TOTAL CONTENT OF PHENOLS AND ANTHOCYANINS IN EDIBLE FRUITS FROM BOSNIA

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Abstract

Content of total phenols and total anthocyanins was estimated in edible fruits from Bosnia by photometric methods. Cyanidin-3-galactoside chloride was used as a standard for determination of total anthocyanins, and galic acid served as a standard for determination of total phenols. Total content of phenols was 12.7 mg/g in elderberry fruits, 10.4 mg/g in bilberry, 9.8 mg/g in blackberry, 8.8 mg/g in wild cherry, 6.1 mg/g in cultivated blackberry, 3.5 mg/g in cultivated strawberry, 2.4 mg/g in average in sour cherry fruits from different locations and the lowest quantity of total phenols was in edible parts of melon, only 0.2 mg/g. Total content of anthocyanins was 6.8 mg/g in wild cherry, 6.7 mg/g in elderberry fruits and 4.5 mg/g in bilberry. Wild bilberry fruits from different locations had in average 3.5 mg/g, cherries from different locations 1.3 mg/g, cultivated blackberries 1.0 mg/g, cultivated strawberries 0.8 mg/g while melon fruit had no anthocyanins at all. Acidity was measured in macerate of edible fruits by direct insertion of electrode. pH values in the macerates were as follows: 3.03 in bilberry, 3.45 in blackberries, 3.59 in sour cherries, 3.92 in wild cherries, 4.44 in elderberries and 6.19 in melon.

KEY WORDS: Total phenols, total anthocyanins, fruits, antioxidants.

Introduction

Diets rich in fruits and vegetables have been considered as excellent sources of antioxidants (1). Polyphenols, vitamins C and E, and carotenoids have been thought to be responsible for most of the antioxidant activity in foods (1 , 2). Considerable epidemiological evidence suggests an association between consumption of diets rich in fruits and decreased risk of some diseases (3, 4, 5). Diet containing high antioxidant activity from fruits can either increase the overall antioxidant capacity or change the relative balances between individual antioxidant components in human body (6). In terms of disease prevention, clinical trials with whole fruits and vegetables are more likely to give positive results (7). It was found that protective effects of antioxidants comes from ingredients other than vitamines, indicating the presence of other potentially important antioxidants in fruits, like polyphenols (flavanone, flavone, isoflavone, flavonol, catehin, flavans, biflavans etc.) and anthocyanins (8). As the amount of individual flavonoids in fruits is usally low, they have often been recorded unspecifically as '' total phenols ''. Most plant-derived phenols exibit strong antioxidant potentials (9). Anthocyanins are group of natural antioxidants widely distributed in fruits and vegetables. The anthocyanins are pigments which give most fruits their red, violet and blue colour (10). Interest in the anthocyanic pigments and their contribution to the colour of many processed products, including jams and juices. Nonethless, anthocyanins have not been used extensively as additives in the food industry because of their instability towards a variety of chemical and physical factors (e.g. pH and light), the difficulty of purification and their limited commercial availability. However, the discovery of more stable acylated anthocyanins will probably see the realization of their considerable potential as safe food additives.

Materials and Methods

Total content of phenols and anthocyanins was determined in bilberry –*[Vaccinium myrtillus L., (Ericaceae)]*, blackberry –*[Rubus fruticosus L., (Rosaceae)]*, wild cherry – *[Prunus avium L., (Rosaceae)]*, elderberry – *[Sambucus nigra L., (Caprifoliaceae)]*, melon – *[Cucumis melo L., (Cucurbitaceae)]*, strawberry – *[Fragaria vesca L., (Rosaceae)]* and sour cherry – *[Prunus cerasus L., (Rosaceae)]* all from Bosnia. Prior to quantification of phenols and anthocyanins, fruit was homogenised in aqueous solution of 10% formic acid and methanol in proportion 1:9 (v/v) and then homogenates were centrifuged at 4000 rpm for 20 minutes, and supernatants were subject of further analysis. Acidity (pH value) was measured in macerate of edible fruits by direct insertion of electrode.

a) Total Phenols Assay

Total soluble phenols in the acetonitrile extracts were determined with Folin- Ciocalteu reagent by the method of Slinkard and Singleton using gallic acid as a standard (11). Preparation of standard solutions of gallic acid: Standard solution of gallic acid (0.5 mg /mL) was prepared in destilled water with addition of several mL 95 % ethanol. This solution was diluted (500, 400, 300, 200, 100 mg/L) for analysis. All measurements were performed at 765 nm. Procedures: On 2.0 mL sample or standard solution (diluted 1 / 10 from above concentrations) was added 10.0 mL Folin and Ciocalteu reagent (1 / 10 diluted) and mixed. The mixture was left to stand from 0.5 to 8 minutes. After that was added 8.0 mL Na_2CO_3 (75 g/L). After 2 hours of standing at room temperature, absorbance was measured at 765 nm.

b) Total Anthocyanins Assay

The total anthocyanins were estimated by a pH differential method (12). Absorbance was measured in a WPA spectrophotometer at 517 nm and at 560 nm in buffers at pH 1.2 and 4.2, using A =

