROWING AREA

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**ABSTRACT:** Individually quick frozen (IQF) fruits of the most abundant raspberry cultivars (Meeker and Willamette) in Western Serbia were randomly taken from the commercial cold storage in Arilje growing area, and analyzed for main phenolics and antioxidative capacity. All samples for freezing were harvested in the full ripening stage in the orchards in close proximity to the commercial cold storage, frozen by conventional freezing, and stored at -18°C, prior to use. Using high-performance liquid chromatography, high content of ellagic acid (from 13,99 to 27,01 mg/100 g fw for Meeker; from 26,15 to 35,22 mg/100 g fw for Willamette) and cyanidin (from 20,75 to 29,97 mg/100 g fw for Meeker; from 32,97 to 64,68 mg/100 g fw for Willamette) were determined. Significant amounts of pelargonidin (up to 7,08 mg/100 g fw), gallic acid (up to 4,42 mg/100 g fw), quercetin (up to 1,56 mg/100 g fw), and apigenin (up to 0,42 mg/100 g fw) were also detected in all samples. Although the raspberries cv. Willamette contain higher concentration of detected phenolics than raspberries cv. Meeker, nevertheless antioxidative capacity is nearly equal for both cultivars examined.
**Key words:** *commercial cold storage, Arilje growing area, Meeker, Willamette, IQF, anthocyanins, phenolics, antioxidative capacity, HPLC-DAD, ellagic acid, cyanidin*

## INTRODUCTION

Red raspberries (*Rubus idaeus* L.) are soft, juicy fruits with a distinct aroma. It is an economically important berry crop that contains numerous bioactive compounds and natural antioxidants with a high free radical scavenging capacity. Berry fruits are extremely perishable and have a short market life. Therefore processing, in particular freezing, is necessary in order to provide raspberries to the market and consumers over the entire year.
In addition to vitamins and minerals, raspberries are also rich in anthocyanins, phenolic acids, and other flavonoids. Anthocyanins are the major contributors to the red colour pigment in berry fruits and are also used by consumers to judge the quality of a fruit. Mullen et al. (2002) have identified as many as 11 anthocyanins in raspberries. Flavonols (kaempferol, quercetin and myricetin) and phenolic acids (*p*-coumaric, gallic, ferulic and ellagic acids) have been also detected in berry fruits, with proposed beneficial effects on human health (Hertog et al., 1992; Horbowicz et al., 2008; Bobinaitė et al., 2012).
According to the International Raspberry Organization, the world's raspberry production in the period 2000-2010. is between 450.000 and 500.000 t (including Russia). In Serbia, the raspberries are being produced on 13.500 ha, and production is varying between 60.000 and 94.366 t. Arilje growing area is the main production center, with one quarter of the total raspberry production in Serbia (Leposavić et al., 2004).
The object of our study was to determine the chemical composition and antioxidative capacity in a commercial cold-storage frozen fruits of the most abundant raspberry cultivars (Meeker (5%) and Willamette (95%)) in Arilje growing area of Western Serbia (Petrović and Leposavić, 2011).

## MATERIAL AND METHODS

### Collecting samples

All samples for freezing were harvested in the full ripening stage in the orchards approximately 1 km away from the commercial cold storage, frozen the same day using the conventional freezing (classic) tunnel, and stored at -18°C (Petrović and Leposavić, 2011). Individually quick frozen (IQF) raspberry commercial packs of 2.5 kg were randomly taken from the cold storage from Arilje growing area of Western Serbia, as follows: three packs of cv. Meeker with harvesting dates: (*i*) 20.06.2010., (*ii*) 09.07.2010., (*iii*) 12.07.2010., and five packs of cv. Willamette with harvesting dates: (*i*) 24.06.2010., (*ii*) 14.07.2010., (*iii*) 17.07.2010., (*iv*) 19.07.2010., (*v*), 22.07.2010. Above-mentioned disproportion in number of packs is due to the different abundance of analyzed cultivars in Arilje growing area. Upon receipt at the Department for Fruit Processing Technology, the raspberries were stored at -18 °C until the analysis.

