QUANTITATION OF ELLAGIC ACID IN BLACKBERRIES

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Abstract

The objective of this study was to evaluate the content of ellagic acid in blackberries, as well as in the juice of different blackberry cultivars obtained by using a specific technology. The analysis of the ellagic acid content in the test samples was performed using the high-pressure liquid chromatography with diode-array detection (HPLC-DAD). The results have shown considerable variations in ellagic acid content in the test blackberry fruit samples, the highest being determined in the fruit of the blackberry- raspberry hybrid cv. "Tayberry" (54.794 mg/100 g fresh weight), and the lowest in blackberry cv. "Čačanska Bestrna" (1.852 mg/100 g fresh weight). The ellagic acid content in the "Tayberry" juice produced using the specific technology was very high and almost identical to that in fruits.

Keywords: ellagic acid, bioavailability, human health.

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Over the last years, there has been an increasing awareness among nutritionists, doctors and most importantly consumers, related to the role of nutrition in improving human health and the quality of life [1]. Dark-coloured small fruits belonging to the families Ericaceae (blueberries) and Rosaceae (blackberries, raspberries and strawberries) are particularly known for their polyphenol content [2], with polyphenols making a significant contribution to the total antioxidant capacity of fruits [3,4]. A growing number of research papers and articles are being published in scientific journals dealing with this subject that show positive effects of plant-based foods on the prevention of different diseases [5–8].

Small fruits are singled out from among these foods due to their beneficial effect on human organism, with their high levels of ellagic acid playing a preventive role against malignant diseases [9–11]. Namely, ellagic acid has been proven to slow down or even prevent the division of malignant cells [12–14]. Moreover, ellagic acid exhibits a potent antioxidant activity, thereby preventing and controlling the spread of cancer [15–17]. The antioxidant properties of ellagic acid allow it to neutralize free radicals, chelate toxic metals and activate antioxidant enzymes, thus contributing to strengthening the body's antioxidant defence systems [18]. In addition, several other beneficial properties have been reported for ellagic acid in particular and phenolics in general, including anti-inflammatory, anti-microbial and anti-allergenic properties, as confirmed by many researchers [19–22].

Given the above facts, modern tendencies in fruit and vegetable production are oriented towards the production of functional foods that have special health effects, involving attempts to increase and preserve the ellagic acid content in fruits or edible parts of plants. Although the high content of ellagic acid in strawberry and raspberry cultivars is a long known fact [23,24], its content in different blackberry species and cultivars has not been sufficiently studied. Therefore, the objective of the present study was to evaluate the ellagic acid content in fruits of different blackberry (*Rubus caesius* L.) cultivars largely grown in the south-western part of Serbia. Another objective was to determine the ellagic acid content in the blackberry juice obtained by a specific technology, in order to determine the potential effect of the technology on the preservation of ellagic acid content in the produced juice. Namely, the ellagic acid as an extremely valuable substance is found mostly in the seed, and hardly a third of its juice content, on average, can be retained using conventional fruit processing methods [25]. The preservation of ellagic acid content in fruit juices is of utmost importance for human health, considering the highest consumer acceptance rate of juice as a processed fruit product that is available for consumption throughout the year.

MATERIAL AND METHODS

Chemicals

Methanol (HPLC, gradient grade), acetonitrile and formic acid (HPLC) were supplied by Merck KGaA (Darmstadt, Germany). The standard substance, includ-

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ing ellagic acid, was purchased from Sigma-Aldrich GmbH (Sternheim, Germany). Water used throughout the experiments was purified using a Millipore, Elix UV and Simplicity Water Purification System (Milford, MA, USA). Tert-buthylhydroquinone, HCl and ethanol were purchased from Centrohem (Centrohem, Stara Pazova, Serbia). All other chemicals and reagents were of analytical reagent grade.

Plant species

This study evaluates the content of ellagic acid in the following samples: fruit of the blackberry-raspberry hybrid cv. "Tayberry" (*Rubus fruticosus*×*Rubus idaeus*), fruit of blackberry cv. "Čačanska Bestrna" (*Rubus caesius* L., cv. Čačak Thornless) and fruit of wild grey–blue blackberries (*Rubus caesius* L.).

