Phenolic Content and Their Antioxidant Activity in Various Berries Cultivated in Romania

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ABSTRACT

Berry fruits are a rich source of phenolic compounds with health benefits. Phenolic compounds occur in berries mainly as a variety of conjugated forms, mostly with sugars. The aim of this work was to evaluate and compare the phenolic content and antioxidant potential in the most common fruits consumed in Romania: blueberry, blackberries, raspberry and cranberries. Folin-Ciocalteu method has been used in order to evaluate total phenolic content of analyzed berries. Antioxidant activity was determinate using ORAC assay which measures the decrease of AAPH-radical level by the scavenging action of the antioxidant substance. In addition, the vitamin C content and total tannins of the berries extracts were determined using spetophomotmetric methods. The phenolic contents and antioxidant potential of analyzed berries did not varied considerably. The highest amounts of TPC and the strongest antioxidant activities were found in blueberry and blackberries (678 GAE mg/100 g FW, 442 mg/100g FW respectively). Vitamin C content was found in higher concentration in raspberries 21.7 mg/100 g FW while the lower concentration was found in blackberry. All berries contain higher levels of bioactive compounds such as polyphenols or tannins which are responsible for their antioxidant potential and bring their nutritional value, being highly recommended for daily consumption.

Keywords: *antioxidants, berries, polyphenols, ORAC, Vaccinum*.

INTRODUCTION

Natural supplements containing berries or vegetables have become an interesting research objective of many researchers because of their high content of antioxidants. Those antioxidants are believed to be responsible of preventing many diseases such as diabetes, cancers and degenerative diseases, linked to free radicals (Pimpão R. C., *et al.* 2013). Berry fruits are known for having antioxidant potential due to the various active compounds from several different classes such as polyphenols, isoprenoids (carotenoids, apocarotenoids, terpens, phytosterols) or organic sulphur compounds (glucosinolates, glutamylcysteine sulphoxide). The most abundant active compounds in edible berries are phenolic acids, tannins, and flavonoids, especially anthocyanins (more than 550 anthocyanins have been reported), which give the nice and very attractive color for many flowers or berries (Wang H., *et al.* 1997).

Phenolic compounds are secondary plant metabolites; their major role is to protect human organisms against oxidative stress induced by free radical species (Chao M. Howard *et al.,* 2004). Flavonoids are polyphenolic compounds which contain a C15 (C6-C3-C6) basic skeleton, and can be found in glycosylated and sometimes in acylated form (Wei Zheng, and Shiow Y. Wang 2003). Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that showed anticarcinogenic and antimutagenic potential (Chung K. T., *et al.* 1998). Many edible fruits which grow natively were adapted and now are cultivated intensively in farms. Also industry is more interested in exploitation and characterization of polyphenols from natural sources in order to use them for their potential bioactive capacity. All potential health-benefits of polyphenolic compounds such as phenolic acids, flavonoids, anthocyanins or tannins can be regarded as biomarkers for chemotaxonomic classification or markers for fruit quality. The most commons berries used for daily consummation are blueberry, blackberries, raspberries, cranberries or blackcurrants. The objectives of this study were to: measure the antioxidant capacity, the total phenolic, total tannins and vitamin C contents in blueberry, blackberries, raspberry and cranberries.

MATERIAL AND METHODS

Reagents

Reagents as 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) (purity 97%), 6- hydroxy-2,5,7,8-tetramethylchroman-2- carboxylic acid (Trolox) (purity 98%), Folin-Ciocalteu's phenol Reagent were purchased from Sigma-Aldrich (Darmstadt, Germany). Methanol, Na_2CO_3 were obtained from local producers.

Plant material

Blueberries *(Vaccinium corymbosum),* raspberries *(Rubus idaeus),* cranberries *(Vaccinium vitis-idaea),* and blackberries *(Rubus fruticosus)* were purchased from local farmers in the west of Transylvania. The samples were stored in a freezer at -20°C until they were analyzed.

