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Analysis and Characterization of Anthocyanins in Raspberry fruits (*Rubus idaeus L.*)

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Abstract. This study focuses on the analysis and characterization of anthocyanins from Raspberry fruit (*Rubus idaeus L.*). The fresh Raspberry fruits were extracted with the solvent of EtOH 96% /0.1% HCl (1:1, ratio) at room temperature for 4 h in the dark. The extraction process was the important step in the isolation and identification of anthocyanins. For this purpose, anthocyanin contents in Raspberry fruits were determined with UV-Vis Spectroscopy. The results showed that the total anthocyanin content (expressed as cyanidin-3-glucoside) in the extract was 277.2 mg/L, which is equivalent to 9.33 mg/100g total anthocyanin of dry material. All statistical analysis was performed using the MS Excel program and SPSS 22.0 statistics software.

Keywords: Anthocyanins, Raspberry fruits, UV-Vis Spectroscopy, Extract

Introduction

Berries contain a significant amount of diverse bioactive compounds, which individually or in combination can have a positive effect on human health. Therefore, berry fruit can be recommended as a natural source of antioxidants.

Small fruits are an excellent source of natural antioxidant substances, which is one of the major reasons for their increasing popularity in the human diet. Extracts of fruits from various blackberry, raspberry and gooseberry cultivars act effectively as free radical inhibitors [1],[2].

Raspberries (*Rubus idaeus L.*) are very popular and attractive fruit from the family *Rosaceae*. Is an important commercial fruit with good flavor and attractive color widely grown throughout the world. They are recognized by consumers as tasty and healthy fruits. These fruits are known as a rich source of dietary antioxidants mainly due to their high level of phenolic compounds, which primarily comprise ellagitannins, and flavonoids, including anthocyanins, and phenolic acids. In recent years, these bioactive

compounds have been associated with various health benefits, including antioxidant, prevention of inflammation disorders, cardiovascular diseases, or protective effects to lower the risk of various cancers, hypertension, overweight and obesity [3],[4].

Also, Raspberries fruits (*Rubus idaeus L.*) as important part of our diet are known for their sensory and nutritional characteristics, because of high content of essential nutrients that are beneficial for biological activity in human health. A high consumption of plant foods, such as raspberries, appears to decrease the risk of obesity, diabetes, heart disease, and overall mortality [5].

Anthocyanins belong to a large group of secondary plant metabolites collectively known as flavonoids, a subclass of the polyphenol family [6]. Anthocyanins are a group of water-soluble pigments that confer the blue, purple, and red color to many fruits and vegetables [7]. Anthocyanins are found abundantly in a variety of berries. The anthocyanins present in blackberries and raspberries are important for the beneficial health effects associated with their antioxidant, anti-inflammatory, and chemo preventative properties [2],[8].

In the present study, we aimed to identify and determine the content of bioactive compounds such as anthocyanins content in different raspberry cultivars from organic and conventional cultivation. and possible beneficial health effects.

Materials and methods

Sampling Preparation

The preparation of samples has been previously described [8],[9]. The extraction process was the important step in the isolation and identification of anthocyanins. The fresh raspberry fruits were extracted and acid hydrolyses using acidified aqueous solvent of EtOH 96% /0.1% HC l (1:1, ratio) at room temperature for 4 h in the dark. Following hydrolysis, samples were cooled to room temperature and 1 mL was subjected to the total anthocyanin analysis.

Total anthocyanin content

Total anthocyanin content was measured with the pH differential absorbance method with some modifications [1],[3]. Absorbance of the extract was measured at 510 and 700 nm in buffers at pH 1.0 (hydrochloric acid–potassium chloride, 0.2 M) and 4.5 (acetate acid–sodium acetate, 1 M). The absorbance of each dilution was measured at 520 and 700 nm using a distilled water as a blank. Absorbance (A) was calculated as follows:

$$A = (A_{520} - A_{700}) \text{ pH}1.0 - (A_{520} - A_{700}) \text{ pH}4.5$$

The anthocyanin concentration (mg/L) was calculated using the following formula:

$$\text{Anthocyanin content} = (A \times \text{MW} \times \text{DF} \times 1000) / (\epsilon \times l)$$

Where MW is the molecular weight of cyanidin-3-glucoside (449.2 g mol⁻¹), DF is the dilution factor, and ϵ is the molar extinction coefficient of cyanidin-3-glucoside ($\epsilon = 26\,900 \text{ L cm}^{-1} \text{ mol}^{-1}$).

Total anthocyanin was calculated in the sample as mg per 100 g of extracts and dry material (FW).

