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THE COMPARISON OF BIOCHEMICAL COMPOSITION OF *ACTINIDIA KOLOMIKTA* AND *ACTINIDIA POLYGAMA* FRUITS

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ABSTRACT

The demand for natural products, which are rich in biologically active compositions, grows constantly. The choice and production of such products can minimize the deficit of importance for human organism components, which are contained only in plant food. The paper contains the laboratory studying results of the chemical composition of the fruits of two *Actinidia* Lindl. cultivars of Federal State Budgetary Scientific Institution Federal Horticultural Research Center for Breeding, Agrotechnology, and Nursery (FSBSI FSC for Horticulture) genetic collection: *Actinidia kolomikta* (Rupr. et Maxim.) Maxim. and *Actinidia polygama* (Siebold et Zucc.) Maxim. All the presented samples are grown in field conditions. The fruits were picked up in the phase of harvest maturity while ripening. The data on antioxidant activity of water and methanol extracts, the content of phenolic compounds sum, soluble solids, and titratable acids in the fruits, and on qualitative composition of secondary metabolites (organic acids, fatty acids, mono-, di- and polysaccharides) are given in the paper. The variation limits of the parameters under study depending on the sample are presented. As a result of the laboratory studies, it was stated that *A. kolomikta* fruits 10 times exceed *A. polygama* fruits on all the stated parameters. Only the results on the soluble solids content in the fruits of both cultivars are approximately at the same level (*A. kolomikta* > *A. polygama* on 1.16%). The positive correlation between antioxidant activity and the general content of polyphenols is confirmed at both cultivars. *Actinidia kolomikta* genotypes Chempion and Lakomka and *Actinidia polygama* ones Tselebnaya and Uzorchataya showed the best results. The correct individual choice of actinidia fruits that are the best ones at the biochemical composition and the content of micronutrients allows supplying the consumers with food products.

Keywords: *Actinidia kolomikta*, *Actinidia polygama*, antioxidant activity, biochemical composition, fruits secondary metabolites

INTRODUCTION

The demand for natural products, which are rich in biologically active compositions, grows constantly. The most useful products are the fruits that contain the complex of biologically active compositions **(Yeomans, Linseisen and Wolfram, 2005)**. In various researches **(Wang, Cao, and Prior, 1996; Zulueta, Esteve and Frígola, 2009)** it is stated that the usage of fruits in the ration makes an essential contribution to the provision of the human organism with antioxidants and useful substances: carotenoids, phenolic compounds, sugars, acids, and others. Fruits and berries consumption decreases the development of chronic and heart diseases **(Tzulker et al., 2007; Xu et al., 2010; Liu et al, 2010)**, reduces the risk of cancer and has a positive effect on comparison with chemotherapy and hormone treatment **(Liu and Dong, 2008; Liu et al, 2010)**.

Nontraditional horticultural crops including actinidia plants play an essential role in the provision of a human organism with micronutrients. Representatives of *Actinidia* Lindl. species become more and more popular in the world thanks to a wide spectrum of possibilities of its fruits usage **(Titlyanov, 1969; Kolbasina, 2007)**. The most frost-resistant species of actinidia: *Actinidia kolomikta* (Rupr. Et Maxim.) Maxim., *Actinidia. arguta* (Siebold et Zucc.) Planch. ex Miq., *Actinidia polygama* (Siebold et Zucc.) Maxim, are of the utmost interest. They outstrip the most famous on the market species of Actinidia: *Actinidia deliciosa,* also known as the kiwi, in all the biochemical parameters **(Kim et al., 2009; Krupa et al., 2011; Zuo et al., 2012; Lee et al, 2015; Leontowiczet al., 2016; Wang et al., 2018).** The advantage of these fruits is not only in bright taste but also in the possibility of eating them with the skin in contrast with actinidia species with tomentose fruits. There are the data of the registered pharmaceutical composition from the extracts of *A. arguta*, *A. kolomikta,* and *A. polygama* for prophylaxis and treatment of some immune and non-allergic inflammatory diseases **(Latocha, 2017)**.

Figure 1 Actinidia plants and fruits.
Note: A – Actinidia plantations. B – *Actinidia Polygama* fruiting, Perchik sample.

On the territory of Russia *Actinidia* Lindl. species are still considered as a rare and nontraditional small-fruit crop. They are not introduced in industrial culture, but at the same time, they are some of the most perspective crops thanks to rich biochemical composition, longevity, and low maintenance of the plants **(Kozak and Imamkulova 2018)**. Ivan Vladimirovich Michurin received the first domestic cultivars thanks to the primary introduction of lianas. Further on, Ella Ioganovna Kolbasina made an essential contribution thanks to her *Actinidacea in situ* and breeding studies **(Kolbasina et al., 2007)**. At present, FSBSI FSC for Horticulture is one of the leading research centers of the Russian Federation that keeps in live conditions and breeds the samples collection of Far Eastern species of actinidia **(Kozak et al., 2017; Burmenko, et al., 2018)**.

