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COMPARATIVE CONTENT OF BIOLOGICALLY ACTIVE SUBSTANCES IN APPLE FRUITS OF DIFFERENT GEOGRAPHICAL ORIGIN

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Abstract

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The purpose of the research was to study the biochemical composition of apple fruits on the basis of the analysis and selection of adaptive varieties and hybrids, suitable for organic gardening of fruits, not inferior to the world's best ones as to the content of PAS. The biochemical composition of 13 apple varieties of different geographical origin was studied by representative methods. The order of the accumulation of elements in the fruit and their total content were established. The total amount of elements in hybrid No. 1 is the highest among all the studied varieties. Antioxidant activity of the apples grown in the conditions of Moscow is 3–5% higher the antioxidant activity of imported varieties. Such perspective varieties as hybrid No. 1, 'Mayak Zagorya' and 'Legenda' are suitable for the production of organic products.

Key words: biochemical composition; apple fruits; organic production; perspective hybrids; physiologically; active substances

Introduction

The significance of apple fruits in human nutrition cannot be overestimated. They contain a large amount of biologically active substances, but, unfortunately, in order to obtain consumable output of the most of their commercial varieties, it is necessary to carry out chemical treatments and apply fertilizers, etc. that reduce their dietary and medicinal properties. In Russia, as elsewhere in the world in recent years a terminology and standards for organic products were developed, many agricultural enterprises start producing them. Unfortunately, widely known local and foreign apple varieties, in spite of their rich biochemical content, are not suitable for the commercial production of organic products.

The purpose of our research is to compare the biochemical composition of the best commercial and local new varieties of apple in order to identify the most suitable for production of organic products ones. In recent years, Russian

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specialized institutions carry out breeding work aimed at obtaining adaptive varieties that are rich in biologically active substances and genetically resistant to apple scab. Central Russia becomes more and more attractive for the production of organic products since it is possible to set intensive plantings with a vigorous root system, penetrating into the deeper layers of the soil on seedling virus-free rootstocks in this region. These plantations can yield industrial harvest without mineral fertilizers (orchards on vegetative bred rootstocks require mineral fertilization and irrigation). In central Russia due to the less intensive pests generations development it is also possible to reduce the number of treatments against them. It refers to such selection varieties of the institute as 'Marat Busurin', 'Legenda', 'Podarok Grafskomu', 'Mayak Zagorya' and the hybrid of institute No. 1.

Materials and Methods

The research carried in 2013–2015 in the Laboratory of Physiology and Biochemistry of Federal State Budgetary Scientific Institution All-Breeding Institute of Technology, Gardening and Nursery, Russia (FSBISI VSTISP). We studied 12 varieties of apple-trees of *Malus domestica* type*:* 3 varieties of vernacular selection (local, old Russian varieties) – 'Antonovka', 'Anis', 'Korichnoe'; 5 genotypes of FSBSI VSTISP selection – hybrid No. 1 and varieties 'Mayak Zagorya', 'Podarok Grafskomu', 'Marat Busurin' and 'Legenda'; 4 varieties of foreign selection – 'Lobo' grown in the Moscow region, 'Golden Delicious', 'Granny Smith', 'Royal Gala' (distributed in a retail network) and one Kitaika – Kerr variety origin of *Malus prunifolia*. In the course of preparation for biochemical studies the samples of fruit were pulverized with the help of homogenizer, weighings were prepared in accordance with procedures for each type of study. All measurements were performed in triplicate. Refractometrical method was used in order to determine the total amount of soluble solids (RDs, %), (GOST 28562–90, 2002) and titratable acidity, in accordance with the requirements of GOST (GOST ISO 750–2013, 2015). Ascorbic acid was extracted from the pulverized fruits by means 6% metaphosphoric acid, followed by 5-minutes centrifugation at 9 rev/min. The content of ascorbic acid was calculated by means of HPLC using a KNAUER liquid chromatograph (GOST 31643-2012, 2013). The total antioxidant activity of aqueous and alcoholic extracts (carbinol) was determined by DPPH in accordance with the official method of analysis (Rivero–Perez et al., 2007; Official Methods of Analysis, 2012). The method is based on the interaction of substances with a stable antioxidant radical chromogen 2,2-diphenyl-1-picrylhydrazil of a blue-violet colour (Gutteridge et al., 1986;Wolfe et al., 2003), using Helios ϒ spectrophotometer in the visible spectrum (λ = 517 nm). In the course of the reaction (interaction of DPPH with aqueous and alcoholic extracts of the fruit) the colour turns to weakly yellow or purple. This is due to the adjoining of hydrogen proton to DPPH molecule with the formation of DPPH – H. 0.0025% DPPH solution was used as the base solution. Antioxidant activity is calculated as a relative value and is determined by the ratio of extinction at the certain period of a reaction (10 minutes). DPPH inhibition rate was calculated using radical carbinol and aqueous solutions according to the following formula:

