Phytochemical and Antioxidant Properties of Fresh Fruits and Some Traditional Products of Wild Grown Raspberry (*Rubus idaeus L.*)

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Abstract

The current study investigated and compared phytochemical and antioxidant activity of fresh fruit and some traditional products of *Rubus idaeus* grown in mountain region of Serbia. The total organic acid, total sugar content, total phenolics, flavonoids, tannins, anthocyanins and vitamin C were evaluated. The antioxidant activities were evaluated using two antioxidant systems 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The fresh fruit contained highest amount of vitamin C (46.62 mg AA g⁻¹) and total organic acids (882.22 mg CA g⁻¹). The sweet preserve had highest content of total phenolics (200.83 mg GA g⁻¹), flavonoids (12.85 mg RU g⁻¹) and tannins (39.11 mg g⁻¹). The juice had the highest total anthocyanin content (107.22 µg mL⁻¹) and total sugar content (25 °Brix). The best antioxidant activity in ABTS assay had juice (IC₅₀ = 4.87 µmol TE g⁻¹), followed by sweet preserve (IC₅₀ = 5.14 µmol TE g⁻¹), almost identical to standard gallic acid. In the DPPH free radical scavenging assay, sweet fruit preserve showed significant better antioxidant activity (IC₅₀ = 41.27 µg mL⁻¹) compared to juice (IC₅₀ = 106.07 µg mL⁻¹) and fresh fruit (IC₅₀ = 294.79 µg mL⁻¹). Our results indicated promising perspectives for usage of *R. idaeus* fresh fruits and traditional products studied with considerable levels of vitamin C, bioactive compounds and antioxidant activity.

Keywords: antioxidant activity; fruit; juice; phytochemical; sweet preserve

Introduction

The *Rubus idaeus* L. (red raspberry) together with about 750 species of the *Rubus* genus belongs to Rosaceae family (Alice and Campbell, 1999). The wild raspberry is a perennial shrub with a height of between 100 cm and 150 cm. The stem is erect, cylindrical, and greyish, with a number of small thorns on the surface. The leaves are pinnate of 5-7 leaflets or sometimes 3, glabrous on the surface and very hairy on the abaxial side. The terminal leaflet is oblong or ovate and shallowly lobed, whereas stipules are fibrous or hairy. The cyme inflorescences are made of flowers that are usually lying down, composed of narrow white, glabrous and whitish petals. The fruit is pale pink or light orange (Tatić, 1972). In Serbia, the wild raspberry can usually be found on slopes, fires, spawns and spurs of beech and other forests, near streams and rivers, at an altitude of 600 to 1200 meters. Raspberry plant requires a lot of light and moisture.

The fruits have been used in traditional and alternative medicine for a long time to cure wounds, colic, diarrhea, and renal illnesses (Zhang et al., 2011). In addition, the red raspberry is an economically important berry crop that contains many phenolic compounds with potential health benefits. Raspberry can be used in fresh or frozen as well as for processing: juice, syrup, wine, natural liqueur, compote, sweet, jam, ice cream, candied fruit, raspberry powder and pulp (Pritts, 2003). The fruits are sweet and sour, very tasty, aromatic and easily digestible. Raspberry is a “honey plant” which contains 77.4 - 90.9% of water, 9.1 - 22.6% of total dry matter, 8.0 - 13.0% soluble matter. Total sugars have 3.4 - 6.9%, of which glucose is 1.1 - 3.3%, fructose is 1.3 - 3.4%

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During last two decades, the interest in consuming fresh fruits has been intensified, due to its content of bioactive nutrients and their importance as food antioxidants. The phytochemicals such as flavonoids and phenolic acids are the most common phenolic compounds with antioxidant activity and may help protect cells against the oxidative damage caused by free radicals (Wada and Ou, 2002). In animal studies involving breast, cervical, colon, esophageal, and prostate cancers, raspberry phytochemicals have been shown to play an important role in lowering oxidative stress, reducing inflammation, and thereby altering the development or reproduction of cancer cells. Among flavonoids, quercetin, kaempferol and myricetin as well as their derivatives (primarily glycosides), may provide health benefits as dietary antioxidants (Siriwoharn et al., 2004). Phenolic acids constitute about one-third of the dietary phenols (Zadernowski et al., 2005), and raspberry is considered to be among the fruits with considerable amount of ellagic acid (Koponen et al., 2007). Most notably, the anthocyanins cyanidin-3-sophoroside, cyanidin-3-(2G)-glucosylrutinoside and cyaniding-3-glucoside, the two ellagitannins sanguin H-6 and lambertianin C are present together with trace levels of flavonols, ellagic acid and hydroxycinnamate (Mullen et al., 2002).

Although literature provides a lot of data about phytochemical composition and the antioxidant activity of raspberries from different cultivation sites, detailed information about wild R. idaeus grown in Serbia and other countries is still missing. Hence, the current study was designed with aim to investigate the phytochemical and antioxidative potential of fresh fruits and traditional products such as juice and sweet preserve. The obtained results can be useful in clarifying the quality of fruits and traditional products in order to their promotion and application as food additive and nutraceutical.

