CHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF BLUEBERRY FRUITS AND JAM

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Abstract

Blueberries have been hailed as excellent source of health-promoting and restoring bioactive compounds: vitamins, phenolic compounds, minerals and sugars. The blueberries could be eaten fresh and raw or processed into jams, syrups, smoothies, yoghurts, beverages or other products. The aim of the present study was to evaluate the content of phenolic compounds, antioxidant properties, water content, acidity, pH and sugar content of blueberry (Vaccinium corymbosum L.) fruits and jam. The antioxidant activities were evaluated using seven different antioxidant assays. It was observed that thermal processing of fruits led to statistical significant alterations for most of monitored parameters. The fresh fruits contains higher water content (86.903%) compared to jam (41.943%). Blueberry jam had higher content of total phenolics, total tannins and total flavonoids compared to fruits and manifested higher antioxidant capacity measured by ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)), FRAP (ferric reducing antioxidant power), total reduction capacity and total antioxidant activity assays. The values of pH, acidity, total anthocyanins and DPPH (2,2-diphenyl-1-picrylhydrazyl radical) assay showed not statistical significant alterations after thermal processing. Methanol extracts of fresh blueberry fruits and jam posses similar scavenging capacities for $O_2^{\cdot-}$ and $\cdot NO$ free radicals. Hence, the obtained results demonstrated that, besides fresh blueberry fruits, the blueberry jams also possess noticeable amount of phenolic bioactive compounds with considerable antioxidant capacity.

Keywords: *antioxidant activity, blueberry, phenolics, jam*

1. INTRODUCTION

During normal metabolic processes oxygen may be reduced to yield partially reduced reactive oxygen metabolites. Many of these reactive oxygen species (ROS) are free radicals and contain an odd number of electrons. ROS are able to initiate different chain reactions in cells, induce lipid peroxidation and oxidize other cellular components, such as DNA and proteins (Su et al., 2019). ROS from different sources may be involved in the etiology of diverse human diseases, such as stroke, coronary artery disease, cancer and rheumatoid arthritis (Prior, 2003). Antioxidants can counteract free radicals and neutralize oxidants. The general endogenous antioxidant system consists of enzymatic and non-enzymatic antioxidants (Su et al., 2019). The interest for natural antioxidants, especially from horticultural products, has increased in recent years. Clinical studies indicated that a higher level of natural antioxidants (vitamins C and E, carotenoids and different phenolic compounds) in everyday diet can protect against many diseases and disorders (Prior, 2003, Olas, 2018).

Berries and other red fruits are characterized by the high amount of antioxidant molecules: phenolic acids, tannins, carotenoids, vitamin A, C, E, folic acid and minerals such as calcium, selenium and zinc. These chemical compounds are a group of secondary metabolites that prevent the fruit from oxidation due to environmental factors, such as oxygen, light, air, and attacks of phytopatogens and herbivorous animals. Phenolic antioxidants interfere with the oxidation process as free radical terminators and sometimes also as metal chelators (Danev et al., 2013; Manganaris et al., 2013; Hidalgo and Almajano, 2017; Šapčanin et al., 2017).

Vaccinium species, including bilberries (*Vaccinium myrtillus* L.) and blueberries (*Vaccinium corymbosum* L.), have been shown to have high phenolic content and strong antioxidant potential

(Bunea et al., 2011, Kevers et al., 2014). Bilberries and blueberries have multiple biological and health-promoting effects, including anticarcinogenic, anti-inflammatory and antimicrobial activities (Johnson and Arjmandi, 2013, Salamon et al., 2011). According to the available literature, blueberries were estimated to be a richer source of different phenolic compounds in comparison to other some other berries such as strawberries (Huang et al., 2012, Kevers et al., 2014), raspberries (Kevers et al., 2014) and blackberries (Huang et al., 2012).

Blueberries are consumed as fresh fruits and also processed into various foods, such as jams, syrups and beverages (Olas, 2018). During the production of jam the fruits undergo to a long thermal heating. During processing degradation and polymerization of phenolic compounds usually occurs and lead to loss of nutritional and health-promoting values and decolourisation. Besides their nutritional properties, the colour of blueberry products is an important factor influencing consumer acceptability and minimizing anthocyanins and other phenolics losses during processing and storage is in primary concern. Different factors, such as temperature and duration of boiling and pasteurization, jam recipe, degree of fruit ripeness are important factors determining the quality of blueberry jam (Amakura et al., 2000).

The aim of the present study was to investigate the concentration of phenolic compounds, antioxidant capacity, water content, acidity, pH and sugar content of blueberry (*Vaccinium corymbosum* L.) fresh fruits and jam.

