SJ IMPACT FACTOR: 2.912 CRDEEP Journals
Global Journal of Current Research Diasamidze & Kalandia Vol. 6 No. 2 ISSN: 2320-2920
Global Journal of Current Research Vol. 6 No. 1. 2018. Pp. 50-53

Full Length Research Paper

The Research of Phenolic Compounds of Raspberry *(Rubus saxatilis L.)* Fruit by HPLC

Mzia Diasamidze* and Aleko Kalandia
Batumi Shota Rustaveli State University, Batumi, Georgia

Article history
Received: 09-07-2018
Revised: 15-07-2018
Accepted: 10-08-2018

Corresponding Author:
Mzia Diasamidze
Batumi Shota Rustaveli State University, Georgia.

Abstract
The research deals with the study of the quantitative and qualitative content (by HPLC method) of phenolic compounds of a wildly grown plant of raspberry fruit (Raspberry Rubus saxatilis L.) using the high-pressure liquid Chromatography (HPLC) and Spectral methods.HPLC analysis has revealed, that the fetus was a major Cyanidin-3 –glucoside, and it reached full maturity period 1248,92±37,47 mg/kg (cyanidin-3-O-glucoside chloride equivalents) calculated on the dry weight. In mature fetus flavonols are 1998,68±29,96 mg/kg (rutin equivalents) calculated on the dry weight, catechins are 112,01±3,36 mg/kg ((+) catechin equivalents) calculated on the dry weight, leucoantocyanins are 19,05±0,6 mg/kg (cyanidin equivalents) calculated on the dry weight. Full maturity period, the number of phenol carbonic acids is 4809,15±144,27 mg/kg, (caffeic acid equivalents) calculated on the dry weight of the unit. The following compounds have also been determined by HPLC method: Cyanidin-3-glucoside, Quercetin-3-rutinoside, Quercetin-3-glucoside, Ellagic acid.

Keywords: *Raspberry (Rubus saxatilis L.), Anthocyanins, Phenolics, Flavonoids, HPLC.*

Introduction

The representatives of *Rubus* (the family of Rosaceae) have long been well-known in the world and their fruits are considered healthy, useful and dietary due to a high content of anthocyanins [1,2], antioxidants [3,4], vitamins, minerals, folic acid, alkaline acid and others [5-7]. Raspberry has long been known for its unique medicinal properties in folk medicine. It was vital for therapeutic purposes, people prepared tea from its fruit and leaves. The roots of plants were once used in India for the treatment of the weakened intestine and dysentery, as well as whooping cough. The leaf infusion was used in the treatment of dysentery and bleeding [8]. Raspberry can be eaten both in raw and processed form; it has a pleasant sweet taste. It can be used with sugar, honey or milk; it can be used as a jelly, compote, juice, syrup, jam and quas. It is also possible to fetch a light blue pigment from the fetus [9, 10]. The studies, conducted by dietologists around the world, have show that raspberries have a positive effect on health. They claim that raspberry help humans get enough vitamin C and potassium [4]. The Western Georgia, namely mountainous Adjara, is rich with wildly grown plants of the family of Rosaceae (the scientific name is Rubus). Representatives of Rubus has practically not been studied in Georgia. However, as the plant is able to collect biologically active substances in different quantities in different agro-ecological conditions, therefore, it is important to study the chemical composition of raw material in terms of its rational use. The goal of the research was to study the quantitative and qualitative content (by HPLC method) of phenolic compounds (antioxidants, flavonols, catechins, leukoanthocyanins, phenolarbone acids) of a wildly grown plant of raspberry fruit, spread in Adjara, Georgia,

Materials and Methods

The fruits with varying degrees of ripeness of Rubus varieties of the Rubus saxatilis L family have been selected as material for the study. The research samples have been obtained in Georgia, particularly, in the Adjara region (Black Sea Coast - Batumi Botanical Garden). At the certain stages of ripening there have been prepared the appropriate extracts and conducted the qualitative and quantitative research of some phenolic compounds with spectral and HPLC methods.