Preparation of materials

The mature fruits were harvested for analysis of ellagic acid content in the wider region of the Mt. Golija Nature Park (43° 20' N, 20° 17' E) in the south-western part of Serbia at the beginning of August, 2011. Immediately after harvest, the fruits were carefully placed into a portable refrigerator and transported to the laboratory, where they were stored at –20 °C until analysis.

The analysis of ellagic acid content in the blackberry samples was performed at the Laboratory of Chemistry, Fruit Research Institute, Čačak, in March 2012, using the high-pressure liquid chromatography with diode-array detection (HPLC-DAD). Apart from the fruit, ellagic acid content was also determined in the juice produced from hybrid cv. "Tayberry" fruits. The objective of this part of the study was to identify the effect of juice production technology on the preservation of ellagic acid in the juice. The used technology has involved cold pressing of blackberry fruits using a wooden press and juice straining through a 0.8 mm sieve. This process ensures retention of a large number of seeds in the juice, with seeds containing the highest percentage of ellagic acid. Furthermore, the juice is a completely natural product, as no preservatives or additives are added.

Sample preparation was performed as follows*:* blackberry samples were prepared for analysis of ellagic acid content according to the method of Hertog M.G.L., Hollman P.C.H., Katan M.B. [26]. The berry fruits were frozen in liquid nitrogen and homogenised by using a stainless steel blender. An aliquot of 15 g of ground fruit or juice was weighed into a 100-mL Erlenmeyer flask and diluted in 20 mL of 62.5% aqueous methanol containing 2 g/L of tert-buthylhydroquinone (TBHQ). Then, the mixture was ultrasonicated for 5 min, and 5 mL of 6 M HCl was added to the extract. Hydrolysis was carried out in a shaking water bath at 85 °C for 2 h. After hydrolysis, the sample was allowed to cool. Then, it was filtered, made up to 50 mL with methanol and ultrasonicated for 5 min. Before quantification by HPLC, the sample was filtered through a 0.45 μm membrane filter.

HPLC-DAD Analysis

Samples were analysed using an Agilent 1260 series HPLC (Agilent Technologies, Santa Clara, CA, USA) linked to a ChemStation data handling system, using a ZORBAX Eclipse Plus C18 column (4.6 mm×150 mm, 3.5 μm particles). Injection volume was 5 μl and the temperature was at 30 °C. Solvent A was 1% formic acid and solvent B was acetonitrile. The used gradient was as follows: 0–10 min, 10% of B in A; 10–25 min, 15–50% of B in A; 25–30 min, 50–80% of B in A; 30–32 min, 10% of B in A. The good purity and separation were achieved in raspberry samples using this gradient (flow rate 0.5 mL/min). The HPLC equipment was used with a diode array detector (DAD). Ultraviolet–visible spectra (ranging from 190 to 540 nm) were recorded for all peaks. Triplicate analyses were performed for each sample. Ellagic acid was detected at 260 nm, and identified according to peak retention time and UV/Vis spectra, which were compared with those of the standard. The quantities of ellagic acid were based on peak areas, and expressed as mg/100 g fw (or mg/100 g juice).

RESULTS AND DISCUSSION

Chromatograms for the test samples subjected to identification and quantification of ellagic acid are presented in Figure 1.

The ellagic acid content of the test samples is given in Table 1.

The results have shown considerable variations in ellagic acid content depending on the type of the sample. The highest content of ellagic acid was determined in hybrid cv. "Tayberry" fruit (54.794 mg/100 g fresh weight, on average), which was considerably higher than that in the fruit of other blackberry species and cultivars. As compared to the data on ellagic acid content in small fruits, the value determined in hybrid cv. "Tayberry" was extremely high. Hertog *et al*. [27] reported average values of ellagic acid content in strawberries and raspberries of only 5.52 mg/100 g and 40.06 mg/100 g fresh weight, respectively. Same authors also assessed the ellagic acid content in blackberry fruit (*Rubus caesius* L.) and found values of 37.60 mg /100 g fresh weight, as confirmed by the present results. The similar results on ellagic content in blackberries were reported by other authors 0.75 do 6.65 mg/100 g [28,29]. The lowest content of ellagic acid in this study was found in blackberry cv. "Čačanska Bestrna" – only 1.852 mg of ellagic acid per 100 g fresh weight. This was a rather unexpected result, parti-