Materials and Methods

Plant material

Plant material (Rubus idaeus L.) was collected from mountain region of 'Golića', Serbia (43.19140735N, 20.25105463E, and 1432.0 m). Plant sample has been deposited at Department of Applied Botany at Faculty of Agriculture, Belgrade and were checked by Prof. Dajić Stevanović.

Fruits of wild raspberry were collected in middle July-August, 2016 at full maturity stage suitable for human diet and preparation of selected nutritional products. Sampling involved 30 individuals from study site. After harvesting, 10 kg of fresh fruits were stored at -20 °C for maximum of one week prior to conducting chemical analysis.

The quantification of total phenolics, flavonoids, tannins, anthocyanins, free organic acids, sugar content, and L-ascorbic acid as well as antioxidant capacity were measured by ABTS$$^+$$ and DPPH$$^+$$ methods in order to compare the quality of fresh fruits and selected traditional products.

Preparation of fresh fruit extract and traditional products

The fruits (10 g) were mixed with 20 mL 80% methanol and homogenized in blender. The obtained mixture was transferred into Erlenmeyer bottle and stored at room temperature for 24 hours in dark. After that, the extract was filtered through a filter paper (Whatman No. 1) and residues were re-extracted by same solvent for tree times and obtained fractions were collected. The portions of the sample were transferred in vials and used for phytochemical analyses and determination of antioxidant capacity.

The selected products, traditional Balkan fruit sweet preserve ("slatko" in Serbian) and juice were prepared according to following procedure. For preparation fruit sweet preserve 1 kg of wild raspberry fruit was mixed with 1 kg of sugar and 200 mL of water. The mixture was cooked on the temperature of 80-90 °C for 20 min. When the cooking was finished, fruit preserve was covered with a wet cloth and was left overnight to cool down before it was put into the glass jars. Traditional juice (syrup) was prepared by crushing of the fresh fruits (1 kg), transferred into Erlenmeyer bottle (1 L) and left overnight. Day after the prepared mixture was filtered through the double gauze and then 1 kg L$$^{-1}$$ of sugar was added. The product was stirred occasionally during next 2-3 days until the sugar was completely dissolved and then poured into the glass bottles. The samples of juice and sweet preserve used for phytochemical analyses and antioxidant activity were prepared according to procedure described for fresh fruits.

Determination of physico-chemical parameters

Determination of free organic acids and total sugar contents

Concentration of free organic acids was determined by volumetric method (Horwitz, 1975). To 10 mL (g) of extract (fresh fruits, juice and "slatko") was added 50 mL of ethanol (70%) and reaction mixture was incubated at 70 °C in water bath for 1 h. The mixture was filtered through Whatman filter paper No. 1 and filtrate was concentrated at 50-60 °C under reduced pressure to the final extract volume of 40 mL. Active charcoal was added to extract following by incubation (30 to 45 min) in the water bath at 70 °C. After incubation, the extract was filtrated to remove active charcoal; the residue was made up to a volume of 100 mL with distilled water. Ten milliliters aliquots of filtrate were sampling for determination of concentration the free organic acids by titration with 0.1 M NaOH. Phenolphthalein (0.1%) was used as indicator. The results were presented as mg citric acid 100 g$$^{-1}$$ fw.

Total sugar content was determined by the refractometric method, using an Abbe refractometer (Model RMT, Optech, Italy) (Bartolomé et al., 1995). A refractometer measures TSS as 'Brix (percent sucrose by weight), in 0.1% graduations.

Determination of phenolic and L-Ascorbic acid contents

Total phenolic content

The total phenolic content in the extracts was determined according to the Folin-Ciocalteu method by Wootton-Beard et al. (2011) with slight modifications. The reaction mixture was prepared by mixing 0.5 mL of methanolic solution of the extract (1 mg mL$$^{-1}$$), 2.5 mL of formic acid and 2.5 mL of 2.5% Folin-Ciocalteu reagent and then 0.5 mL of 25 g L$$^{-1}$$ Na$_2$CO$_3$ was added. The reaction mixture was incubated at 70 °C for 30 min. After incubation, the mixture was cooled down to room temperature and absorbance was measured at 765 nm. The total phenolic content in the extracts was then calculated using calibration curve of gallic acid.

Preparation of traditional Balkan fruit sweet preserve

The fruit preserve was prepared according to following procedure. First, fruits were mixed with 1 kg sugar and 200 mL water. The mixture was cooked on the temperature of 80-90 °C for 20 min. When the cooking was finished, fruit preserve was covered with a wet cloth and was left overnight to cool down before it was put into the glass jars. The traditional sauce was prepared by crushing of the fresh fruits (1 kg), transferred into Erlenmeyer bottle (1 L) and left overnight. Day after the prepared mixture was filtered through the double gauze and then 1 kg L$$^{-1}$$ of sugar was added. The product was stirred occasionally during next 2-3 days until the sugar was completely dissolved and then poured into the glass bottles. The samples of juice and sweet preserve used for phytochemical analyses and antioxidant activity were prepared according to procedure described for fresh fruits.
10% water-soluble Folin-Ciocalteu reagent and 2.5 mL 7.5% NaHCO₃. The samples were then incubated at 45 °C for 15 min. Blank was prepared in the same way, only methanol was added instead of the extract. The absorbance of the samples and the blank was measured on the spectrophotometer at λmax = 765 nm (Jenway 6105; Bibby Scientific Limited, Staffordshire, UK). The same procedure was repeated for gallic acid (GA) to calculate the equivalent concentration of total phenols (mg of GA g⁻¹ fw).