2. MATERIALS AND METHODS

2.1. Fruits and jam

Fresh fruits of blueberry were picked up at full maturity (dark blue colour) in commercial orchard in vicinity of town of Šabac, western Serbia. Samples were kept refrigerated and transferred to laboratory. On arrival, the fruits were manually washed, and damaged fruits were eliminated. One gram of fruits was extracted in 10 ml of 70% (v/v) methanol overnight. The extracts were filtered and centrifuged at 8000 rpm for 15 minutes. Extracts were used for further chemical analysis.

Jam was prepared in laboratory conditions under atmospheric pressure. The jam formulation was 64.2 % fruit, 35 % sugar and 0.8 % pectin. Firstly, fruit purée was prepared by blending blueberry fruits in commercial blender. Thence, fruit purée blended with sugar (sucrose) was stirred and boiled. The mixture was allowed to boil for 10 minutes and pectin solution was added. When cooked mass was finished and jam was filled into hot glass jars, capped and kept pasteurized at 80 °C/10 min. The jam allowed to cool at room temperature and stored in the dark at 20 °C until analysis. For analysis, extraction of bioactive compounds from jam was extracted with 70% methanol following the same procedure used for blueberry fruits extraction.

2.2. Chemical analysis

2.2.1. Determination of water content, pH, total titratable acidity and total sugars content

Water content was established according to the AOAC (2005) method through convective drying at 105 °C for until achieving a constant weight. The pH was measured at room temperature using 2ml juice with 25 ml distilled water by a digital pH meter. Total titrable acidity (TTA) was determined using 0.1 M NaOH to the titration end-point of pH 8.2 and values were expressed as percentage citric acid equivalents (AOAC, 2005). Total sugars were determined according to AOAC (2005) by phenol – sulphonic acid method. The intense yellow-orange colour resulted was measured at 490nm with a spectrophotometer.

2.2.2. Determination of phenolic compounds content

The concentrations of total phenolics (TP) and total tannins (TT) in fresh blueberry fruits and jam were determined by Folin-Ciocalteu colorimetric method according to Nagavani and Raghava Rao (2010). Twenty microliters of the extracts were mixed with 2 ml of diluted Folin-Ciocalteu reagent. Ten minutes later, 0.4 ml of 20% solution of sodium carbonate were added. After 1 h of incubation at

ambiental temperature, the absorbance was measured spectrophotometrically at 765 nm (UV/VIS spectrophotometer, model Evolution 220, Thermo Fisher Scientific, USA). TT content was determined after removal of tannins by their adsorption on insoluble matrix PVPP (polyvinylpolypyrrolidone). Calculated values were subtracted from TP content. Quercetin was used for calibration of a standard curve. The results were expressed in milligrams of quercetin equivalents per gram of fresh fruit weight (mg QE/g). Triplicate measurements were taken and mean values were calculated. The total flavonoid (TF) content in the fruit extracts were estimated by the aluminum chloride spectrophotometric assay (Saha et al., 2013). Total flavonoid content was determined from the regression equation of the quercetin calibration curve, and expressed as mg quercetin equivalent (QE) per gram of weight of fresh fruits or jam. The content of total anthocyanins (TA) in the obtained methanol extracts of selected fruits was measured spectrophotometrically by measuring the difference in the absorbance between solutions of pH 1.0 and pH 4.5 at absorption on 510 and 700 nm (Giusti and Wrolstad, 2001). Values are expressed as mg cyaniding-3-glucoside equivalents per gram of fresh fruit weight (mg C3GE/g).

2.2.3. Antioxidant assays

Scavenging of free radicals was tested in a DPPH (2,2-diphenyl-1-picrylhydrazyl) acetone solution (Lai and Lim, 2011). The degree of decoloration of solution indicates the scavenging efficiency of the substance added. Methanol solution of known trolox concentrations were used for calibration and the results were expressed as mg trolox equivalents per g of dry plant material (mg TE/g). The ABTS assay was based on a method developed by Miller et al. (1993). Ferric-reducing antioxidant power (FRAP) assay was carried out according to the procedure described in the literature (Valentão et al., 2002). The standard curve was constructed using different concentrations of trolox, and the results were expressed as mg trolox equivalents per gram of dry plant material (mg TE/g). The total antioxidant activity (TAA) of plant extracts were evaluated by phosphomolybdenum method as reported by Kalaskar and Surana (2014). A total reducing capacity (TRC) was performed according to the method of Oyaizu (1986). Methanol extracts of fruits and jam were mixed with mL of potassium ferricyanide in phosphate buffer (pH = 6.6) and incubated at 50°C for 20 min. Trichloroacetic acid (10% w/v) was added and centrifuged for 10 min. Supernatant was mixed with ferric chloride (0.1% w/v) and absorbance was measured at 700 nm. Trolox was used as a standard. Results were expressed as mg TE per gram of sample. Inhibition of NO radical was performed by the method as described in Orčić et al. (2011). The radical inhibition capacity was expressed as % of inhibition compared with the blank control. The superoxide free radical scavenging activity was carried out by NBT (nitroblue tetrazolium) test (Kalaskar and Surana, 2014). The percent inhibition of superoxide anion generated was calculated using the following formula: scavenging activity (%) = (1 - absorbance of sample/absorbance of control) x 100.