FRUITS		**Total phenols mg/g fresh weight**
Sambucus nigra L., (Caprifoliaceae)	elderberry fruits (Cazin)	12.7
Vaccinium myrtillus L., (Ericaceae)	bilberry (Fojnica)	10.4
Rubus fruticosus L., (Rosaceae)	blackberry (Stijene Cazin)	9.8
Vaccinium myrtillus L., (Ericaceae)	bilberry (Vranica - Busovača)	9.1
Prunus avium L., (Rosaceae)	wild cherry (Sarajevo)	8.8
Vaccinium myrtillus L., (Ericaceae)	bilberry (Konjic)	8.3
Prunus avium L., (Rosaceae)	wild cherry (Sarajevo)	7.4
Prunus avium L., (Rosaceae)	wild cherry (Konjic)	7.1
Rubus fruticosus L., (Rosaceae)	cultivated blackberry (Cazin)	6.1
Fragaria vesca L., (Rosaceae)	cultivated strawberry (Mostar)	3.5
Prunus cerasus L., (Rosaceae)	sour cherry (Sarajevo)	2.6
Prunus cerasus L., (Rosaceae)	sour cherry (Travnik)	2.1
Cucumis melo L., (Cucurbitaceae)	melon	0.2

TABLE 1. Total content of phenols in fruits

FRUITS		Total anthocyanins mg/g fresh weight
Prunus avium L., (Rosaceae)	wild cherry (Sarajevo)	6.8
Sambucus nigra L., (Caprifoliaceae)	elderberry fruits (Cazin)	6.7
Vaccinium myrtillus L., (Ericaceae)	bilberry (Fojnica)	4.5
Vaccinium myrtillus L., (Ericaceae)	bilberry (Busovača)	3.4
Vaccinium myrtillus L., (Ericaceae)	bilberry (Konjic)	2.7
Prunus avium L., (Rosaceae)	wild cherry (Sarajevo)	1.5
Prunus cerasus L., (Rosaceae)	sour cherry (Sarajevo)	1.5
Prunus cerasus L., (Rosaceae)	sour cherry (Travnik)	1.1
Rubus fruticosus L., (Rosaceae)	cultivated blackberry (Cazin)	1.0
Fragaria vesca L., (Rosaceae)	cultivated strawberry (Mostar)	0.8
Rubus fruticosus L., (Rosaceae)	cultivated blackberry (Cazin)	0.7
Prunus avium L., (Rosaceae)	wild cherry (Konjic)	0.6
Cucumis melo L., (Cucurbitaceae)	melon	0.0

TABLE 2. Total anthocyanins in friuts

$[(A_{517}-A_{560})_{PH1.2} - (A_{517} - A_{560})_{PH4.2}]$ with a molar extinction coefficient of cyanidin-3-glucoside of 29 600. Results were expressed as milligrams of cyanidin-3-glucoside equivalents per 100 g of fresh weight.

Results and Discussion

Total content of phenols, total anthocyanins and pH values in edible fruits are showed in Tables 1, 2 and 3. Results showed that total content of phenols in edible portion of fruits was in the order: elderberry fruits > bilberry > wild cherry > cultivated blackberry > cultivated strawberry > sour cherry > melon. Results showed that total content of anthocyanins in edible portion of fruits was in the order: wild cherry > elderberry fruits > bilberry > wild cherry > sour cherry > cultivated blackberry > cultivated strawberry. Melon fruit had no anthocyanins at all.

Fruits	pH
Melon – Sarajevo	6.19
Elderberry fruits - Cazin	4.44
Wild cherry - Konjic	3.92
Wild cherry - Sarajevo	3.76
Wild cherry (fresh) - Sarajevo	3.60
Sour cherry (fresh) - Sarajevo	3.59
Blackberry – Stijena Cazin	3.45
Bilberry - Busovača	3.05
Bilberry - Fojnica	3.03
Bilberry - Konjic	3.00

TABLE 3. pH values in fruits

In a Table 3. results showed that measured pH value was highest in melon, and furthermore decreased in the next order: elderberry > wild cherry > sour cherry > blackberry > bilberry. These results suggests that the major phenols from the analyzed friuts are presented in elederbery fruits and bilberry. The major anthocyanins are presented in wild cherry and elderbery fruits. The potential effectiveness of fruit supplements in promoting various aspects of health depends upon their botanical and chemical composition and on the concentrations of active ingredients that they contain. Comparison of different cultivars of the same fruit and between different fruits showed broad variations in both phenolic content and in vitro antioxidant activity as estimated by the oxygen radical absorbance capacity assay - ORAC (13) and ferric reducing antioxidant power assay – FRAP (14). The fruit extracts antioxidant quality was better than the vitamin antioxidants and most pure phenols, suggesting synergism among the antioxidants in the mixture. Fruits had significantly better quantity and quality of polyphenol antioxidants than vegetables. Some fruits, specifically apples and cranberries, have phenol antioxidants that can enrich lower density lipoproteins and protect them from oxidation. (15). Total phenols tend to have a higher correlation with antioxidant properties than total anthocyanins. For anthocyanin-rich fruits such as blackberries and cherries, anthocyanins will be the largest contributor to total phenols (16). Our results for a content of total phenols and total anthocyanins in the analyzed edible fruits are in accordance with literature data (13). Quantification of total phenols and total anthocyanins in edible fruits from Bosnia is necessary for estimation of these fruits as a potentially antioxidant dietary suplements. Our data thus provide a base for potentially epidemiological examination the relation between edible fruits from Bosnia and risk of some disease.

Conclusion

- Total content of phenols was the highest in elderberry fruits and follow bilberry, blackberry, wild cherry, cultivated strawberry, sour cherry and the lowest in melon.
- Total anthocyanins content in wild cherry was the highest and follows elderberry fruits , bilberry, sour cherries, cultivated blackberries and cultivated strawberries.
- Melon fruit had no anthocyanins at all.
- Fruit acidity depended directly on the concentration of aliphatic and aromatic organic acids in the fruits. There was greater statistic-mathematical correlation between pH values in the macerate of fruits and total content of phenols than between pH values of the fruit macerate and total anthocyanins content.

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