Original article

Bioactive Compounds and Antioxidant Activity of Feijoa (Feijoa Sellowiana Berg) Cultivated in Subtropical Zones of Georgia

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Abstract. Feijoa is one of the most interesting fruit cultures in Georgia. Feijoa is a cross-pollinated plant. Therefore, it produces a huge variety under a selectionist point of view. New forms differ not only in their structure but also in chemical composition. 10 samples of Feijoa were chosen from different regions of Georgia and their chemical characteristics were determined. Moreover, DPPH method was used for determining their antioxidant activity. Our investigation has revealed that out of ten samples four were the most interesting for their chemical properties such as the amount of total phenols, flavonoids, catechins and antioxidant activity.

Key words: Feijoa; Antioxidant activity; Catechins; Flavonoids; Phenols; DPPH method.

Introduction

Feijoa (Feijoa sellowiana berg) is one of the most interesting fruit cultures. In Georgia, this fruit initially was introduced for decorative purposes, but later, in 1930 it was cultivated in a much bigger scale. Generally, Feijoa is a tree-like plant, but there are several forms of it that resemble a bush. The height of the plant is 2-4 meters and the size of the fruit varies from 3-8 cm in length and 2-5 cm in diameter (1). Subtropical fruits have an essential part in the food ration of a human being and Feijoa is one of the best examples of it. In fact the raw material is full of bioactive compounds, most of which are natural antioxidants (2). Antioxidants block free radicals and take part in the regulation of oxidation-reduction reactions in the human organism (3). Besides the phenols (4-8), the fruit of Feijoa contains pectin compounds, sugars and organic acids (9), vitamins (PP,B_1,B_2) and other microelements Cr, P, V, Mn, Ni, S, Ti, Fe (10-12). Moreover, recent studies underline antioxidant, antibacterial and anti-fungicidal properties of Feijoa leaves and fruit (9, 13-18).

It's well known that aqueous extract of the Feijoa fruit possesses antioxidant activities. It should also be noted that its acetonic extract can exert anti-cancer activities in different cancer cell models, with low toxicity on normal cells (19). In order to study different types of newly emerged Feijoa, we have focused this research on the variation of its bio-morphological features and antioxidant potential. In general, the methods for the determination of antioxidant activity can be divided into several groups like photometric, fluorimetric, electrochemical, chemiluminescence and other very specific methods. In most cases, radical reactions are used between, specific, colored radical and the extract that has a potential antioxidant activity and the obtained absorbance determined using a spectrophotometer. The most common methods are ORAC - determination of the ability of absorption of oxygen radical,

TRAP- determination of the antioxidant property of retention of free radicals, FRAP- the ability to decrease the amount of iron, TEAC- the antioxidant capacity of a substance compared to the standard, Trolox. Antioxidant activity is also determined using organic radicals such as DPPH (2,2-Diphenyl-1-pic arylhydrazine) and ABTS (2,2-Azino-bis(3-ethylbenzenethiazoline-6-sulfonic acid) method, etc. (9, 14, 20). It is well known that DPPH is the most popular antioxidant assay for plant extracts. This method was first described for the first time in 1958 (21) and then it was modified several times. DPPH assay is known for being fast, easy and precise test-method. It is widely used not only for determining the capability of retaining free radicals but also determining antioxidant activity in food products and juices (9, 14). It should be noted that DPPH is a stable free radical with maximum absorbance at 517 nm. Extract that is dissolved in methanol is initially violet, but after the reduction, it becomes yellow (9, 18). To this end, we have chosen one of the types of Feijoa called choasoana and its emerged forms.

Materials and Methods

Materials

Cuvette Changer (Mettler Toledo UV5A), C18 Cartridge Solid Phase Extraction (SPE) Waters Sep-Pak C18 (500 mg), Chemicals – stability radical- 2,2-Diphenyl-1-picrylhydrazyl (Aldrich-Germany), Acetonitrile, Methanol, Acetic Acid (Merck-Germany), AlCl3, Folin Ciocalteu reagent (preparation), Standards –Gallic acid, Quercetin.