For this purpose, anthocyanin contents in Raspberry fruits were determined with UV-Vis Spectroscopy (Genesys10S UV-Visible). Cyanidin-3-glucoside is commonly used to create the standard curve. The results showed that the total anthocyanin content expressed as cyanidin-3-glucoside.

Statistical analysis

All data were expressed as the mean \pm standard deviation of triplicate experiments. All statistical analysis performed using the MS Excel program and SPSS 22.0 statistics software. Differences were tested for significance using the ANOVA procedure, with a significance level of $p < 0.05$.

Results and Discussion

Results for total anthocyanins content on Raspberries fruits (*Rubus idaeus L.*) are summarized in Table 1. and Figure 1.

Table 1. Total anthocyanin content of raspberry extracts and dry material (mg/100 g dry material)

Sample	TAc (CyGE mg/100 g)
Extract of Raspberry fruits	277.2 \pm 1.01 ^b
Dry material of Raspberry fruits	9.33 \pm 0.8 ^a

^{a,b}: means \pm SD in the same column differ significantly at level $p < 0.05$; Tac-total anthocyanin contents; CyGE—cyanidin-3-glucoside equivalents. Values represent means SD of three measurements (n = 3).

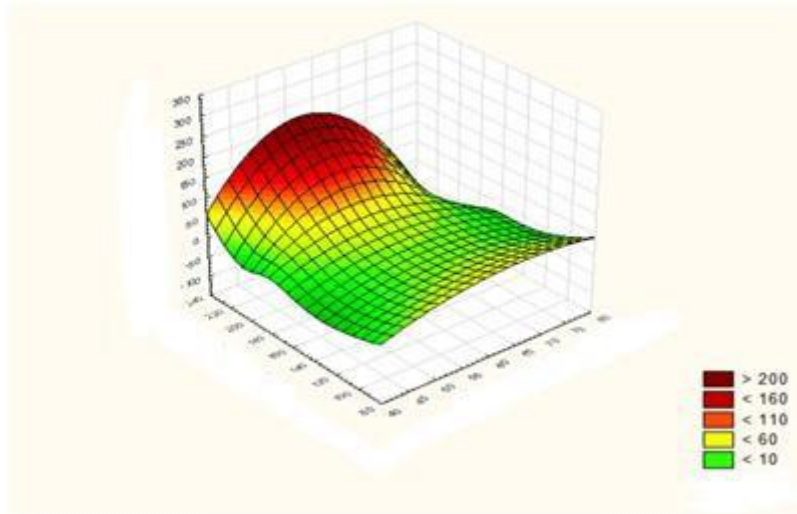


Figure 1. Total anthocyanin content of raspberry extracts and dry material (mg/100 g dry material)

The results showed that the total anthocyanin content (expressed as cyanidin-3-glucoside) in the extract of the Raspberries fruits (*Rubus idaeus L.*) was 277.2 mg/L, which is equivalent to 9.33 mg/100 g total anthocyanin of dry material.

This is the first study to characterize anthocyanin content of the Raspberry fruits (*Rubus idaeus L.*) in Kosovo. Similar results for anthocyanin content of the Raspberry fruits (*Rubus idaeus L.*) have been reported by others researchers de Ancos et al. [10] therefore anthocyanins are regarded as important antioxidants in berry fruits. However, in humans the bioavailability of dietary anthocyanins is low [1].

Pantelitis et al. [1] in their work report total anthocyanin content of the Raspberry fruits (*Rubus idaeus L.*) was observed 35-49 mg/100g dry material. Similar results for anthocyanin content have been reported **Szymanowska et al.** [3] who found the content of anthocyanins content of the Raspberry fruits (*Rubus idaeus L.*) are 58.7-42.9 mg/100g dry material, these values are much higher from our findings.

Conclusion

Raspberry fruit (*Rubus idaeus L.*) is valuable fruit crop, highly evaluated for the nutritional and sensory value. In our work, we evaluated the content of anthocyanins in selected varieties of Raspberry fruit (*Rubus idaeus L.*) as mentioned, this is the first study to characterize anthocyanins content of the Raspberry fruits (*Rubus idaeus L.*) in Kosovo. In addition, the total anthocyanin value of Raspberry fruits (*Rubus idaeus L.*) sampled help to give a realistic picture of what values might be expected from growing available Raspberry fruits (*Rubus idaeus L.*) cultivars as well as from new ones developed through breeding in the near future. These data will help provide a baseline for researchers studying the health effects of phytochemicals in Raspberry fruit (*Rubus idaeus L.*) as well as product developers for nutraceutical, natural colorant, and other industries interested in these properties and health benefits from health properties of anthocyanins.

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