Total flavonoid content

The aluminum chloride method was used for the determination of the total flavonoids content of the extracts (Brighente et al., 2007). The samples were prepared by mixing 1 mL of the methanolic solution of the extract (1 mg mL⁻¹) and 1 mL of 2% AlCl₃ dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was measured spectrophotometrically at λmax = 415 nm. The same procedure was repeated for rutin (RU) to calculate the equivalent concentration of flavonoids (mg of RU g⁻¹ fw).

Total tannins content

The total amount of tannins was determined by spectrometry measurement (Hosu et al., 2014). The samples were prepared by mixing 2 mL of the extract, 3 mL of concentrated HCl and 1 mL of distilled water. The content of the first sample was incubated for 30 min at 100 °C whereas 0.5 mL of ethanol was added to the second sample. The absorbance of the samples was determined spectrophotometrically at λmax = 470, 520 and 570 nm. The differences (ΔA) between the samples values obtained were determined at the same wavelengths (ΔA470, ΔA520, ΔA570). The values for wavelengths ΔA470, ΔA520 and ΔA570 were calculated as follows: TTC (g L⁻¹) = 15.7 × minimum (ΔA520).

Anthocyanins content

Samples containing 0.5 mL of extract, 0.5 mL of 0.1% ethanolic - HCl solution and 10 mL of 2% aqueous - HCl solution were used to determine the total anthocyanins content (Hosu et al., 2014). The procedure was as follows: 4.4 mL of distilled water was added to the first sample, while 4.4 mL of 13% sodium bisulphate was added to the second sample and diluted at a ratio of 1:1. The absorbance of the samples was determined at a wavelength of λmax = 520 nm using a starting solution made up of 4.9 mL distilled water, 0.5 mL of 0.1% ethanolic-HCl solution and 10 mL of 2% aqueous - HCl solution. The values obtained (ΔA) were multiplied by the coefficient 875. The total amount of anthocyanins in a sample is expressed in μg mL⁻¹ of fw.

L-ascorbic acid content

Analysis of ascorbic acid was performed as described in protocol by Stevens et al. (2007). Fruits were ground in liquid nitrogen and conserved at -80 °C until analysis. Around 500 mg of grounded raspberry fruits were homogenized in 2 mL tubes with 600 μL of 6% trichloroacetic acid (TCA). During this process, it was paid attention that powder does not melt at any time of preparation. Afterwards, the tubes were shaken in grinder for one minute and then put on vortex for 20 s. The next step was centrifugation (15 min, 4 °C, 13,200 rpm). Supernatant was used for further analysis. For each sample two assays were carried out: one to measure total ascorbic acid (including the addition of DTT) and one to measure reducible ascorbic acid (omission of DTT from the assay). After addition of DTT (total ascorbic assay) and phosphate buffer (reduced ascorbate assay), the 96-micro-well plate was incubated at 37 °C for 20 min. In wells with DTT was added 10 μL of N-ethylmaleimide. In each well was added 80 μL of coloring reagent. The coloration agent consisted of a mixture of two solutions. Preparation A was composed of 18 mL ortho-phosphoric acid (85%), 31.5 mL ultrapure water, 2.3 g trichloroacetic acid and 0.3 g ferric chloride (FeCl₃). Preparation B was a dilution of 2.0 g 2,2-Bipyridyl in 50 mL of ethanol (70%). 6.6 mL of preparation A and 2.4 mL of preparation B were combined imminently before analysis due to instability of the mixture. 80 μL of the coloration agent was placed in each well to start the coloration reaction. The micro-plate was then placed in the micro-plate reader where it was agitated and kept for complete reaction during 50 min at 37 °C. Absorbance was measured at a wavelength of 525 nm. Commercial L-ascorbic acid was used to generate the standard curve.

In vitro antioxidant activity assays

DPPH free radical scavenging assay

For the estimation of anti-radical potential, DPPH free radical scavenging activity of all the extracts was conducted using DPPH method (Takao et al., 1994). Working solution of extracts was carried out by dilution stock solution (2 mg mL⁻¹) of extracts. DPPH was dissolved in methanol to obtain a concentration at 8 μg mL⁻¹. To 1 mL of DPPH solution, 1 mL of various concentrations of the extracts or the standard solution was added separately. The reaction mixtures were incubated at 37 °C for 30 min, following by absorbance measured at 517 nm using methanol as blank reference. The DPPH scavenging activity (% of extracts and standards AA, gallic acid (GA), butylated hydroxytoluene (BHT), α-tocopherol, quercetin was determined using the following equation (1):

\[ \text{%inhibition} = \left( \frac{\text{Ac} - \text{As}}{\text{Ac}} \right) \times 100 \]  

(1)

Where Ac was absorbance of control reaction and As the absorbance in presence of the sample.