Plant material

All the analyzed material was gathered in the same period (October-November), and they were always collected from the same regions of western Georgia (Adjara, Guria, and Samegrelo). We have also taken into account how ripe the material was and conducted analysis in the manner that biochemical composition of the fruits was not altered by the difference in the time of their storage. We sorted the raw material according to their size, position on the tree and other features. 10 samples were gathered for the analysis, and 10 grams of each was used for investigation. Also, It should be noted that the raw material was processed without using metal tools.

Plant extracts

For the quantitative analysis of Phenolic compounds, Flavonoids and Catechins 10 grams of skin and fruit were taken from each of the selected samples. Extraction was performed using ethyl alcohol: water in various concentrations (80:20, 70:30), on water bath at 70° C (for Flavonoids) and 40° C (for Catechins and Phenols), the process was repeated three times for 30 minutes. For the qualitative analysis extraction was performed only on the skin. The extract was then put in vacuum distillatory and then filtered using C18 Cartridge Solid Phase Extraction (SPE) Waters Sep-Pak C18 (500 mg).

Determination of antioxidant activity using DPPH assay.

For determination of antioxidant activity-radical retention to 1 ml of the sample 3 ml of DPPH (0.1 mM DPPH-0.004 g/100mL in ethyl alcohol) were added and after 30 minutes optical density was evaluated in the spectrophotometer. Therefore, DPPH and 96% ethyl alcohol were used as blanks for the activity of free radical inhibition.

The following) formula has been used to determine the activity of free radical inhibition (DPPH) is provided below: In % = $A_C - A_S/A_C$*100, Ac indicates absorption of DPPH/Alcohol solution, and As indicates absorption of the extract.

Determination of phenols using Folin-Ciocalteu's Reagent.

Folin-Ciocalteu's spectrophotometric method is widely used in the determination of phenols (22). Extraction of the samples was conducted using 80% ethyl alcohol, at the temperature of 70° C. 0.5 or 1.0 milliliters of the extract were transferred to 25 ml volumetric flask and 5.0 ml of water and with 1.0 ml of

Folin-Ciocalteu reagent were added. After 8 minutes at 25° C, 10.0 ml of 7% Na_2CO_3 were added, and the flask was then filled with water (final volume 25 ml) and left at room temperature for 2 hours. The determination is conducted at 750 nm. As control 1 ml of extragent (80% ethyl alcohol) is used. After obtaining the values, calculations were performed using a calibration curve of Gallic Acid as described by Ceccarini et al. (23). The following formula X = (D K V F)*1000/m was used to determine phenols. Where, X is the amount of phenols mg/kg; D is the optical density; K is the coefficient; F is the factor of dilution; V is the volume of extract in ml; m is the mass in grams of the raw material used for extraction (24).

Quantitative analysis of Flavonoids.

For the quantitative analysis of flavonoids, the spectral method was used (25). 1 ml of extract was added to 10 ml volumetric flask containing 5 ml of water and 0.3 ml of 5% $NaNO_2$. After 5 minutes 0.3 ml of 10% $AlCl_3$ was added and then 2 ml of 1N NaOH. The determination was conducted at 510 nm in HPLC-UV. Results were expressed in mg/l of Catechine (or Ruthin).

HPLC-UV Waters (Breeze, USA) HPLC system equipped with a model 1525 pump and a UV detectors (2489), preparation collector. The UPLC analyses were carried out using an UPLC system model Waters Acquity H MS-QDa and PDA detectors with electrospray ionization and ion trap analyzer. C18 reversed-phase column was used (100 × 2.1 mm, ACQUITE BEH C18, 1.7 μm). Methanol and ethyl acetate were used as a solvent and during extraction (Merc, Germany HPLC grade). For the preparation of mobile phases for chromatography water with 0.1% formic acid as solvent A and acetonitrile with 0.1% formic acid as solvent B were used (Merc, Germany HPLC grade). In order to filter the samples before chromatography C18 Cartridge Solid Phase Extraction (SPE) Waters Sep-Pak C18 (500 mg) was used.