Extraction

Frozen fruits were blended to a puree using commercial blender. Subsamples (5g) of puree were then homogenized for 1 min in 20 ml of extraction solution containing ethanol/water/HCl (40:57:3 v/v/v) ((-15)–(–18)°C) to the smallest particle size using a laboratory homogenizer. Homogenates were filtered and the filtrates were centrifuged for 5 min at 5000 rpm. Aliquots (4ml) of supernatant were evaporated to dryness using a concentrator (RVO 400 SD) with no radiant heat and re-suspended in 1 ml of aqueous 5 % formic acid solution. All samples were passed through 0.45 µm filters (Acrodist LC PVDF Syringe Filters Waters) prior to HPLC analysis. In cases of flavonols, catecins, leucoanthocyanin's and phenol carbon acids extraction of samples was carried out with 70% ethanol at 70-8°C temperature. For quantitative analysis of anthocyanin's sample extraction was carried out with acidic ethanol (3% hydrochloric acid) (-15)–(-18) °C. For qualitative analysis following reagents have been used [11, 12], and their composition has been

defined by spectric method by setting standard calibre curve. Flavonols quantified as - Rutin (400 nm), Catecins - (+) Catecin (500 nm), leucoanthocyanins – cyanidins (550 nm), phenol carbon acids – on caffeine acid (325 nm), anthocyanin's- cyanidine-3-glucosid chloride (528 nm) equivalents[13]. The results are shown in tables 1, 2, 3.

HPLC analysis of flavonoids

Samples (20 µL) were analyzed using a Waters HPLC system equipped with a model 525 pump, UV/Vis detector. Separation was carried out using a 4,6x150 Symmetry C 18 column (Waters Corp, Milford, MA, USA) with a 3,9 mmx20mm C 18 guard column. The mobile phase was a linear gradient of 5 % formic acid (A)band methanol (B) from 2 % B to 60 % B for 60 min at 1 ml min-1. The system was equilibrated for 20 min at the initial gradient prior to each injection. Detection wavelengths used were 510 nm for anthocyanin's and 370 nm for flavonols. Total anthocyanin derivatives were calculated as the sum of individual anthocyanin. Flavonols were quantified as rutin equivalents.

Results and Discussion

The content of phenolic compounds in raspberry fruit depends on the stage of maturity and the environmental conditions where the plant grows. [1, 14, 15]. Raspberry, being an edible berry, is also useful due to the high content of phenolic compounds, which increase its healing effect as well [16]. It has been found out that in quantitative terms the highest number of flavonoids in the fetus is in the immature fruit of green color (3503,01 ± 105,1 mg / kg), and in the end of vegetation it is reduced by almost 50%. The results are discussed in Table 1.

Table 1. Content of flavonoids in Raspberry fruits according to vegetation

Raspberry fruit	Flavonols, mg/kg 70% C_2H_5OH		Catecins, mg/kg 70% C_2H_5OH		Leucoanthocyanins, mg/kg, 70% C_2H_5OH	
	Raw weight	Dry weight	Raw weight	Dry weight	Raw weight	Dry weight
Immature green-pink hue	701,04±21,03	3503,01±105,1	22,8±0,7	115,2±3,45	4,8±0,14	20,07±0,6
Immature light pink	377,86±11,34	2671,31±80,14	11,7±0,4	80,05±2,4	5,0±0,15	40,3±1,2
Immature pink	257,79±7,73	1987,88±59,64	12,9±0,38	94,87±2,85	6,94±0,2	72,7±2,18
Mature crimson	255,68±7,67	1998,68±29,96	13,8±0,41	112,0±3,36	15,7±0,5	19,05±0,6

The table shows that the content of flavonoids, catechins and leucoanthocyanins is reduced at all four stages of maturity, according to the vegetation; the number of flavonoids in ripe fruits is 1998.68 ± 29,96 mg / kg calculated on the dry weight, catechins are 112,0 ± 3,36 mg/kg of dry weight and leucoanthocyanins are 19,05 ± 0,6 mg / kg of dry weight. Together with the qualitative identification of phenol carbonic acid in the raspberry fruit there has been determined the quantitative content according to vegetation (Table 2).