2,2′-azino-di(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) decolorization assay

The ABTS⁺ radical cation decolorization assay is spectrophotometric method widely used for determination of the antioxidative activity of substances. The ABTS⁺ scavenging activity was measured according to procedure described in work of Jakovljević et al. (2016). In brief, the ABTS⁺ radical cation was first produced by reacting ABTS stock solution (7 mM) with potassium persulfate (2.45 mM). The mixture was then placed in the dark at room temperature for 12 to16 h before using. Under this condition, ABTS⁺ can be stable in this form for more than 2 days. The ABTS⁺ solution was diluted with double-distilled water to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Aliquots of 30 μL of the sample extracts different
concentrations (from 2 mg mL\(^{-1}\) to 3.91 μg mL\(^{-1}\)) were then added to 2.7 mL diluted ABTS\(^{+}\) solution, and mixture was incubated at room temperature for 30 min. Absorbance was determined spectrophotometrically at 734 nm. For the control, 1.0 mL of methanol was used instead of extract. AA, GA, chlorogenic acid (GCA), protocatechuic acid (PCA), and beta-resorcylic acid (BRA) were used as standards. The percentage of inhibition was calculated using the Eq. (1) and results are expressed as IC\(_{50}\) value.

**Statistical analysis**

Statistical analyses were performed with the software packages SPSS for Windows (version 17.0). All measurements were carried out in triplicate, and the results are presented as mean ± S.E.M. Statistical analysis was performed via Pearson’s correlation coefficient, as well as, ANOVA, followed by the Tukey HSD test which was used to test the differences in quality parameters (the content of organic acids, total sugar and ascorbic acid).

**Results and Discussion**

The total sugars and organic acids content have significant impact on fruit flavor and quality. Due to, the total organic acids (total acidity), total sugar and ascorbic acid content in fresh fruit, juice and sweet fruit preserve of *R. idaeus* from Serbia were examined (Table 1). As the Table shows, the fresh fruit sample had a considerable amount of TOA (882.22 mg CA g\(^{-1}\) fw). Previous findings on the amount of organic acid in the raspberry fruits are obtained by testing the different raspberry cultivars growing in different region, but only one study investigated the *R. idaeus* and two cultivars from Serbia. Milivojević et al. (2011) found significant lower amount of organic acids in fruits of *R. idaeus* and cultivars ‘Willamatte’ and ‘Meeker’ from Serbia (0.15 mg CA g\(^{-1}\) fw, 0.24 mg CA g\(^{-1}\) fw and 0.18 mg CA g\(^{-1}\) fw, respectively). Gulcin et al. (2011) revealed titratable acidity of domesticated and three wild ecotypes of raspberry fruits (from Coruk region, Turkey) in amount of 1.35% and 1.05-1.11%, respectively. Tamer (2012) found that the total acidity of four raspberry cultivars (from Bursa, Turkey) ranged 0.54-0.87 g CA 100 g\(^{-1}\) fw. Mazur et al. (2014) detected titratable acidity of several cultivars from Western Norway in interval 1.76-2.23 g 100 g\(^{-1}\) fw. The study of Zorenc et al. (2017) showed that two raspberry cultivars ‘Amira’ and ‘Polka’ (from Slovenia) had the TOA content of 20.2 and 19.9 mg g\(^{-1}\) fw. Comparing the current result with above-mentioned it can be concluded that tested *R. idaeus* fruits from Serbia is much better source of organic acids than mentioned raspberry cultivars. According to statement of Poyrazoglu et al. (2002) the differences in the organic acid content between raspberry fruits might have arisen from genetic factors, climatic factors, and/or cultural practices. In the case of traditional product prepared from fruits, such as juice and sweet fruit preserve, the current study showed that both of them had significantly lower amount of TOA (396.29 and 250.73 mg CA g\(^{-1}\) fw respectively) than fresh fruits. The obtained results could be explained by addition of sugar (sucrose) during their processing. This is opposite to results of Tamer (2012) who revealed an increase in total acidity content (about 2- to 3-fold) in samples of marmalade prepared from raspberry cultivars. The author was explained these results by addition of citric acid during marmalade preparation.

Beside organic acids, sugars also have a significant impact on the sensory quality and flavor of the fruit (Wang et al., 2009). The highest amount of total sugar was found in the juice (25.74 °Brix), followed by in fruit preserve (16.12 °Brix), and at least in fresh fruit sample (7.02 °Brix) (Table 1). The existence of a significantly higher TSC in traditional products than in fresh fruits could be explained by adding the sucrose during preparation of products. The study of Milivojević et al. (2011) revealed small amount of sucrose in *R. idaeus*, ‘Willamatte’ and ‘Meeker’ (from Serbia) (6.9 mg g\(^{-1}\) fw, 6.4 mg g\(^{-1}\) fw and 5.3 mg g\(^{-1}\) fw). According to the literature data (Mikulić-Petkovsek et al., 2012), the wild raspberry fruit grown in Slovenia had some higher TSC than tested fruit sample. In addition, the two cultivars (‘Amira’ and ‘Polka’) from Slovenia had higher TSC (34.3 mg g\(^{-1}\) fw and 31.9 mg g\(^{-1}\) fw) than current *R. idaeus* fruits. Gulcin et al. (2011) found total soluble sugars in amount of 15.56% in domesticated and 19.58-22.03% in three wild ecotypes of raspberry fruits (from Coruk region, Turkey). As in the case of organic acids, these differences could be explained by different environment conditions: light exposure, growing of plants on open, sunny and warmer habitats (Wang et al., 2009). Fruits from plant individuals, which grew in conditions of low light intensity, have lower sugar content.