HPLC was used for quantitative and qualitative analysis of the catechins in the samples. Several systems were used for the extract, methanol : water (80 : 20, v/v), acetone : water (80 : 20, v/v), methanol : acetone (50 : 50, v/v), methanol : acetone : water (40 : 40 : 20, v/v), ethanol :acetone : water (40 : 40 : 20, v/v). The most optimal partition was observed using system -solvent A 2% CH3 CN-solvent B 80% CH3 CN. 0–6 min, 100–0%; 6-9 min. 68%–32%; 9–21 min. 100–0%;

For UPLC-MS analysis 2 μm of extract was used in the following systems: solvent A and solvent B, column ACQUITY UPLC BEN C18.

At the first stage of sample preparation, the samples were concentrated from 100 to 50 mL in vacuum at a temperature of not more than 40° C (before alcohol removal). Obtained samples were subjected to Sep-pak C18 filter. Elution was performed with the mixture of water and 0.1 % formic acid. Elution of phenolic compounds was performed with 4 mL of ethyl acetate. The process allowed us to greatly reduce the presence of non-target compounds. Final fraction was concentrated and dissolved in 90-10 ratio A-B solvent mixture mentioned above.

2.0 μL of each sample was injected and analyzed at 30° C. The elution program at 0.20 mL/min was 10% B (0-2 min), 10-60% B (2-14 min), 60% B (14-16 min) followed by a 2 min wash with 100% B and a 5 min equilibration step. The detection wavelengths were set at 285 and 360 nm. Electrospray ionization in positive and negative mode was used. Samplers temperature 10° C; MS- scan 100- 1100 da; probe 600° C; negative 0.8 kV, Capilarity 1.5 kV, C -20, 40V).

Results and Discussion

Determination of technical characteristics.

The following characteristics of Feijoa choasoana were analyzed: average mass, volume, height, and diameter of the fruits (Table 1).

The biggest sample (96.1 g) was found in Guria n.78, followed by those of Adiara (Batumi Botanical Garden) n.88 (87.9 g) and n.89 (83.4 g). Samples gathered in Samegrelo were relatively small compared to others. The volume of the raw material was relatively the same in all samples from different regions (from 41,0 ml^3 to 55,0 ml^3), the only sample that wasn't in this range was n.73, (30.1 ml^3). The average height of the material was between 3.2 a 6.3 cm. The highest samples were n.78, 74 (6,3 and 5,9 cm). The

Table 1. Technical characteristics of the selected samples of Feijoa.

Region	Sample number	Mass (gr)	Volume (ml)	Height (cm)	Diameter (cm)
Adjara	88	87,9±1,2	49,1±3,8	5,1±0,01	3,9±0,002
	89	83,35±2,8	43,9±2,6	4,5±0,006	3,4±0,01
	90	61,22±1,8	41,0±1,8	4,8±0,004	3,1±0,3
Guria	74	55,83±0,6	40,1±0,9	5,9±0,07	4,2±0,02
	78	96,1±1,3	46,04±0,8	6,3±0,09	3,9±0,1
	71	84,4±2,0	48,13±1,5	4,7±0,09	3,6±0,03
Samegrelo	61	87,9±0,04	5,46±1,9	3,2±0,05	2,2±0,008
	73	30,51±0,1	30,3±0,1	4±0,008	3,9±0,05
	83	36,34±0,08	30,34±0,01	4,3±0,11	3,9±0,04
	87	43,42±0,05	55,0±0,009	5,2±0,01	3,7±0,001

Table 2. Biochemical Characteristics of Feijoa

Region	Sample number	Dry Compound Brix, %	Dry Compounds after drying, %	Acidity, %	pH
Adjara	88	9,6±0,5	13,1±0,4	1,1±0,06	3,33
	89	8,4±0,5	12,2±0,3	1,2±0,01	3,30
	90	8,5±0,4	13,1±0,9	1,6±0,2	3,31
Guria	74	11,4±0,08	15,6±0,1	2,0±0,02	3,27
	78	9,2±0,1	15,6±0,04	2,0±0,04	3,26
	71	9,2±0,04	15,2±0,01	1,4±0,04	3,29
Samegrelo	61	8,1±0,4	20,2±0,1	1,5±0,2	3,30
	73	9,1±0,01	21,3±0,06	2,2±0,1	3,26
	83	9,1±0,08	13,5±0,06	1,7±0,1	3,27
	87	9,3±0,1	14,4±0,02	1,2±0,05	3,32