Table 2. The content of phenol carbohydrates in the fruit of Rubus saxatilis L. in accordance with vegetation

Raspberry fruit	Phenolarbone acids in mg / kg 80% C_2H_5OH	
	Raw material	**Dry material**
Immature light pink	209,67±6,29	1496,89±44,9
Immature pink	457,89±13,74	3405,21±102,16
Mature crimson	**578,94±17,37**	**4809,15±144,27**

The amount of phenol carbonic acid increases in parallel to the ripening of the fetus. In particular, the immature light pink fruit contains 1496.89 ± 44,9 mg / kg, the mature crimpson raspberry fruit contains 4809,15 ± 144,27 mg / kg, calculating the dry weight. The quantity of anthocyanin pigments has been determined in the parallel to the maturity of the fetus of Rubus saxatilis L.

Table 3. The content of anthocyanins in the fruit of Rubus saxatilis L. in accordance with vegetation

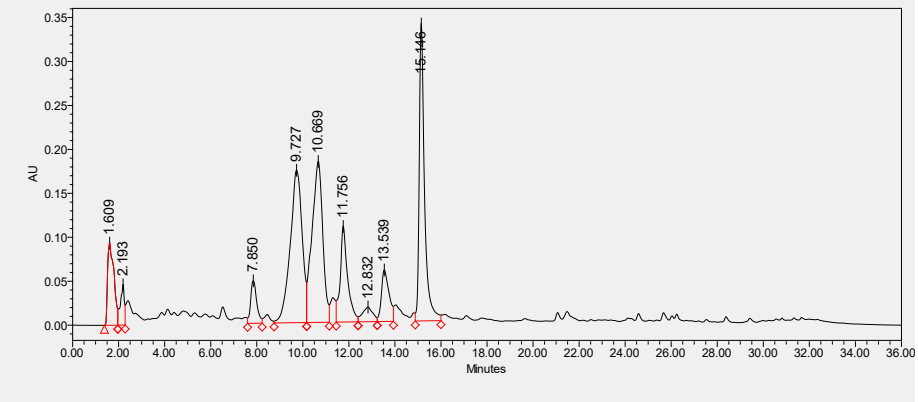
Raspberry fruit	anthocyanins in mg/kg, 3% HCl 40% C_2H_5OH	
	Raw material	**Dry material**
Immature, light pink	21,15±0,63	142,11±4,26
Immature, pink	52,89±1,59	375,07±11,25
Mature, crimson	**150,08±4,5**	**1248,92±37,47**

The studies have shown that the amount of anthocyanins increases and reaches the maximum during the full maturity of the fetus. Particularly, the immature, light pink fetus contains 142.11 ± 4,26 mg / kg, the immature pink fetus contains 375,07 ± 11,25 mg / kg, and in the period of full maturity it is 1248,92 ± 37,47 mg / kg, calculating the dry weight. The results of the qualitative analysis (chromatograms) are given in Figure 1-3.

Raspberry fruits are not distinguished by the high content of flavonoid glycosides in the maturity period, however, they are quite diverse in qualitative terms. In the fetus of Rubus saxatilis L. at least 8 compounds have absorption on 285 nm. Among them,

quercetin-3-glucosides and quercetin-3-rutinosides were identified with the help of authentic compounds. It should be noted that these compounds have significantly been changed during the plant growth and development. The number of flavonoids in the immature fetus is greater than in the mature one, their qualitative composition changes as well.

Fig 1. HPLC chromatogram of flavonoids of immature, light pink fetus of Rubus saxatilis L. (Detection 285 nm)



Among the phenolcarboxylic acids, only alkaline acid has been identified by extract chromatography of the ripe Rubus saxatilis L. fetus. The concentration of Alkaline acid is lower than that of other acids (Fig. 2).

Fig 2. HPLC chromatogram of phenolcarboxylic acid of immature, light pink fetus of Rubus saxatilis L. (Detection 309 nm)