The concentration of Vitamin C (AA) found in fresh fruit sample was 46.62 mg 100 g\(^{-1}\) (Table 1). Based on results found in literature (Mapson, 1970), this value is approximately 2-fold higher than the usual amount of AA found in raspberry fruits (25 mg 100 g\(^{-1}\) fw). The obtained result is similar to data recorded for wild raspberries fruit growing in Macedonia (Karakashova et al., 2012), as well as for cultivated varieties from the territory of Norway (17.4 mg AA 100 g\(^{-1}\) fw - 46.9 mg AA 100 g\(^{-1}\) fw) (Mazur et al., 2014). Pantelidis et al. (2007) reported that cultivated raspberry varieties are also a rich source of AA. Our results on *R. idaeus* fresh fruits are in line with previous studies showing that berries are an important source of vitamin C.

**Table 1. The content of organic acids, total sugar and ascorbic acid in Rubus idaeus samples**

| Samples            | AA (mg 100 g\(^{-1}\) fw) | TSC (°Brix) | TOA (mg CA g\(^{-1}\) fw) |
|--------------------|---------------------------|-------------|---------------------------|
| Fruits             | 46.62±2.25                | 7.02±0.04   | 882.22±24.15              |
| Juice    (syrup)   | 9.73±1.12                 | 25.7±1.68   | 396.29±16.11              |
| Sweet fruits preserve | 4.25±0.15            | 16.12±1.55  | 250.73±10.33              |

AA = ascorbic acid; TSC – total sugar content; TOA – total organic acids. Results are expressed as mean values ± SD from three independent experiments. Mean values in the same column with superscript with different letters are significantly different (p < 0.05) (ANOVA, followed by the Tukey HSD test).
However, it is important to note that the current result is significantly better compared to amount AA found in fresh raspberries (7.60 to 18.11 mg 100 g⁻¹) cultivated in Bursa (Turkey) (Tamer, 2012). Gulcin et al. (2011) found very low amount of AA in wild (2.4 mg kg⁻¹) and domesticated raspberry fruits (5.34 mg kg⁻¹). The concentration of AA in traditional products prepared from fresh fruits i.e. juice and sweet fruit preserve was ranged from 9.73 to 4.25 mg 100 g⁻¹ fw, respectively. The obtained values were significantly lower compared to the fresh fruit sample. This observation is in accordance with findings of other researchers. Tamer (2012) revealed that AA content in raspberry marmalade was lower than in fresh fruit of different raspberry cultivars. Depending from cultivars, its content was ranged 3.88 to 6.45 mg 100 g⁻¹. It is well known that AA is a thermolabile compound (Rauha et al., 2000), so in the juice, the AA concentration decreased 4.8-fold and in the sweet fruit preserve for 10.9-fold. The thermal degradation of vitamin C had strong temperature dependent. The best temperature for minimizing reduction of water-soluble vitamins is 70°C (Kadalak et al., 2017).

Many researchers revealed that berry fruits are great dietary sources of bioactive compounds (phenolic compounds such as phenolic acids, flavonoids-flavonols, anthocyanins, tannins, and ascorbic acid). These compounds may act as strong antioxidants and, thus, could help in the prevention of many diseases (Skrovankova et al., 2015). The current study showed that fresh fruit of R. idaeus growing in Serbia contained the TPC value of 362.30 mg GAE 100 g⁻¹fw (Table 2) which is in agreement with other studies. Literature revealed many results of TPC obtained with Folin-Ciocalteu reagent for raspberry fruits. Wang and Lin (2000) showed that TPC ranged from 208 to 268 mg GAE 100 g⁻¹ fw in red raspberries. Similar content of total phenolics was confirmed by Mazur et al. (2014) in fruits of several cultivars from Norway (183.1 - 287.7 mg GAE 100 g⁻¹ fw). Mikulíč-Petkovsek et al. (2012) revealed that wild grown raspberry (from Slovenia) contained 223.2 mg GA kg⁻¹ fw compared to cultivated raspberry which contained 107.6 mg GA kg⁻¹ fw. Pantelidis and co-workers (2007) reported that 50%-methanolic extracts of fruits of a few raspberry cultivars grown in Greece contained between 1052 and 2494 mg GAE 100 g⁻¹ dw. According to work of Bobinaite et al. (2012) the TPC values of methanolic extracts of raspberries from Lithuania (‘Pokus’ and ‘Bristol’) varied from 278.6 to 714.7 mg GAE 100 g⁻¹ fw. Chen and co-workers (2013) showed the TPC values of 0.1% (v/v) methanolic extract of raspberries (grown in northern China) were 215.54 to 619.35 mg 100 g⁻¹ fw. Kostecka-Gugala et al. (2015) revealed the TPC, expressed as chlorogenic acid equivalents, in red raspberry fruits (cv. ‘Sokolica’ and ‘Laszka’) in amount of 175.90 in seasons 2013, to 549.02 mg 100 g⁻¹ fw in season 2012. Sariburun et al. (2010) observed the TPC in water and methanolic extracts of several raspberry cultivars (from Turkey) in amount of 1040.9-1822.0 mg GAE 100 g⁻¹ fw, and 1787.3-2062.3 mg GAE 100 g⁻¹ fw, respectively.