### Soluble solid content (SSC), dry matter content (DM), sugar content, and titratable acidity (TA)

The SSC of the fruit was determined on a manual refractometer (3828, Carl Zeiss, Germany). The dry matter content was determined by drying at 105 ºC until constant mass. Titratable acidity (TA) was determined by neutralization of fruit extract with 0.1 N NaOH to pH 8.2, using phenolphthalein as indicator. Acidity was expressed as mg malic acid/100 g fresh weight. Sucrose, inverted sugars, and total sugars content were determined by Luff-Schoorl method (Tanner and Brunner, 1979).

### Determination of anthocyanin content

The monomeric anthocyanin pigment content of the aqueous extracts was determined using the pH-differential method described previously (Torre and Barritt, 1977; Prior et al., 1998; Liu et al., 2002). Pigment content was calculated as micrograms of cyanidin-3-glucoside equivalents/100 g fresh weight (mg cyn-3-glu/100 g fw), using an extinction coefficient of 26.900 L/cm/mol and molecular weight of 449,2 g/mol.

### Determination of flavonoid contents and total phenolics

Total flavonoid content was determined by a colorimetric method described previously (Zhishen et al., 1999; Liu et al., 2002). The results are expressed as micrograms of catechin equivalents/100 g fresh weight (mg CE/100 g fw).

The total phenolic content was determined using a modified Folin-Ciocalteu colorimetric method (Singleton et al., 1999; Liu et al., 2002), with results expressed as micrograms of gallic acid equivalents/100 g fresh weight (mg GAE/100 g fw).

### Antioxidant activity

Antioxidant properties were determined by the ABTS and DPPH assays. $ABTS^{\bullet+}$ radical cation scavenging activity was determined according to the method described by Re et al. (1999). Antioxidant activity was determined using the DPPH method reported by Brand-Williams et al. with modifications (Sanchez-Moreno et al., 1998). Results were expressed as Trolox equivalent antioxidant capacity (mM TE/100 g fw).

### Extraction and HPLC-DAD analysis

Samples were prepared according to the method of Hertog et al. (1992). Samples were analyzed using an Agilent 1260 series HPLC (Agilent Technologies, Santa Clara, CA, USA) linked to a ChemStation data handling system, using a ZORBAX Eclipse Plus C18 column (4.6 x 150 mm, 3.5 µm particles). Injection volume was 5 µL and the temperature was set at 30 ºC. Solvent A was 1% formic acid and solvent B was acetonitrile. The gradient used was as follows: 0–10 min, 10% of B in A; 10–25 min, 15–50% of B in A; 25–30 min, 50-80% of B in A; 30–32 min, 10% of B in A. By using this gradient (flow rate 0.5 ml/min), a good purity and separation was achieved in raspberry samples. The HPLC equipment was used with a

diode array detector (DAD). Ellagic acid (EA) and gallic acid (GA) were detected at 260 and 280 nm, respectively; apigenin and quercetin were detected at 329 and 360 nm, respectively; anthocyanins (cyanidin (CYA) and pelargonidin (PEL)) were detected at 520 nm. Phenolic compounds were identified according to peak retention time and UV/Vis spectra by comparing them with those of the standards. The quantities of the different phenolic compounds were based on peak areas, and expressed as mg/100 g fw.

### Statistical analysis

For all the experiments, three samples were analyzed and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) to examine differences among the cultivars, using Statistica 7 (StatSoft, Inc., Tulsa, OK, USA). The pairwise comparisons between different parameters were performed out using Duncan's test ($p < 0.05$).

## RESULTS AND DISCUSSION

### Raspberry samples

Raspberries cvs. Meeker and Willamette were analyzed after four months of storage at -18°C. Given that the raspberry fruits ripen successively, the harvest is conducted on several occasions. The raspberry harvest season for the cvs. Meeker and Willamette lasts from 21 to 40 days, depending on the way of growing and agro-ecological condition of the raspberry growing area (Petrović and Leposavić, 2011). Taking this into account, randomly taken IQF raspberry packs of cvs. Meeker (from 20.06.2010. till 12.07.2010.) and Willamette (from 24.06.2010. till 22.07.2010.) from the commercial cold storage completely cover the entire harvesting periods.

Every single IQF raspberry pack was divided in three subsamples, in order to provide three replicate experiments. All results in this study are presented as mean value of all cultivar subsamples ± standard deviation (9 and 15 subsamples for Meeker and Willamette, respectively).

### Chemical properties

Chemical properties of berry fruits contribute to fruit flavour. High sugars and high acids are required for good berry flavour.