Extraction methods

For sample extraction, 1g of each berries, were extracted with 10 methanol/formic acid (99:1, v/v) ml by grinding the sample 1 min using an Ultra-Turrax T25 homogenizer (IKA Werke, Staufen, Germany). The pellets were centrifuged at 3500 rpm for 10 min and re-extracted until the extraction solvents became colorless. The obtained extracts were concentrated at 40˚C under reduced pressure (Rotavapor R-124, Buchi, Switzerland) and filtered through 0.45 µm Millipore nylon filter. The samples were stored at -20˚C prior to future analysis.

Phenolic Content

The phenolic content (PC) of the analyzed extracts was determined by Folin-Ciocalteu reagent with minor modifications. The Folin Ciocalteu reagent (FCR) is called also Folin`s phenols reagent, Folin Denis reagent and Gallic Acid Equivalence method (GAE) (Singleton *et al.,* 1999). This reagent contains phosphomolybdate and phosphotungstate and was used first time for the analysis of proteins taking advantage of the reagent's activity toward protein tyrosine (containing a phenol group) residue (Folin and Ciocalteu, 1927).

Each sample (25 µl) was mixed with 1.8 ml distilled water. Next step was adding and mixing 120 µl of Folin-Ciocalteu reagent followed, after 5 minutes by addition of 340 µl Na2CO3 (7.5% in water) in order to create basic conditions (pH ~10) for the redox reaction between phenolic compounds and Folin-Ciocalteu reagent. After 90 min incubation at room temperature, the absorbance was read at 750 nm by microplate reader (BioTek Instruments, Winooski, VT), against the blank. Total phenolic content of analyzed berries were expressed as gallic acid equivalents, mg of GAE/ 100 g FW. All analyzed were performed in triplicate using a 24 wells microplate.

Total Tannins

The amount of total tannins (TT) was evaluated according to *Susana M. A. et al., 2013*, Ribereau-Gayon and Stonestreet, 1966. The analyzed samples were diluted to 1/50 in water. 2.0mL of the obtained solution was mixed with 1.0 mL water and 3.0mL of 12M HCl. The mixture was divided in two aliquots. First aliquot was heated for 30min in boiled water and cooled on bath ice (A_1), while the second one was kept at room temperature (A_2). Last 0.5mL of 95% ethyl alcohol was added to each sample. The absorbance was read at 550nm for each tube, $AbsA_1$ and $AbsA_2$. Total tannins content (g/L) was calculated using the equation:

$$TT = 19.33 \times (AbsA_1 - AbsA_2)$$

Oxygen Radical Absorbance Capacity (ORAC) Assay

The antioxidant potential of all analyzed berries was determinate by ORAC assay according to procedures previously described by Huang et al. 2002. The oxygen radical absorbance capacity (ORAC) measures the peroxyl radical scavenging activity using a strong antioxidant standard (Huang et al. 2002).

Briefly, 25 µl of each extract were mixed with 150 µl sodium fluorescein solution 4 x 10-3 µM in phosphate buffer (75 mM, pH 7.4) and incubated for 30 min, at 37°C. Generates peroxyl radicals were obtained by initiated the reaction by the addition of 25 µl AAPH solution. The fluorescence of analyzed samples was monitored kinetically for 30 min, at 37°C, excitation wavelength 485±20 nm and emission wavelength 525±20 nm using a fluorescence microplate reader (BioTek Instruments, Winooski, VT). The obtained results were calculated using a regression equation between standard (Trolox) concentration and the net area under fluorescence decay curve (AUC). The net AUC corresponding to a sample was calculated by subtracting the AUC corresponding to the blank. ORAC values were expressed as µmol Trolox /g sample. All determinations were done in triplicate.