With regard to TPC, the juice and sweet fruit preserve samples contained 108.30 and 200.83 mg GAE 100 g⁻¹ fw, respectively. These values are significantly higher compared to fresh fruits, which could be explained by processing operations. However, most of the literature data about antioxidants of fruits, vegetables, and grains have shown that food processing operations reduced the antioxidants of the processed foods. Rickman et al. (2007) and Serrano et al. (2011) found that some of the phenolic compound was decreased in thermal processing. Le Bourvellec et al. (2018) revealed that thermal processing could cause significant reduction in chemical composition of foods including phenolic compounds and antioxidant activities. Hassani et al. (2015) observed the mean decrease percentage of ellagic acid, total flavonoids, total polyphenols as well as antioxidant activity after jam processing. Van Boekel et al. (2010) reported that cooking of vegetables causes the breakage of cell wall component and subsequent release of molecules and cause the leaching of water-soluble polyphenol into the surrounding water or may destroy polyphenols by high temperature. Nevertheless, positive effect of thermal processing/cooking on amount of TPC is also reported in literature. Processing and heating during jam making (at 104-105 °C) reduces the TPC of some varieties of cherries and plums, whereas no significant change occurred in raspberries, plums, and some varieties of cherries (Kim and Padilla-Zakour, 2004). Sablani et al. (2010) reported that canning of raspberries (100 °C, 28 minutes) and blueberries (100°C, 22 minutes) increases the phenolic content and antioxidant activity by 50% and 53%, respectively.

The TFC measured in fruit, juice and sweet fruit preserve was 4.93, 9.25 and 12.85 mg RU 100 g⁻¹ fw, respectively (Table 2). Similar to TPC, the TFC was 2-fold and 3-fold higher in juice and sweet fruit preserve compared to fresh fruit. The obtained result for fresh fruit is in good agreement with previous findings. Gulcin et al. (2011) showed the TFC in domesticated and wild raspberry fruits (Coruk region, Turkey) were 2.62 and 1.77-6.09 QE, respectively. The study of Sariburun et al. (2010) revealed that water and methanolic extracts of five raspberry cultivars (from Turkey) possessed TFC in amount of 15.4 - 41.3 mg CTE 100 g⁻¹ fw, which is much higher compared to tested R. idaeus fruits from Serbia.

The TAC determined in fruits, juice and sweet fruits preserve samples was 4.73, 107.22 and 106.67 mg mL⁻¹ respectively (Table 2).

### Table 2. The content of phytochemicals in R. idaeus samples

| Sample              | TPC (mg GA) | TFC (mg RU) | TAC (mg TA/mL) |
|---------------------|-------------|-------------|-----------------|
| Fruit               | 36.23 ± 0.43 | 4.93 ± 0.27 | 4.73 ± 0.35     |
| Juice (syrup)       | 108.83 ± 0.67 | 9.25 ± 0.12 | 107.22 ± 1.77   |
| Sweet fruit preserve| 200.83 ± 2.64 | 12.85 ± 0.22 | 106.67 ± 1.42   |

**TPC** - total phenolic content; **TFC** - total flavonoid content; **TAC** - total anthocyanin. Results are expressed as mean values ± SD from three independent experiments. Mean values in the same column with superscript with different letters are significantly different (p<0.05) (ANOVA, followed by the Tukey HSD test).
According to Kostecka-Gugal et al. (2015), the TAC among red raspberries ranged from 29.69 (’Sokolica’, season 2013) to 81.13 mg (’Willamette’, season 2012). The TAC in red raspberries, expressed also as cyanidin 3-glucoside equivalents per 100 g fw, varied from 45.4 to 99.5 mg (Wang and Lin, 2000) and from 35.1 to 49.1 mg (Pantelidis et al., 2007). Koponen and co-workers (2007) concluded that TAC was typically below 100 mg 100 g⁻¹ fw in red raspberries, regardless the method or cultivar. The EtOH-water extracts from varieties of R. idaeus (’Ljulin’, ’Veten’) was studied by Kureza-Baranowska et al. (2014) and it was found TAC of 1328.2 and 889.1 mg 100 g⁻¹ dw. The fruits of R. idaeus ‘Ljulin’ contained cyanidin 3-O-sophoroside as the main anthocyanin, while in the fruits of the ‘Veten’ variety, cyanidin 3-O-glucoside, cyanidin 3-O-rutinoside and cyanidin 3-O-sophoroside were the dominating cyanidin glycosides (Kureza-Baranowska et al., 2014). The study of Sariburun et al. (2010) showed that water and methanolic extracts of raspberry cultivars from Turkey contained between 22.4 - 69.6 mg cyan-3-glu 100 g⁻¹ fw and 16.3 - 34.8 mg cyan-3-glu 100 g⁻¹ fw, depending from solvent type.