Table 1. Chemical properties of frozen raspberries from commercial cold storage in Arilje growing area

| | ANOVA | Meeker | Willamette |
|---|---|---|---|
| dry matter content - DM (%) | ns | 14,17 ± 1,04 a | 14,47 ± 0,95 a |
| soluble solid content - SSC (%) | ns | 9,09 ± 1,26 a | 9,95 ± 1,37 a |
| total sugars (%) | ns | 5,54 ± 1,07 a | 5,83 ± 0,90 a |
| inverted sugars (%) | ns | 4,72 ± 0,96 a | 4,92 ± 0,85 a |
| sucrose (%) | ns | 0,79 ± 0,11 a | 0,86 ± 0,09 a |
| titratable acidity (%) | *** | 1,73 ± 0,21 b | 2,15 ± 0,13 a |
| pH | ** | 2,86 ± 0,06 a | 2,78 ± 0,06 b |
| sugar/acid ratio | ns | 3,30 ± 0,97 a | 2,71 ± 0,41 a |

*Values with a different letters denote statistically significant differences (Duncan's test, $p < 0.05$).*
*ns,*,**,***: non significant or significant at $p < 0.05, 0.01, 0.001$, respectively.*

For instance, high acid and low sugar level result in a tart berry, while high sugar and low acid level result in a bland taste. Moreover, low levels of acid and sugar result in tasteless berries (Wang et al., 2009). Chemical properties of Meeker and Willamette commercially available frozen samples are presented in Table 1. As can be noticed, there are no statistically significant differences between the cultivars examined. As for the titratable

acidity, cv. Willamette has a higher total acids content compared to cv. Meeker, that is already reported (Finn and Lawrence, 2001).
In comparison, the results of chemical properties in our study are in excellent agreement with reports by other researchers (Finn and Lawrence, 2001; Stanisavljević et al., 2002; Kafkas et al., 2008).

**Contents of bioactive compounds and antioxidative capacity**
The total anthocyanins, total flavonoids, and total phenolics were measured for all the samples. The results are given in Table 2.
The total anthocyanin and total flavonoid contents showed great variation in different raspberry cultivars (Willamette had higher anthocyanin and flavonoid contents, followed by Meeker). On the other hand, regarding the total phenolic content there is no statistically significant difference between the cultivars examined. These results are in great agreement with results by other researchers (Anttonen and Karjalainen, 2005; Wang et al., 2009; Sariburun et al., 2010). Anttonen and Karjalainen (2005) reported that the total anthocyanin and total phenolic contents ranged from 0 to 51 mg/100 g fw and from 192 to 359 mg/100 g fw, respectively, for seventeen raspberry cultivars grown at two different farms in Finland. Bobinaitė et al. (2012) found in raspberry cv. Meeker that the total anthocyanins and total phenolics were 44,3 and 388,8 mg/100 g fw, respectively. However, it is known that the contents of bioactive compounds in fruits and vegetables depend on various factors, such as genotypic differences, pre-harvest climactic conditions and post-harvest handling procedures.

Table 2. Total anthocyanin, total flavonoid and total phenolic contents (mg/100 g fw), and free radical scavenging parameters (mM TE/100 g fw) of frozen raspberries from commercial cold storage in Arilje growing area

| | ANOVA | Meeker | Willamette |
|---|---|---|---|
| total anthocyanins | *** | 44,04 ± 4,05 b | 87,04 ± 10,09 a |
| total flavonoids | *** | 43,17 ± 4,82 b | 69,41 ± 9,78 a |
| total phenolics | ns | 358,77 ± 39,81 a | 346,99 ± 42,37 a |
| ABTS | ns | 278,71 ± 18,96 a | 255,93 ± 37,08 a |
| DPPH | ns | 27,15 ± 1,83 a | 27,34 ± 1,54 a |

*Values with a different letters denote statistically significant differences (Duncan's test, $p < 0.05$). ns,*,**,***: non significant or significant at $p < 0.05$, 0.01, 0.001, respectively.*

The results for the antioxidant capacity (ABTS and DPPH assays) are also given in Table 2. As can be noticed, no statistically significant differences were found amongst these two raspberry cultivars. Since there are a large number of different types of antioxidant compounds that might contribute to the total antioxidant capacity, it is not clear which components are responsible for the observed antioxidative capacity. The antioxidant capacity of raspberry fruits appears to be largely influenced by the polyphenolics, rather than anthocyanins and flavonoids. These data are in agreement with other reports in the literature (Sariburun et al., 2010; Novaković et al., 2011).
Analyzing HPLC-DAD chromatograms of aqueous methanol extracts of frozen raspberries cvs. Meeker and Willamette, six peaks were clearly identified. Some of the peaks are presented in Figure 1. Identified compounds were quantified and summarized in Table 3.