$$AA = A_0 - A_{10} / A_0 \times 100,$$

where A_0 – optical density of a radical solution, A_{10} – optical density of radical solution with a sample 10 minutes later.

The amount of chlorogenic and gallic acids in fruits was calculated by the method of high performance liquid chromatography (HPLC) using a KNAUER liquid chromatograph (Robbins, 2003; Millin and Marcs, 2007). 150 mm-long RP-HPLC leg, filled with Diasfer 110-C18 sorbent with 5 microns granulation, eluant composition and 0.03% trifluoroacetic acid – acetonitrile (70:30) – were used. The density was identified by retention time, the contents of individual substances was determined by calibration. The preparation of the samples designed for the determination of the mineral (cindery) composition was carried out in accordance with GOST 26929-86 (1986). Since the elements in fruits tissues are distributed inhomogeneously, prior EDS analysis the ashes were carefully triturated in a mortar and evenly spread on the analyzer table. The mass fraction of elements in the fruits ash was determined by energy-dispersive spectrometry using EDS analyzer with scanning electron JEOL JSM 6090 LA Japanese microscope. The microscope has 4 nm resolution at an accelerating voltage of 20 kV (image in secondary electron), zoom ranges from × 10 to × 300 000, beam current – up to 200 nA, elemental analysis – from B to U. Operating distance during elemental analysis (WD) amounts to 10 mm. The analysis of EDS spectrum and elements distribution can be easily performed. The receipt of spectrum and elements distribution data and display of images on the screen of scanning electron microscope occur together. The elemental composition was evaluated by the weight fraction of 13 elements – Na, Mg, Si, P, S, K, Ca, Cr, Mn, Fe, Cu, Zn and Se – which were reliably diagnosed.

The results are calculated on the basis of the analysis in quintuplicate (n = 5). The quadratic mean deviation did not exceed 1.2–6.9%. The samples of fruits for analysis were harvested at the stage of full maturity, thoroughly rinsed with deionized water. The average 50 g weighing of fruits segments was dried out in exsiccator at 80°C into airly dry state.

The dried samples were mineralized in a Naberterm muffle furnace (Germany) at T = 400°C. The ash derived was dispersed by ultrasound at a frequency of 18 kHz for 15 minutes. The dispergat was evenly spread on the objective table, covered with carbon tape. Energy-dispersive spectrometer makes it possible to perform quantitative X-ray microanalysis with a choice of area for analysis: at a point or areally generate element distribution maps. The system performs qualitative and quantitative analyses of the existing elements on the x-ray spectra obtained by scanning the electron beam on the observed image. The results of X-ray microanalysis are presented in the form of standard protocols, consisting of the sample area microstructure image, tables of data at the atomic or weight ratio, spectra and histograms. Figure 1 shows an example of spectrum. Ratio error of chemical analysis is as follows: less than 10% when the element content ranges between 1 and 5%; less than 5% error when the content ranges between 5 and 10%; less than 2% error when the element content is more than 10%. 100 ash areas of each sample were examined. Local analysis amounted to 3 mm, the scanning area – not less than 12 microns. Experimental data processing was performed by the method of mathematical statistics in *Office Excel 2003*.