2.3. Statistical analysis

Results were expressed as a mean value of determinations of 3 independent samples made in triplicates. Statistical significance was tested by analysis of variance followed by comparison of means by Bonferroni test ($p < 0.05$) calculated using STATISTICA for Windows version 13.2 (StatSoft, Tulsa, OK, USA).

3. RESULTS

The water content, pH, titratable acidity and total sugar content of blueberry fruits and jam is shown in Table 1. Higher water content ($p < 0.05$) was observed in fresh blueberry fruits (86.903%) comparing to jam (41.943%), since the evaporation of water occur during thermal processing of jams. Higher total sugar content was verified for the jam (55.632 g/100g) compared to the fruits (8.382 g/100g). There was no statistical difference ($p > 0.05$) between the pH and titratable acidity of the fresh raw fruits and jam.

The amounts of polyphenolic compounds (total phenolics, tannins, flavonoids and anthocyanins) are summarized in Table 2. According to performed statistical analysis, the difference among TP, TT and

TF contest in fruits extracts and jam extracts was significant ($p < 0.05$).The range of TP varied between 985.17 (fruits) and 2292.00 (jam) mg GAE/ g FW. The concentration of TT was from 736.75 (fruits) to 2105.50 (jam) mg GAE/ g FW and the content of TF was from 84.00 to 908.33 mg QE/ g FW. There was *no* important *difference* in TA content between the fruits extracts and jam extracts (99.78 to 117.73 mg C3G/g FW, respectively).

Table 1. Physical and chemical characteristics of blueberry fruits and jam

Parameter	Fruit	Jam
Water content (%)	$86.903^{a} \pm 0.355$	$41.943^{b} \pm 0.225$
Total sugar content (g 100 g^{-1})	$8.382^{b} \pm 1.197$	$55.632^{a} \pm 2.365$
pH	$3.159^{a} \pm 0.046$	$3.286^{a} \pm 0.006$
Titratable acidity (g citric acid 100 g^{-1})	$1.152^{a} \pm 0.192$	$0.920^{a} \pm 0.021$

Values within the same row with the same lowercase letters are at the same level of significance

The antioxidant capacity of different extracts involves more than one chemical mechanism and may vary with test performed. Therefore, more than one assay for comparing the mode of antioxidant activity need be used and a single method could be inadequate. For this reason, antioxidant capacities of extracts made from blueberry fruits and jam was tested with seven different assays (Table 3). The jam extracts showed the higher value of TAA (157.366 mg TE/g FW), TRC (44.785 mg TE/g FW), ABTS free radical scavenging activity (8.714 mg TE/g FW) as well as DPPH free radical scavenging activity (12.018 TE/g FW). The lower antioxidant capacity obtained by TAA, TRC, ABTS and DPPH tests was observed in fruits extracts (58.746, 8.968, 2.966 and 6.042 mg TE/g FW, retrospectively). No significant differences between extracts were observed in FRAP assay and in ability to neutralize superoxide ($O_2^{\cdot -}$) and nitric oxide ($\cdot NO$) radicals.

Table 2. Content of polyphenolic compounds in blueberry fruits and jam

Parameter	Fruit	Jam
Total phenolics (mg GAE 100 g^{-1} FW)	$985.17^{b} \pm 250.66$	$2292.00^{a} \pm 184.57$
Total tannins (mg GAE 100 g^{-1} FW)	$736.75^{b} \pm 211.30$	$2105.50^{a} \pm 57.56$
Total flavonoids (mg QE 100 g^{-1} FW)	$84.00^{b} \pm 31.30$	$908.33^{a} \pm 71.95$
Total anthocyanins (mg G3CE 100 g^{-1} FW)	$99.78^{a} \pm 10.17$	$117.73^{a} \pm 8.88$

Values within the same row with the same lowercase letters are at the same level of significance