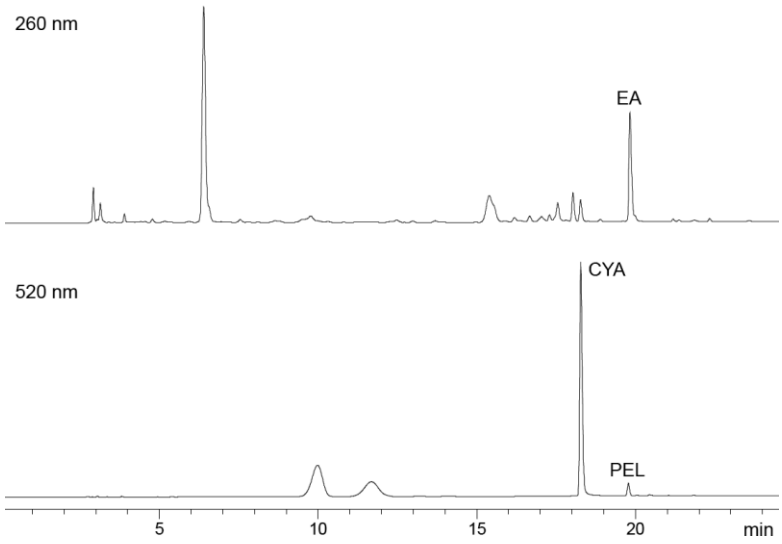


Figure 1. HPLC chromatogram for the quantification of ellagic acid (EA) monitored at 260 nm (top), and cyanidin (CYA) and pelargonidin (PEL) monitored at 520 nm (bottom)

It was found that the amount of quercetin in Meeker is five times lower than that in Willamette. Surprisingly, certain amount of apigenin is found in both cultivars. Kaempferol and myricetin were not detected in any of the samples. Relatively high content of free ellagic acid was found (19,59 and 28,90 mg/100 g fw for Meeker and Willamette, respectively). These results are comparable with the findings previously reported (Anttonen and Karjalainen, 2005; Bobinaitė et al., 2012). The main anthocyanin found in raspberry is cyanidin (23,99 and 53,42 mg/100 g fw for Meeker and Willamette, respectively), followed by pelargonidin. Comparable results are reported in the literature (Horbowicz et al., 2008; Wang et al., 2009).

Table 3. Contents of main phenolics (mg/100 g fw) in frozen raspberries from commercial cold storage in Arilje growing area

| | ANOVA | Meeker | Willamette |
|---|---|---|---|
| apigenin | *** | 0,23 ± 0,06 b | 0,36 ± 0,05 a |
| quercetin | *** | 0,18 ± 0,03 b | 0,96 ± 0,37 a |
| gallic acid | *** | 2,73 ± 0,21 b | 3,68 ± 0,55 a |
| ellagic acid | *** | 19,59 ± 5,07 b | 28,90 ± 2,77 a |
| cyanidin | *** | 23,99 ± 4,10 b | 53,42 ± 11,32 a |
| pelargonidin | *** | 2,47 ± 0,60 b | 5,16 ± 1,42 a |

*Values with a different letters denote statistically significant differences (Duncan's test, $p < 0.05$). ns,*,**,***: non significant or significant at $p < 0.05, 0.01, 0.001$, respectively.*

## CONCLUSIONS

In conclusion, commercially available frozen raspberries cvs. Meeker and Willamette were randomly taken from the commercial cold storage in Arilje growing area of Western Serbia, and analyzed by high performance liquid chromatography. Numerous bioactive compounds, such as apigenin, quercetin, ellagic acid, gallic acid, cyanidin, and pelargonidin were identified and quantified, in both cultivars examined. Furthermore, free radical scavenging capacity assays were performed and it was found that antioxidative capacity is nearly equal for both cultivars examined, although the raspberries cvs. Meeker and Willamette contain different concentrations of detected phenolics.

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