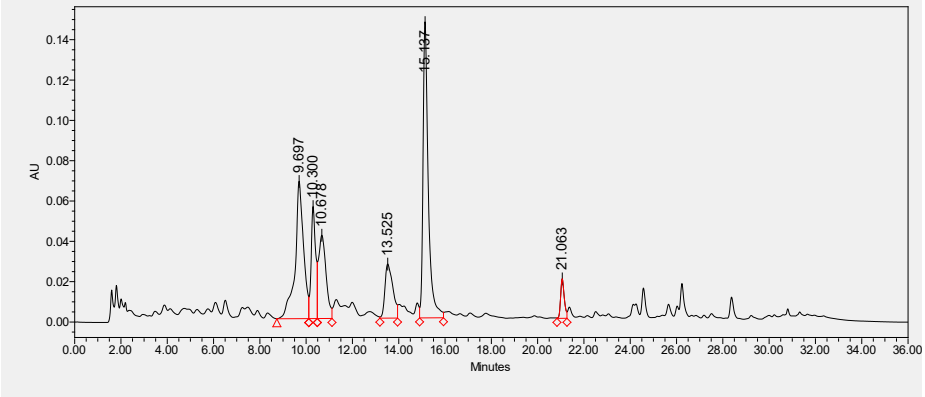
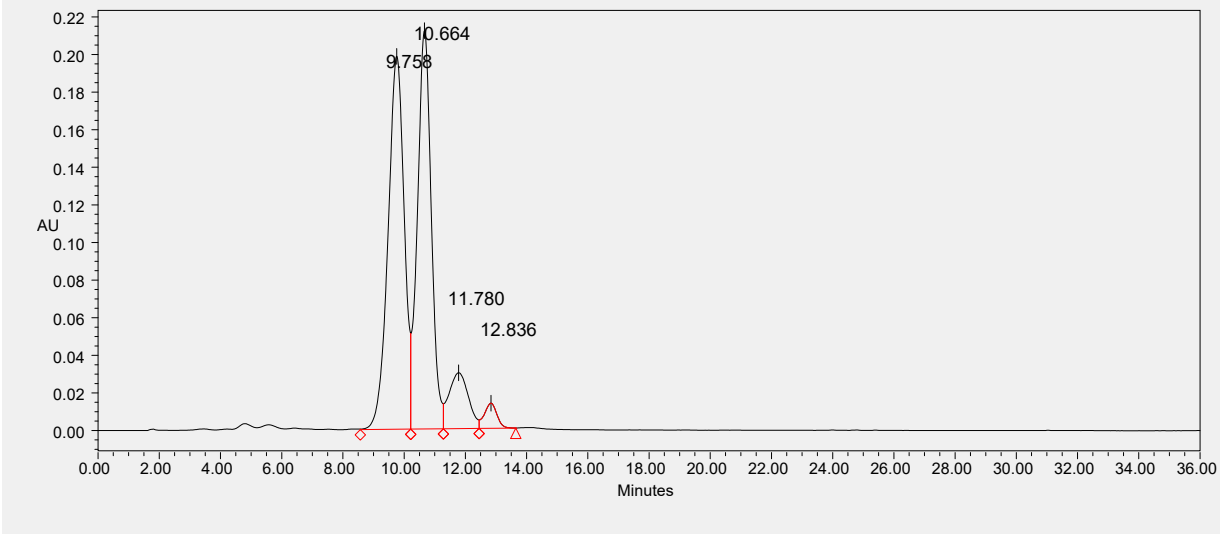


Fig 3. Chromatography of anthocyanins of immature light pink, immature pink and mature crimson fetus of Rubus saxatilis L. (Detection 518 nm)



Three anthocyanins have been identified in the fetus of Rubus saxatilis L. Among them quercetin-3-glucosides, quercetin-3-rutinosides, cyanidin-3-glucoside is dominates (Figure 3).

Conclusions

The qualitative and quantitative content of the fetus of Rubus saxatilis L. flavonoid compounds (flavonols, leucoanthocyanins and catechins) has been analyzed, particularly: by the high pressure liquid chromatography method there have been indentified quercetin-3-rutinoside and quercetin-3-glucoside in the raspberry fruits. It has been found that the highest number of flavonols, leucoanthocyanins and catechins in the fetus of Rubus saxatilis L. is in the immature fetus. The number of flavonols decreases during the ripening of the fetus and it is reduced by almost 50% in the end of vegetation. The research has shown that the fruits of Rubus saxatilis L. phenol carboxylic acid is presented in the form of alkaline acid. The qualitative and quantitative content of anthocyanins in the raw fetus of Rubus saxatilis L., spread in the Adjara region, has been studied and it was found that the fruits are rich in anthocyanins and their bulk is cyanide-3-glucoside. The amount of anthocyanins increases with the ripening of the fetus.

Acknowledgement

The authors express their gratitude to Shota Rustaveli National Science Foundation (Grant GNSF/ST8/513) for financial provision.

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