Despite the studies of actinidia fruits' biochemical composition, the question of the secondary metabolites content and identification get increased attention only in the last decades. However, there is no enough publicly available information about the biochemical parameters of *A. polygama* fruits. Local and industrial cultivars are mostly studied. The comparative data of the biochemical composition of *Actinidia kolomikta* and *Actinidia polygama* fruits, grown in the Central region of Russia, are fragmentary.

Our work aims to compare the biochemical composition of *A. kolomikta* and *A. polygama* fruits from FSBSI FSC for Horticulture genetic collection to specify the best sources with the highest biochemical potential for further breeding.

Scientific hypothesis

The study of the biochemical composition of rare fruit and small-fruit crops fruits is actual. Actinidia is a valuable material for studying thanks to the rich content of biologically active substances and unique organoleptic characteristics of its fruits. We suppose that *Actinidia kolomikta* and *Actinidia polygama* fruits should have different cross-species and intraspecific biochemical compositions.

We planned to reveal the differences in the chemical composition of two Actinidia species: *A. kolomikta* (Rupr. Et Maxim.) Maxim. and *A. polygama* (Siebold et Zucc.) Maxim., grown in the conditions of the Moscow region, on six parameters that form the quality and the nutritional value of the fruits. Based on the held laboratory experiments the best samples of the quality content will be revealed.

MATERIAL AND METHODOLOGY

The research were held in 2019-2020 on *Actinidia* Lindl. experimental plantings of (Federal Horticultural Research Center for Breeding, Agrotechnology, and Nursery (FHRCBAN), Moscow region, Michnevo).

The collection was formed on argillaceous sod-podzolic hard soil. The total area of the plantation is 2 ha, it was planted on the 4 x 2 m (Figure 1).

Samples

The studied objects were the fruits of six cultivars of *A. kolomikta* (Rupr. Et Maxim.) Maxim.: Chempion, Lakomka, Sestra, Vinogradnaya, Uslada, Prazdnichnaya and the fruits of six cultivars of *A. polygama* (Siebold et Zucc.) Maxim.: Ostropryanaya, Celebnaya, Osennyaya, Perchik, Uzorchataya, Krasna Devica.

The samples were taken at the fruits ripeness stage.

The biochemical researches were held at the Laboratory of Physiology and Biochemistry of Federal State Budgetary Scientific Institution Federal Horticultural Research Center for Breeding, Agrotechnology, and Nursery.

Chemicals

All chemical substances chosen for the analysis were of an analytical sort and were bought from Sigma Aldrich (USA).

Instruments

Homogenizer IKAA11 basic (Germany), centrifuge Sigma 2-16P (Germany), pH meter HI 2211 HANNA (Germany), shaker Lab-PU-01 (Russia), GC-MS chromatograph JMS-Q1050GC (JEOL Ltd, Japan) with capillary column DB-5HT (Agilent, USA), spectrophotometer Helios ɣ (Thermo scientific, England).

Laboratory Methods

SSC was determined via refractometric method according to **GOST ISO 2173 (2013)**. TTA was estimated via the potentiometric method according to **GOST ISO 750 (2013).**

The total phenolics amount was determined according to the method described by **Velioglu et al. (1998).**

The scavenging activity on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined spectrophotometrically according to the method described by **Brand-Williams et al., (1995).**

We used a method described by **Robbins (2003)** for the derivation of samples**.**

The substances identification was done according to NIST-5 National Institute of Standards and Technology (USA).

Sample preparation

300g of fruits were prepared from the representative not less than 500g probe. The mass was homogenized using the analytical homogenizer. Then it was extracted by double distilled water and by pure methanol and centrifugated at 4000 g within 10 min. The supernatant was used for measurements purposes. To study the fruits' metabolic profile a methanol extract was used.

Basic chemical analyses

General biochemical parameters, i.e. soluble solids content (SSC) and total titratable acidity (TTA) were studied. SSC was determined via the refractometric, method, the values were expressed in%. TTA was estimated via the potentiometric method by pH meter via titrating with 10 N. NaOH and expressed in the equivalent of apple acid, %.

Total phenolic compounds analysis

The total phenolics amount was determined with Folin–Ciocalteu reagent according to the method**.** A standard curve with gallic acid was used. Different concentrations of gallic acid were prepared in distilled water, and absorbance was recorded at 750 nm. 100 µL of a diluted sample (1:10) was dissolved in 500 µL of Folin–Ciocalteu reagent and

1000 µL of distilled water. The solutions were mixed and incubated at room temperature for 1 min. After 1 min, 1500 µL of 20% sodium carbonate (Na_2CO_3) solution was added. The final mixture was shaken and then incubated for 2 h in the dark at room temperature. The absorbance was measured at 750 nm using a Helios Y UV–vis spectrophotometer and the results are expressed in mg of gallic acid (GEA) calculated on the wet weight of plants.