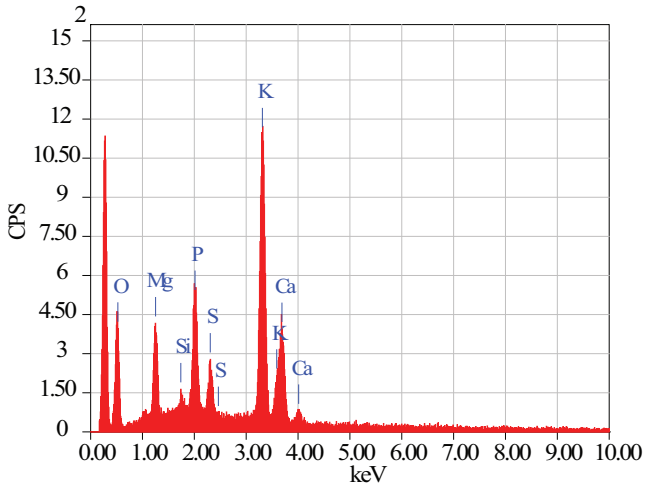

Fig. 1. General view of x-ray spectral lines that shows the presence of elements in the study area

Results

The results of the biochemical analysis of apples are presented in Table 1.

The content of phenol compounds, chlorogenic and gallic acids was defined by the method of high performance liquid chromatography (Figure 2).

The mass fraction of soluble solids in the primordial varieties of apples ranges from 9.38% (‘Antonovka’) to 13.94 (‘Korichnoe’). In the apples varieties of FSBSI VSTISP selection (hybrid No. 1, ‘Mayak Zagorya’, ‘Podarok Grafskomu’, ‘Marat Busurin’ and ‘Legenda’) the proportion of soluble solids ranges from 10.08% to 11.82%. The content of soluble solids in ‘Lobo’, ‘Golden Delicious’, ‘Granny Smith’ and ‘Royal Gala’ apple varieties ranges from 12.68% to 14.86%.

The registered shares of titratable acidity (the total amount of organic acids, contained in apples) ranged from 0.41% to 0.45% (‘Golden Delicious’ and ‘Korichnoe Polosatoe’) respectively to 1.20% (‘Antonovka’). The average

Table 1
Biochemical characteristics of the apple varieties under study, $\dot{X}$ (2014–2016)

Samples	RDs, %	Titratable acidity, %	Antioxidant activity, $EC_{50\%}$		Ascorbic acid mg 100 g^{-1}
			Aqueous extract	Ethanolic extract	
Antonovka obyknovennaya	9.38	1.20	10,12	20.25	6.46
Anise	11.76	0.60	23.04	31.6	7.18
Korichnoe Polosatoe	13.94	0.45	23.04	31.6	6.64
Kitaika	11.42	0.81	18.63	37.01	9.11
Hybrid No. 1	11.68	0.60	16.54	27,66	7.83
Mayak Zagorya	10.81	0.65	16,17	24.95	6.83
Podarok Grafskomu	10.42	0.64	14.46	26.82	7.57
Marat Busurin	11.82	0.62	16,15	28.98	6.61
Legenda	11.28	0.66	17.71	23.46	7.07
Lobo	12.72	0.60	22.75	23.85	7.38
Golden Delicious	12.68	0.41	17.02	26.51	5.77
Granny Smith	14	0.74	14.84	27.75	5.94
Royal Gala	14.86	0.54	14.62	26.62	7.91

Note: EC_{50} – antioxidant extract concentration which results in 50% inhibition of DPPH radicals