Vitamin C content was determined by titration with a 0.05-M iodine solution according to Moor *et al.*, 2005. 25 g of berries were mixed with a 100 ml oxalic acid solution (6%) and homogenized for 5 minute. The mixtures were filtered in order to obtain a clear extract. Further 2 ml of starch solution 1 % was mixed with 10 ml of clear extract and then was titrated until a change of color, which does not disappear during 30 seconds. The content of vitamin C mg was calculated based on the following equation and expressed as mg/100g FW.

$$C = 400 * (Vsample / Vstandard)$$

Where *Vsample* – volume of the iodine solution titrated in a sample, ml;

Vstandard – volume of the iodine solution titrated in a standard solution, ml

RESULTS AND DISCUSSIONS

Total phenolic content (expressed as mg GAE/100g FW) in fruits of blueberry, raspberry, blackberry and cranberries are summarized below. Blackberry and the blueberry were characterized by the highest phenol content compared to other species (Fig. 1). The total phenolic (TPC) values for analyzed berries ranged from 200.3 to 678 mg GAE/ 100 g FW. The results obtaining for total phenolic assay are comparable to previous findings which had reported values between 251-310 mg GAE/100 g for some cultivated blueberries and between 577 and 614 mg GAE/100 g for wild Italian blueberries (Giovanelli et al., 2009). Also the total phenolic value of blueberry 678 mg GAE/ 100 g FW was found to be similar with 690.2 mg GAE/ 100 g FW reported previously Benvenuti et al., 2004) but two fold higher than values reported *by Q. You et al. 2011* (48.9-362 mg GAE/ 100 g FW). However higher contents in other blackberries cultivars have been observed and reported by Deighton et al. 2000. The difference among the analyzed berries and published data can be explained by different extraction method, berries environmental growth conditions, degree of maturity at harvest, genetic differences (Zadernowski et al. 2005).

Ascorbic acid. The obtained values for vitamin C content relieved a significant difference among the different analyzed berries. The higher concentration was found in raspberries followed by cranberries and blueberry (Fig. 2). These data are in agreement with data reported previously for raspberry (16.6-37.7 mg/100 g FW) (G.E. Pantelidis, 2007, Ancos, Gonzalez, & Cano, 2000). Deighton N., 2000, reported values range 12.3–

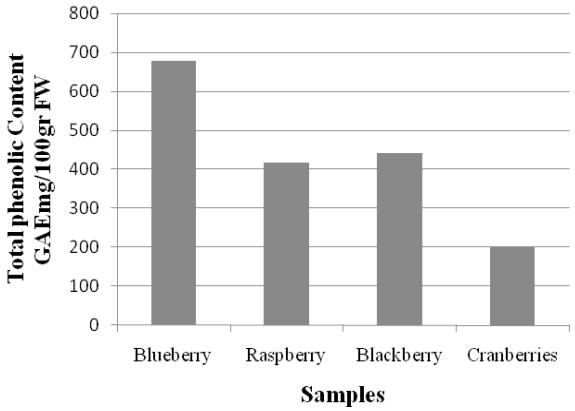

Fig. 1. Total phenolic content in fresh blueberry, rasberry, blackberry and cranberries.

16.4 mg/100 g FW for blackberries which is in good correlation with obtained results.

Oxygen radical absorbance capacity (ORAC) assay. This assay is probably the most widely used HAT-based assay and measure the scavenging capacity of antioxidants against peroxyl radical. The ORAC values for analyzed berries ranged from 19.3 to 36.4 µmol Trolox/ g FW. As shown in Fig.3.,

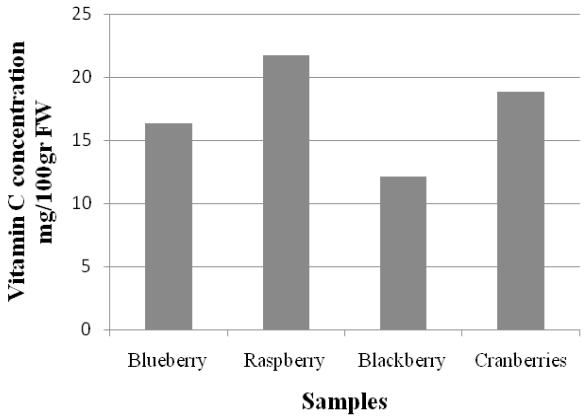

Fig. 2. Vitamin C content of fresh blueberry, rasberry, blackberry and cranberries.