The results of the current study found TTC in all tested samples, in following order: fruit preserve (39.11 mg mL⁻¹), juice (36.71 mg mL⁻¹) and fresh fruit (0.40 mg mL⁻¹) (Table 2). From obtained results, it is clear that fresh fruit sample contained about 100-fold lower amount of tannins compared to raspberry preserve and juice. Obtained results of Hussein et al. (2017) showed that R. idaeus fruit (from market of Sulaimanya city, North of Iraq) contain 853 µg g⁻¹ of tannic acid, which is lower compared to current fresh fruit. Gaspertoti et al. (2010) described two main raspberry ellagittannins: sanguin H-6 and lambertianin C. It was found that the content of these compounds in fresh raspberry fruit, depending on the cultivar, ranges from 360 to 750 mg kg⁻¹ for sanguin H-6 and from 280 to 630 mg kg⁻¹ for lambertianin C. The higher amount of TTC found in the juice and sweet preserve in relation to fresh fruit is in line with literature data. The increase in amount of free ellagic acid and stability of ellagic acid glicosides during processing and storage in raspberry jams has been reported by Amakura et al. (2001). In addition, Zafrilla et al. (2001) revealed an increase in amount of ellagic acid (about 2.5-fold) during raspberries jam processing. Ellagic acid monomers are probably better absorbed than high molecular weight ellagittannins, and therefore jam processing could increase ellagic acid bioavailability.

**Antioxidative potential of raspberry**

Measurement of antioxidant activity using in vitro assays is paramount in the evaluation of various food products and nutraceuticals for determining antioxidant benefits. The most popular and frequently used spectrophotometer methods for determining the antioxidant capacity in foods and chemical compounds are ABTS or DPPH methods (Kusoski et al., 2005). Therefore, these two oxidant system were selected for the current study.

The DPPH antioxidant activity of fresh fruit, juice and sweet fruit preserve as well as some standards is presented in Fig. 1. In the DPPH free radical scavenging assay, sweet fruit preserve showed significant better antioxidant activity with IC₅₀ = 41.27 µg mL⁻¹, compared to juice (IC₅₀ = 106.07 µg mL⁻¹) and fresh fruit samples (IC₅₀ = 294.79 µg mL⁻¹). However, all samples had significant lower antioxidant activity in relation to tested standards. Tamer (2012) found that DPPH activity of fresh fruit extract (0.3 mg mL⁻¹) ranged from 24.50% to 38.63%. Sariburun et al. (2010) reported DPPH antioxidant activity of water and methanolic extracts of raspberry fruit cultivars (from Turkey) in range 64.14 - 89.13 µmol TE g⁻¹ fw and 81.17 - 127.59 µmol TE g⁻¹ fw, respectively. Venskuonis et al. (2007) described the DPPH antioxidant activity of ethanolic extracts of 31 raspberry plants (from natural habitats of Lithuania) in range 52.9 - 92.6%. Bobinaite and co-workers (2012) found radical scavenging capacity of 57.9% in methanolic extract of raspberry fruit ‘Meeker’ (from Lithuania) Kostecka-Gugal et al. (2015) investigated the antioxidant activity of fresh fruit extract (0.3 mg mL⁻¹) from several Polish cultivars of floricane and primocane-fruited red raspberry and found significant differences in DPPH antioxidant activity: 30.0-66.28% (in season 2012), and 28.05-56.89% (in 2013 vegetation period). The better antioxidant activity of sweet preserve and juice than fresh fruit is opposite to results found in literature. Veda et al. (2006) reported that heating and oxidation caused a decrease in the antioxidant activity. For example, antioxidant activity of marmalade significantly decreased and was in interval 1.79% to 12.11%.

The ABTS antioxidant activity of fresh fruit, juice and sweet fruit preserve was in following order: IC₅₀ = 15.07 µmol TE g⁻¹ fw, IC₅₀ = 4.87 µmol TE g⁻¹ fw and IC₅₀ = 5.14 µmol TE g⁻¹ fw, respectively (Fig. 2). The best antioxidant activity was observed in juice followed by in sweet preserve and fresh fruit sample. In relation to standards, the activity of juice and sweet preserve was in line with GA (IC₅₀ = 4.33 µmol TE g⁻¹ fw), about 4- to 5-fold better than activity of