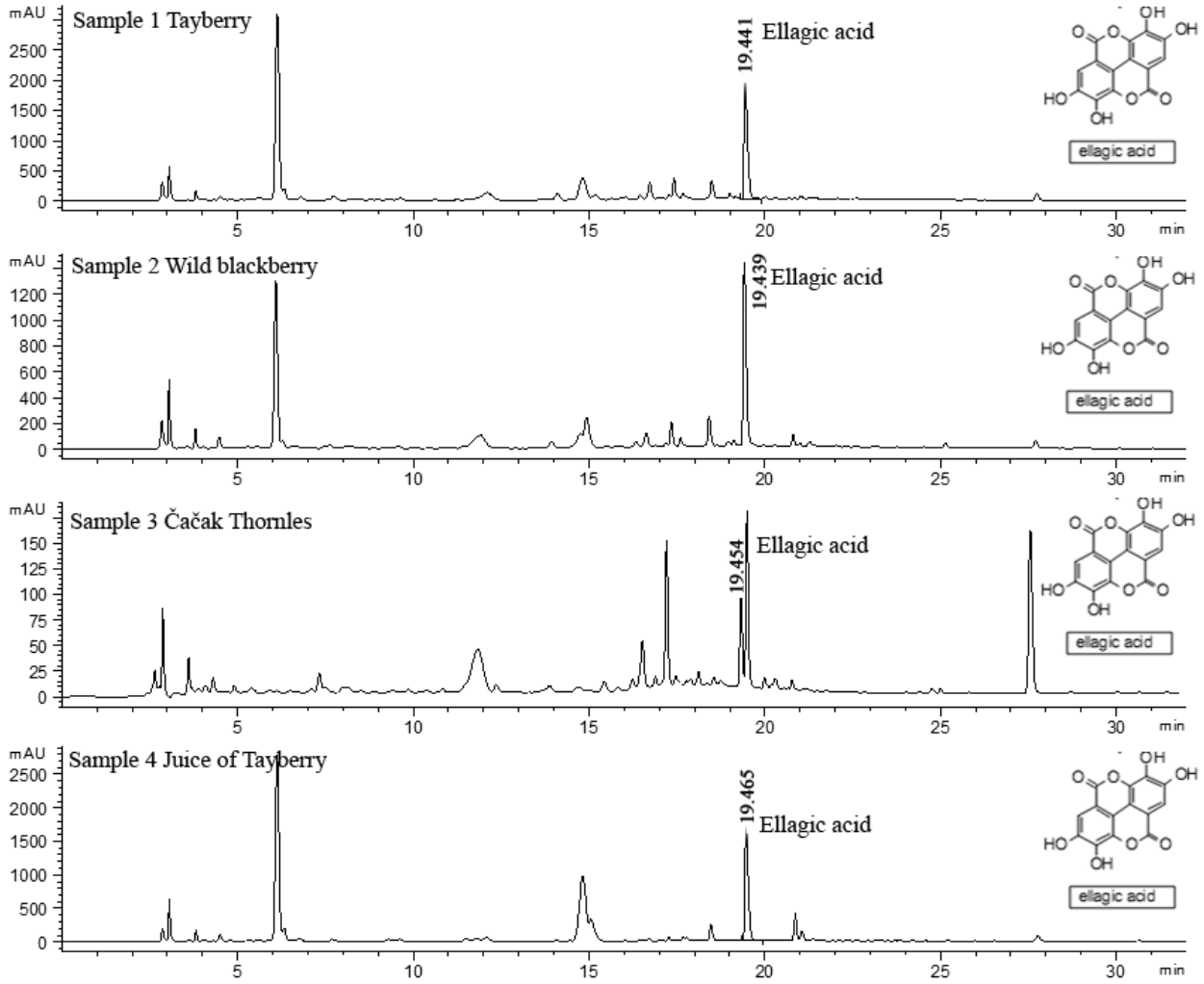

Figure 1. Chromatograms for the test samples using the high-pressure liquid chromatography with diode-array detection (HPLC-DAD).