diameter ranges were from 2.2 to 3.9 cm. Thus, the best raw material, according to their physical properties and other characteristics, were n.88, 89, 90, 71, 74, 78, 61,73, 83, 87. These samples have been characterized for their content in flavonoids, phenol, catechin, and antioxidant activity. These samples were also analyzed for their biochemical characteristics.

According to the literature the insight of the fruit should be 3.5 times heavier than the mass of its skin. This difference changes with the proportions of the fruit. The average height of the fruit is 5.5 cm, width- 3.7 cm, and the number of seeds is equal to 80-100 (26).

The highest value of the refractometric index was in the samples from Guria n.74 (11,4%) and Adjara n.88 (9,6%) (Table 2). The results of the drying showed that samples n.n.61,73 from Samegrelo have the highest amount of dry compounds 20,2 and 21,3% respectively, followed by Gurian samples and Adjarian (Batumi Botanical Garden) samples.

There is a little difference in acidity in all samples (1,1-2,2%). The values of samples n.74,78, 73 were relatively high. Also, the pH values of all the samples from each region were relatively low (range 3.2-3.3).

Determination of Phenols, Flavonoids, and Catechins.

Determination of the total amount of phenols was performed by the Folin-Ciocalteu's spectrophotometric method (Table 3). The highest amount of phenols was found in samples in n.78 from Guria (1.404 mg/g), n.83 from Samegrelo (0.670mg/g),

Table 3. Phenols, Flavanoids and Catechins in Feijoa samples (mg/g)

Region	Sample number	Total Catechins	Total Flavanoids	Total Phenols
Adjara	88	0,364	0,58	2,464
	89	1,101	1,21	5,340
	90	1.120	1,15	5,060
Guria	74	0.710	0,81	3,504
	78	1,404	1,45	6.367
	71	0.356	0,61	2,745
Samegrelo	61	0,115	0,12	0,528
	73	0.299	0,48	2,110
	83	0,670	0.80	3,600
	87	0,580	0,78	3,517

Table 4. Catechins in Feijoa fruit (mg/g)

Region	Adjara		Guria		Samegrelo	
Feijoa Sample	89	90	74	78	83	87
(+)Catechin	0.40	0.48	0.28	0.56	0.28	0.22
(-)Epicatechin	0.31	0.40	0.20	0.49	0.20	0.16
(-)Epigallocatechin	0.16	0.20	0.11	0.21	0.08	0.08
(-)Gallocatechin	0.04	0.08	0,08	0,08	0.04	0.06

and n.90 from Adjara region (1.120 mg/g). The least amount of phenols was in sample n.61 (0.155 mg/g) from Samegrelo region.

The analysis on flavonoid content showed that it was 25% of the total phenol mass. The highest amount of flavonoids according to their regions were determined in samples n.89 (Adjara), n.78 (Guria), and n.83 (Samegrelo), the least amount was n.61 (Samegrelo).

Using UPLC-MS method the following Catechins: (+)C Catechins ($C_{15}H_{14}O_6$), (-) Epicatechins ($C_{15}H_{14}O_6$), (-) Gallocatechins($C_{15}H_{14}O_7$), (-)EGC-Epigallocatechins ($C_{22}H_{18}O_{10}$), were identified. In addition, using UPLC-MS method Phenilcarbonic acids were identified – Gallic acid, Caffeic acid, Chlorogenic-Acid, and Flavonoids – Guajavarin, Hyperin, Avicularin, Quercitrin, Quercetin.

The total amount of catechins range from 0,115 to 1,404. They are 1/5-1/6 of the mass of phenols.

The content of (+) Catechins, Epicatechins, Epigallocatechin, and Galocatechins was determined in the best samples of each region, Samegrelo (n.83,87), Guria (n.74,78), and Adjara (n. 89,90) (Table 4).