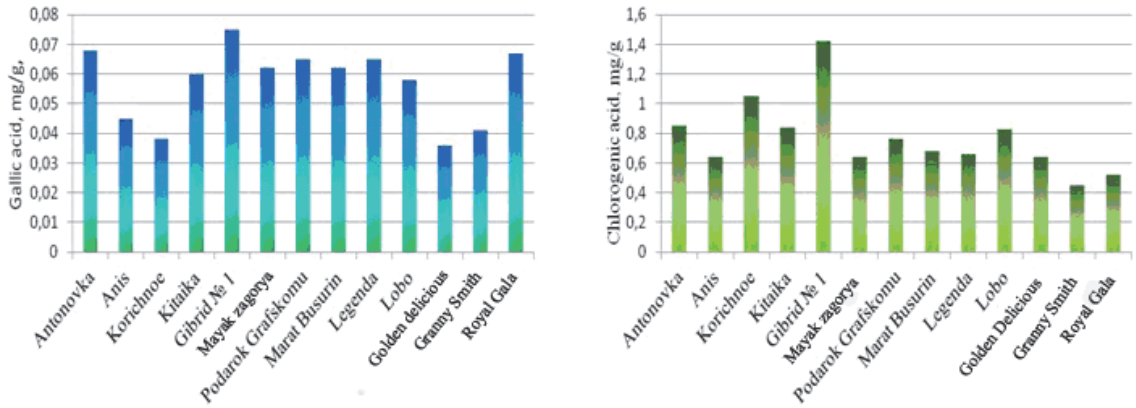

Fig. 2. Chlorogenic and gallic acids content in apples

share of titratable acidity in the apples grown in Moscow Region (Central Russia) ranges within 0.60–0.66%.

The antioxidant activity of ethanolic (carbinol) apple extract is on average 10% higher than the auqeous extract that shows their ability to dissolve both in water and in alcohol. Varieties 'Anise', 'Korichnoe' (23.04_{auq} – 31.61_{ethan})% and 'Kitaika-Kerr' (18.63_{auq} – 37.01_{ethan})% are characterized by high antioxidant activity. The aqueous extract antioxidant activity of the apple fruits of FSBSI VSTISP selection ranged from 11.46% to 16.54%, ethanolic extract – from 23.46% to 28.98%. The highest value was registered in the case of variety 'Lobo' grown in the conditions of the Moscow region. Such varieties as 'Golden Delicious', 'Granny Smith' and 'Royal Gala' showed high antioxidant activity – 24.75_{aq} – $33.85_{ethan.}$, 14.67_{aq} – 17.02_{ethan} and 26.51_{aq} – 29.75_{ethan}, respectively. The average antioxidant activity of the apples grown in the conditions of the Moscow region is higher than the one of imported varieties by 3–5%.

Ascorbic acid is a recognized antioxidant. Its content in the varieties grown in the conditions of the Moscow region is 1.2 times higher than in the imported varieties.

Discussion

It was established that there is relationship between the antioxidant activity of aqueous extract, titratable acidity ($r = 0.58549$) and antioxidant activity and the ascorbic acid content ($r = 0.468335$). The content of the most important biologically active substances in varieties 'Golden Delicious', 'Granny Smith' and 'Royal Gala' does not exceed the content of the same substances in new varieties and selection hybrids of the Institute.

The following apple varieties are characterized by the highest gallic acid content – hybrid No. 1 (0.075 mg g^{-1}), 'Antonovka', 'Kitaika', 'Mayak Zagorya', 'Podarok Grafskomu', 'Marat Busurin', 'Lobo' and 'Royal Gala' (0.063 mg g^{-1} on average). The content of gallic acid in varieties 'Granny Smith' and 'Golden Delicious' is 1.3–2 times higher than in hybrid No. 1. The content of chlorogenic acid in the fruits is on average 25–45% higher than the content of gallic acid. The maximum content of chlorogenic acid was found in hybrid No. 1(1.4 mg g^{-1}), 'Antonovka' and 'Kitaika-Kerr' (0.82 mg n^{-1}), 'Podarok Grafskomu' and 'Marat Busurin' (0.78–0.81 mg g^{-1}). The content of chlorogenic acid in apple varieties 'Royal Gala' and 'Granny Smith' is 2–3 times lower than in hybrid No. 1.

The order of mineral elements accumulation in fruits ash (in Table 2) is the following: K > P > Ca > Mg > S > Si > Na > Cu > Zn > Fe ≈ Se > Mn > Cr. The content of calcium in variety 'Mayak Zagorya' is more than two times higher. Hybrid No. 1 is characterized by the high content of the following electric elements: sodium, silicon (2.5 to 3 times higher), phosphorus, potassium, copper, zinc and especially selenium (more than by 20% higher than in the imported varieties).