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**Fig. 1.** Antioxidant activity (IC₅₀ values) of R. idaeus and some natural and synthetic antioxidants in DPPH assay
GCA (IC₅₀ = 17.23 μmol TE g⁻¹ fw) and PCA (IC₅₀ = 20.66 μmol TE g⁻¹ fw) and more than 100-fold better than activity of BRA (IC₅₀ = 169.50 μmol TE g⁻¹ fw). In addition, the current results are much better than those existing in literature. Milivojević et al. (2011) found antioxidant activity in ABTS assay for R. idaeus (1.41 mg AA g⁻¹ fw), ‘Willamette’ (3.20 mg AA g⁻¹ fw) and ‘Meeker’ cultivar (1.32 mg AA g⁻¹ fw) from Serbia. Sariburun et al. (2010) found that ABTS activity of water and methanolic extracts of raspberry cultivars (Turkey) ranged from 64.36 – 83.66 μmol TE g⁻¹ fw to 72.92-117.07 μmol TE g⁻¹ fw, respectively. Venskutonis et al. (2007) reported the radical scavenging activity of ethanolic extract of 31 raspberry plants (from Lithuania) in interval 52.5-97.8%.

Çeĉik and Özen (2010) revealed antioxidant capacity of 8.9 to 21.5 μmol TE g⁻¹ fw for wild and cultivated red raspberries (Rubus idaeus L.) obtained at different altitude range.

Statistical analysis

Relationships among phytochemicals and antioxidant activity of fresh fruit sample and traditional products: juice and sweet preserve are presented in Table 3. The TPC was in a very strong positive correlation with TTC (r = 0.962**), TTC (r = 0.859**) and TAC (r = 0.827**) at p < 0.01 level, in a two-tailed Pearson correlation. Similarly, strong positive correlation between TFA and TTC (r = 0.886*) as well as between TFA and TAC (r = 0.861*) was found. However, the strongest correlation was found between TTC and TAC (r = 0.998*). The amount of Vitamin C was in a very strong negative correlation with all tested phytochemicals, in following order: TTC (r = -0.996*), TAC (r = -0.991*), TPC (r = -0.890*) and TFC (r = -0.899*). Antioxidant activity of samples studied in DPPH assay was in a very strong positive correlation with TOA (r = 0.999*), Vitamin C (r = 0.990*) and TTC (r = 0.943*) and in negative correlation with TTC (r = -0.980*), TAC (r = -0.967*), TFC (r = -0.943*) and TSC (r = -0.673*). In ABTS antioxidant assay, radical cation scavenging activity of samples was in positive correlation with TOA (r = 0.972*) and Vitamin C (r = 0.980*) and in negative correlation with TTC and TAC (r = -0.979*), TTC (r = -0.884*), TTC (r = -0.852*) and TSC (r = -0.792*).

Table 3. Linear correlation coefficients between phytochemicals and antioxidant capacities of R. idaeus

| TPC  | TTC | TSC | TAC | Vitamin C | TOA | TFC | TAC | DPPH | ABTS |
|------|-----|-----|-----|-----------|-----|-----|-----|------|------|
|      |     |     |     |           |     |     |     |      |      |
| TPC  | 1   | -   | -   | -         | -   | -   | -   | -    | -    |
| TTC  | 0.962* | 1  | -   | -         | -   | -   | -   | -    | -    |
| TSC  | 0.859* | 0.886 | 1  | -         | -   | -   | -   | -    | -    |
| TAC  | 0.827* | 0.861 | 0.998* | 1   | -   | -   | -   | -    | -    |
| Vitamin C | -0.890* | -0.899* | -0.966* | -0.991* | 1  | -   | -   | -    | -    |
| TOA  | -0.932* | -0.940 | -0.986 | 0.974* | 0.994* | 1   | -   | -    | -    |
| TFC  | 0.392* | 0.471 | 0.803* | 0.838* | -0.767* | -0.696* | 1   | -    | -    |
| DPPH | 0.943* | -0.947 | -0.980 | -0.967* | 0.990 | 0.999* | 0.673* | 1    | -    |
| ABTS | -0.852* | -0.884 | -0.979* | -0.979* | 0.980 | 0.972 | -0.792 | -0.966* | 1    |

**Total phenolic contents, *total flavonoid contents, †total tannins, ‡total anthocyanins, §total organic acids, ††total sugar content, ‡‡DPPH scavenger activity, ‡ABTS radical cation scavenger activity.

Pearson Correlation Sg. (2-tailed). $^*$ Correlation is significant at the 0.05 level. $^**$ Correlation is significant at the 0.01 level.

Conclusions

This is the first study focused on the comparison of phytochemical and antioxidative potential of fresh fruit and traditional products (juice and sweet preserve) of R. idaeus L. grown in mountain region ‘Golija’ (Serbia). The study confirmed a considerable level of bioactive compounds such as total phenols, flavonoids, tannins, anthocyanins and Vitamin C in studied samples. Statistical analyses showed significant differences among tested samples in content of bioactive compounds mentioned above, with exception of anthocyanine (in juice and sweet preserve). Moreover, the power antioxidant activity of R. idaeus L. samples was found, particularly in juice and sweet preserve. The current results indicate promising perspective for the exploitation of the
fresh fruits and traditional products of wild grown R. idaeus as food antioxidant diet and nutraceutical. Bearing in mind that the wild raspberry grows without addition of fertilizers, pesticides and other additives compared to cultivated raspberry it could be an excellent nutritional alternative.

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