The obtained results showed that there are no significant differences between these samples. Samples from all regions had high levels of Catechins and Epicatechins, whereas lower levels were observed for Epigallocatechin and Gallocatechin. The highest amount of Catechin was found in sample n.78 from Guria (0.56 mg/g,) and the lowest amount was in sample n.87 from Samegrelo (0.22 mg/g). Epicatechins and Epigallocatechin have the highest levels in samples n.78,n.90,- 0.49 mg/g, and 0.21 mg/g, respectectivly, and the lowest levels in samples n.87,83 – 0.16 mg/g. Finally, samples n.78,74, and 90 (0.08 mg/g) contain the highest levels of gallocatechin whereas samples n.89,83 (0.04 mg/g) the lowest levels.

Determination of antioxidant activity.

For the antioxidant analysis, 1 gram of Feijoa raw fruit was prepared in 100 ml of Ethyl alcohol. To determine the antioxidant properties of the extract 0.1mM of DPPH solution was added to the sample and after 30 minutes optical density was determined at 517 nm.

The indicator of the antioxidant activity of the fetus, gathered in different regions, has been determined and calculated for mg samples AA 50% inhibition (0.1mM of DPPH). The highest rate of inhibition was recorded in the sample n.78, harvested in the Guria region, 5,88 mg of which can produce 0.1mM of DPPH 50% inhibition. The lowest rate was observed in the sample n.88 in Adjara and the sample n.61 in Samegrelo (the fetus of 8.77 and 8.96 mg for 0.1mM of DPPH 50% inhibition). The antioxidant activity of other samples varied from 6.15 mg to 6.93 mg of the fetus for 0.1mM of DPPH for 50% inhibition (Table 5).

According to our previous investigation based on the ecological zones, any reliable statistics on differences in size and form have not been revealed (27). In the present studies, as shown in Table 3, the qualitative and quantitative content of flavonoids in the fetus of Feijoa is different according to regions. For example, the highest concentration of catechins and flavonoids are found in the Samegrelo region; the lowest amount was in the Guria region. Interesting a study conducted by Georgian scientist have revealed that geographical environment and genotype might play a prominent role in the composition of phenolic compounds (28).

The antioxidant activity of the Feijoa fruit is directly proportional to its chemical composition, namely, the phenolic compounds. Among the studied forms, the highest rate has been observed in the sample n.78. At the same time, the content of total phenolic compounds, as well as the content of flavonoid, glycosides and catechins is quite high (6.367, 1.45, 1.404 mg/g respectively). In the samples n.88,61 with the lowest antioxidant activity, the total amount of phenolic compounds (0.528, 2.464 mg/g respectively), flavonoid glycosides (0.58, 0.12 mg/g respectively) and the total amount of catechins (0.364, 0.115 mg/c respectively) is the lowest. The other samples, studied by us, obey the principle of regularity between the antioxidant activity and the components of the phenolic compounds.

The antioxidant activity is probably due to the presence of catechins, flavonoids, and other phenolic compounds. The raw material, containing antioxidants, can be easily dissolved in water and can represents a very important product for the diet. This is the characteristic of the Feijoa that is found in Georgia. They have very distinctive taste, and aroma. Because of the high amount of phenols, it is possible to produce functional food products, that are very good for health in general.

Our studies revealed that the fruits of different genotypes of Feijoa cultivated in Georgia contain a high amount of antioxidants important for human consumption. Finally, it should be noted that Feijoa has the following activities: antimicrobial, anti-inflammatory, antitumor, gastroprotective and hepatoprotective (3, 14, 29, 30).

Table 5. Antioxidant activity of Feijoa Samples mg Inhibition 50% of 0.1mM DPPH

Region	Sample number	Inhibition (50 %)
Adjara	88	8.77
	89	6.93
	90	6.87
Guria	78	5.88
	74	6.92
	71	6.90
Samegrelo	61	8.96
	73	6.76
	83	6.19
	87	6.44

Conflict of Interest: All authors have declared that they have no conflict of interest associated with this research manuscript.

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