Table 3. Antioxidant capacity and free radical scavenging activity of blueberry fruits and jam extracts

Parameter	Fruit	Jam
FRAP (mg TE g^{-1} FW)	$8.416^{a} \pm 0.702$	$9.342^{a} \pm 0.340$
ABTS (mg TE g^{-1} FW)	$2.966^{b} \pm 0.746$	$8.714^{a} \pm 0.672$
DPPH (mg TE g^{-1} FW)	$6.041^{b} \pm 1.000$	$12.018^{a} \pm 0.483$
TRC (mg TE g^{-1} FW)	$8.968^{b} \pm 2.455$	$44.785^{a} \pm 1.394$
TAA (mg TE g^{-1} FW)	$59.746^{b} \pm 3.926$	$157.366^{a} \pm 7.497$
$O_2^{\cdot-}$ inhibition (%)	$67.680^{a} \pm 14.393$	$52.843^{a} \pm 4.053$
·NO inhibition (%)	$57.303^{a} \pm 8.725$	$52.808^{a} \pm 1.200$

Values within the same row with the same lowercase letters are at the same level of significance

4. DISCUSSION

The water content of the fresh blueberry fruits was two-fold higher comparing the jam. Similar results were observed by Mendes Rodrigues et al. (2017). The difference in water content is expected because of the heating and water evaporation involved during jams preparing procedure. Moisture has a great impact on the shelf life of horticultural products (Pavlova et al., 2013, Iwuagwu and Okonkwo, 2014). Processing of jam resulted in water removal and thus increased concentration of primary and secondary metabolites in final product. Values for the pH and titratable acidity obtained from the fresh blueberries and jam used are in good agreement with, and within the ranges of, those reported in previous studies (Ścibisz and Mitek, 2009, Mendes Rodrigues et al., 2017, Zhang et al., 2020). Sugar content in blueberry fruits was in agreement with rusluts of Zhang et al. (2020) and slightly lower comparing to results of some other authors (Milivojević et al., 2012, Okan et al., 2018, Fotirić Akšić et al., 2019). Jams contained much higher content of total sugars due to added sucrose during preparation of jams.

Regarding TP content in fresh blueberry fruits results of our study are in agreement with works of other authors (Ribera et al., 2010, Bunea et al., 2011, Milivojević et al., 2012, Kevers et al., 2014, Gonçalves et al., 2015) who found detected similar or lower TP contents in blueberry fruits ranging from 150.00-820.00 mg GAE/100g. It is well known that environmental, agronomic and genetic factors play a significant role in the phenolic composition, content and nutritional quality of blueberry fruits (Di Vittori et al., 2018). Our results of TT content in examined blueberry fruits were much higher than those in the study of Gonçalves et al. (2015), who reported for fresh blueberry tannins content in the range between 150.00 and 380.00 mg/100 g. The TF data's obtained are comparable to previous findings which reported values between 84.33 and 103.18 mg/100 g for some cultivated blueberries (Bunea et al., 2011). Reported data (fresh weight) for TA content in cultivated blueberry fruits exhibit a wide range, from 61.58 to 325.74 mg/100 g FW (Vollmannová et al., 2009, Ribera et al., 2010, Bunea et al., 2011, Yuan et al., 2011, Gonçalves et al., 2015). Our results for TA content in fresh fruits are in agreement with findings of other researchers.

During processing blueberry fruit into jam TP, TT and TF contents significantly increased. These results are not in accordance with the literature showing phenolic compounds losses during the processing of blueberries (Howard et al., 2010), strawberries (Levaj et al., 2012), blackberries (Oanacea and Călin, 2016), red raspberries (Oanacea and Călin, 2016) to jam. Results of TP, TA and antioxidant capacity for blueberry jam obtained in this experiment is significantly higher than those of Diaconeasa et al. (2019).

It is well-known that blueberry fruits and its products are rich source of antioxidants and potent scavenger of free-radicals. Our results are in agreement with findings of other researchers who

measured antioxidant capacity of fresh blueberry fruits and jams with different assays (Bunea et al., 2011, Yuan et al., 2011, Milivojević et al., 2012, Okan et al., 2018)

5. CONCLUSIONS

The results showed that fresh blueberry fruits and jam are rich source of phenolic compounds and possesses strong antioxidant activity. The antioxidant capacity and free radical scavenging activity of blueberry fruits and jam extracts varied according to the assay applied. Processing procedure did not have any impact on decreasing of bioactive compounds. Contents of most measured phenolic compounds were significantly higher in jam comparing to fruits. The present results suggest that blueberry jam may represent important sources of bioactive compounds in the diet with strong antioxidant capacity.

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