Hybrid No. 1 contains the highest amount of elements among all the studied varieties but it is slightly inferior to the internationally recognized Golden Delicious variety. The elements form a series, characteristic of all varieties: K > P > Ca > Mg > S > Si > Na > Cu > Zn > Fe ≈ Se > Mn > Cr. The main share of mineral elements corresponds to K, the percentage of which in the fruits ranges from 21.36 to 25.59 wt% (variety 'Golden Delicious'). P share varies from 1.18 to 5.91 wt% (hybrid No. 1) in fruits ash. The share of Ca in the apple fruit ash ranges from 0.01 to 0.25 wt% (in 'Mayak Zagorya' fruits). The share of Mg in the fruits ash ranges from 2.04 to 3.73 wt% ('Granny Smith' fruit) (20). The share of Na in the fruits is not high and varies from 0.11 to 0.17 wt% (hybrid No.

Table 2
The ash content of apple fruits, mass %, Ẋ (2014–2016)

Element	The varieties under study						
	Antonovka	Hybrid No. 1	Mayak Zagorya	Granny Smith	Golden Delicious	Royal Gala	Variation coefficient %
Na	0.12	0.17	0.13	0.15	0.11	0.11	18.2383936
Mg	2.34	2.04	2.53	3.73	3.39	3.04	22.8905899
Si	0.14	0.32	0.11	0.15	0.18	0.11	46.8391136
P	4.32	5.91	3.64	5.46	5.28	1.18	40.3877517
S	0.36	0.49	0.38	1.04	1.07	0.34	56.4391118
K	21, 36	25.42	22.81	23.27	25.59	23.56	6.81182668
Ca	0.39	0.45	0.87	0.45	0.31	0.43	40.6838102
Cr	0.01	0.02	0.04	0.03	0.01	0.01	63.2455532
Mn	0.02	0.01	0.15	0,002	0.04	0.01	145.073683
Fe	0.0 3	0.0 7	0.05	0.04	0.04	0.02	41.3376342
Cu	0.09	0.25	0.11	0.02	0.01	0.01	113.928012
Zn	0.08	0.31	0.05	0.04	0.02	0.05	118.586719
Se	0.04	0.105	0.05	0.05	0,085	0,066	37.5439748
$\Sigma_{elements}$	29.28	35.51	30.89	34.43	36.14	28.94	

1). The share of S ranges from 0.34 to 1.07 wt%. Variety 'Golden Delicious' is characterized by the maximum sulphur share. The share Fe in the fruits varies from 0.02 to 0.07 wt% (hybrid No. 1). Cu and Zn shares don't exceed 0.5 wt%. The maximum share of Zn (0.21 wt%) and Cu (0.31 wt%) is registered in the fruits of hybrid No. 1. The proportion of manganese ranges from 0.01 to 0.15 wt% ('Mayak Zagorya'). Maximum content of selenium (0.105 wt%) was found in the mineral part of hybrid No. 1 fruits. The high coefficient of variation, which is characteristic for the content of such elements as S, Cr in ash, and especially Cu, Zn and Mn indicates that their accumulation is associated on the one hand with environmental conditions, on the other hand with adaptive properties of a particular type or variety. The average coefficient of variation in apple fruits (from 28.2 to 46.8%) is characteristic of such biologically significant elements like Na, Si, Fe, Si and Ca. Their accumulation limits are conditioned by their varietal characteristics. The low coefficient of potassium variation – 6.81% indicates the stability of this element accumulation in a crop and depends little on variety. The following correlations between elements were established: high correlation between Ca and Mg (r 0.75); average correlation between the accumulation of Ca and Cu (r 0.62).

Conclusion

As the result of the performed research, the comparative data on biochemical values was obtained (total antioxidant activity, content of ascorbic, gallic and chlorogenic acids, mineral status) for 13 varieties of apples of different environmental and geographical origin. By the complex of biologically active substances accumulated in fruits, there are promising hybrid No. 1, Mayak Zagorya and Legenda marked off.

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