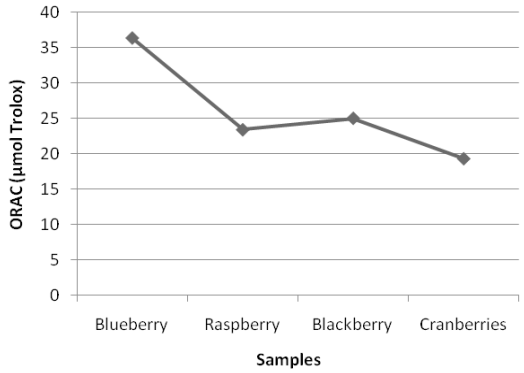

Fig. 3. Antioxidant Activity (ORAC) of fresh blueberry, rasberry, blackberry and cranberries.

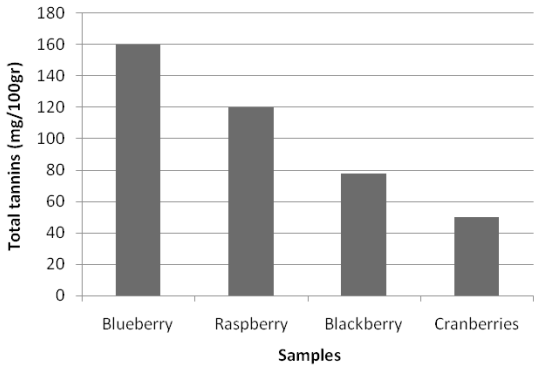

Fig. 4. Total tannins content in fresh blueberry, raspberries, blackberries and cranberries.

blueberry extract received the highest antioxidant ORAC value. For cranberries extract, the values obtained 19.3 µmol TE/g are comparable with those reported by Wei Zheng and Shiow Y. Wang, 2003 (40.4 µmol TE/g). Regarding the ORAC values for raspberries and blackberries, literature data ranged from 20.0-28.2 and 20.3-24.6 µmol Trolox/ g FW respectively, those results being comparable with our values (Shiow Y. Wang and Hsin-Shan Lin, 2000).

According to Prior et al., 1998 the normal intake of antioxidants in humans is in the range of 1.2-1.7 mmol Trolox/ day, estimation according ORAC method. Hence, a serving of 40-50 g blackberries or blueberries could provide the necessary ratio of antioxidants per day. The ORAC assay is based upon the inhibition of the peroxyl-radical-induced oxidation initiated by thermal decomposition of azo-compounds such as AAPH. The fluorescent reagents used in the ORAC assay were initially β-phycoerythrin and later fluorescein (Cao et al. 1995; Ghiselli et al. 1995; Ou et al. 2001). β-phycoerythrin seems to have inconsistent reactivity toward peroxyl radicals and exhibited non-specific protein binding to condensed tannins, why it was replaced by fluorescein (Ou et al. 2001).

Total tannins concentration. The blueberry and raspberry contained the highest amounts of tannins, 160 and 120 mg/100g FW, respectively, as shown in Fig.4. These values are comparable with those reported in a recent study by Susana M. A. et al. 2013.

CONCLUSIONS

Wild blueberries, raspberries, blackberries and cranberries contain large amounts of phenolics and also have high antioxidant activity. Significant variations in total polyphenolic content and antioxidant activity was found in analyzed berries, blueberries being the riches source of phenolics and tannins while raspberries, richest sources of vitamin C. The results of this study reveal the health benefits of these berries, consumed in daily diet, as a good source of antioxidants.

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