Table 1. Ellagic acid content of the test samples

Sample	Ellagic acid content (mg/100 g fw)
Hybrid berry cultivar "Tayberry"	54.794±0.536
Rubus caesius L. (wild blackberry)	35.701±0.986
Blackberry cultivar "Čačanska Bestrna"	1.852±0.063
"Tayberry" juice	53.999±1.985 (mg/100 g juice)

cularly in view of the fact that the content of ellagic acid in grey-blue blackberries and hybrid cv. 'Tayberry' fruits was 20-fold and almost 30-fold higher, respectively. Such high differences in ellagic acid content between the samples speak in favour of the proposition that ellagic acid content shows substantial variation among different blackberry cultivars and small fruits in general, as confirmed by a number of studies [30,31]. In the research of authors Hakkinen *et al*. [30] determined values for the content of ellagic acid in fruits of different berries was in the range from 23.8 to 68.6 mg/100 g, and in the research of Vrhovsek *et al*. [31] the value for the same parameter ranged from 1.2 to 39.3 mg/100 g.

An important part of this research involved determination of ellagic acid content in the juice obtained from the fruit of the blackberry-raspberry hybrid cv. "Tayberry". The value identified in the juice slightly deviated from that of the fruit of this cultivar. This result is in disagreement with the literature data reporting a considerably lower content of ellagic acid in the juice than in the fruit used for juice production [25]. The successful preservation of the ellagic acid content in the juice obtained from the fruit of the blackberry–

–raspberry hybrid cv. "Tayberry" can be primarily attributed to the technology used for juice production, as an important factor in preserving valuable substances in fruits. The present findings also indicate an important role of blackberry juice production technology in preserving the valuable substances present in blackberries, particularly in view of the fact that storage of red raspberries and blueberries over a period of 9 months at –20 °C may result in a 30–40% reduction in ellagic acid content [30]. Therefore, the cold pressing procedure used in juice production is of extremely high importance since it significantly contributes to preserving the content of ellagic acid.

CONCLUSIONS

The results obtained in this study suggest considerable variations in ellagic acid content in blackberry fruit, depending on blackberry species and cultivars. A high fruit content of ellagic acid was found in blackberry–raspberry hybrid cv. "Tayberry", which should be taken into account when selecting, purchasing and processing small fruits. Both the juice and the juice production technology used in the research suggest a significant content of ellagic acid. The research has shown that ellagic acid content was highest in the seeds. The ellagic acid content of the juice produced from hybrid cv. "Tayberry" was high, almost identical to that of the fruits, suggesting the need for juice processing technology (involving homogenization of blackberry seeds) to take a targeted approach to the preservation of the nutritional quality of both natural ingredients and fruit flavour. In terms of human health improvement and commercially speaking, this finding is of high importance, considering the highest consumer acceptance rate of juice as a processed fruit product that is available for consumption throughout the year. Regular intake of blackberries and their juice has a preventative effect on human health, thereby considerably reducing the risk of different diseases.

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IZVOD

KVANTIFIKACIJA ELAGINSKE KISELINE U KUPINI

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(Naučni rad)

Rad je koncipiran sa ciljem da se ispita sadržaj elaginske kiseline u plodovima različitih sorti kupine, kao i u soku dobijenom iz plodova kupine primenom posebnog tehnološkog postupka, koji se ogledao u homogenizaciji semenki u plodu kupine. Istraživanja pokazuju da je sadržaj elaginske kiseline najveći u semenu. Sadržaj elaginske kiseline u ispitivanim uzorcima određen je kvantitativno primenom tečne hromatografije uz korišćenje DAD detektora (HPLC-DAD). Dobijeni rezultati su pokazali da najveću vrednost elaginske kiseline ima plod hibridnog kultivara kupine i maline „*Tayberry*“ i iznosi je 54,794 mg/100 g, a najmanju sorta kupine „Čačanska bestrna“, svega 1,852 mg/100 g suve materije. Sadržaj elaginske kiseline u soku dobijenom iz plodova hibridnog kultivara „*Tayberry*“ je bio visok, gotovo isti kao i u plodovima, što upućuje na zaključak da se kod tehnološkg postupka proizvodnje soka mora obratiti posebna pažnja u cilju očuvanja nutritivne vrednosti prirodnih sastojaka, kao i arome voća. Plodovi jagodastog voća, posebno maline i kupine, bogati su polifenolnim jedinjenjima koja bitno doprinose ukusu, aromi i obojenosti plodova. Istraživanja sve više ukazuju da elagina kiselina ima izraženo antimikrobno, antioksidativno, antikancerogeno dejstvo i konzumiranjem ovog voća bitno se doprinosi očuvanju zdravlja a samim tim i smanjuje se rizik od nastanka bolesti.

Ključne reči: Elagina kiselina • Biodostupnost • Zdravlje ljudi