Total antioxidant capacity scavenging activity on the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined spectrophotometrically according to the method**.** The principle of the analysis was based on the color change of DPPH solution from purple to yellow as the radical was quenched by antioxidants. The homogenized leaves were mixed with distilled water and methanol. The samples were put on the shaker Lab-PU-01 (Russia) for 6 hours, and then they were filtered and the antioxidant activity was measured in 10 minutes after interaction between the extract and the reagent. The absorbance was recorded at 515 nm to determine the concentration of the remaining DPPH. All measurements were performed in triplicate. The radical-scavenging activity was calculated as a percentage as follows:

DPPH radical-scavenging (%)= [(AC - AAt) / AC]· 100, where;:
AC – DPPH solution absorption;
AAt – absorption at the antioxidant presence.
The lower absorbance of the reaction mixture indicates a higher level of free radical scavenging activity.

Metabolic analysis by gas chromatography-mass spectrometry

The metabolites analysis was fulfilled using the method of gas chromatography-mass spectrometry (GC-MS) via GCMS chromatograph. Capillary column DB-5HT; length 30 m, inner diameter – 0.25 mm, the film thickness – 0.52 um, and gas-carrier – helium) was used. The temperature gradient during the analysis was within 40 – 280°C, the injector and interface temperature – 250°C, the ionic source – 200°C. Gas flow in the column was equal to 2.0 mL/min, split-flow injection mode, sample injected in volume 1 – 2 mcl of the evaporated extract. The analysis was held for 45 min. The derivation was held using silylation reagent N,O bis (trimethylsilyl) trifluoracetamide (BSTFA). The substances identification was done according to NIST-5 National Institute of Standards and Technology (USA) retention behavior and mass spectra the scanning range was 33-900 m/z. The substance identification credibility was within 75-98%.

Statistical Analysis

All the analyses were performed in triplicate. The results were expressed as mean values (n = 3) in standard deviation (SD). Statistical analyses were carried out through the Excel package (Microsoft Excel, v. 2016).

RESULTS AND DISCUSSION

The laboratory studies showed that the difference of SSC in the fruits of the species under study was not big. On average, SSC depended on the species and varied from 17.78% (Uzorchataya) to 20.25% (Krasna Devica) at *A. polygama* (Figure 2.) and from 15.97% (Prazdnichnaya) to 22.50% (Chempion) at *A. kolomikta* (Figure 3).

On average, the SSC value of the fruits was 19.80% at *A. kolomikta* and 18.64% at *A. polygama*.

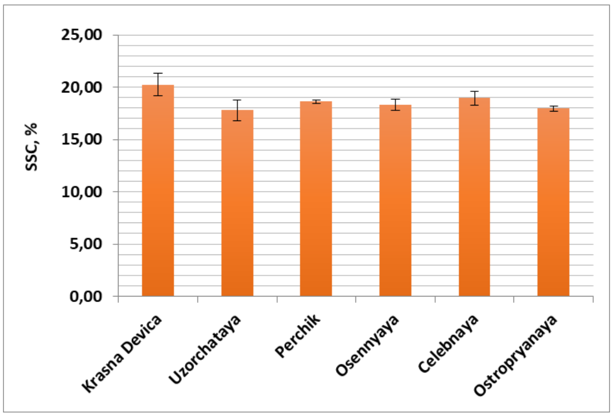

Figure 2 Soluble solids content in *Actinidia polygama* (Siebold et Zucc.) Maxim. fruits at average in 2019 – 2020; each value represents the mean of three independent experiments (±SD).

A. kolomikta fruits samples Chempion and Sestra showed the highest values (Figure 3).

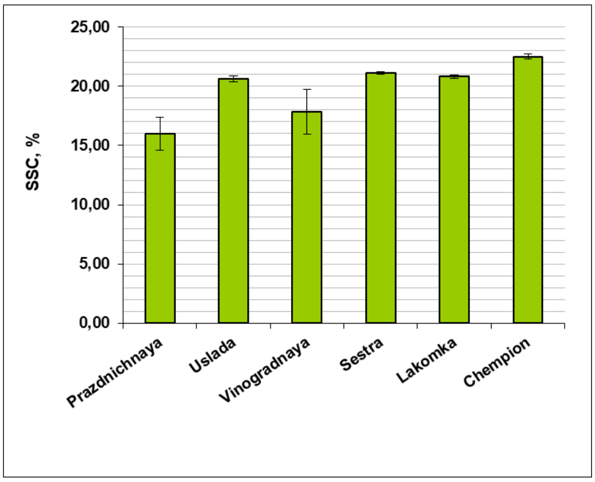

Figure 3 Soluble solids content in *Actinidia kolomikta* (Rupr. et Maxim.) Maxim. fruits at average in 2019 – 2020; each value represents the mean of three independent experiments (±SD).

It is known that the fruits characteristic taste is determined by the ratio of the sugars and organic acids content **(McBride, Johnson, 1987)** that is considered to be especially useful as an index of acceptability for a lot of kinds of fruit **(Esti et al., 1998)**. The sugar content, acidity, the content of vitamin C, and other nutrients are essential parameters to evaluate the quality and taste; high levels of SSC, TAC, and SAR often indicate the best taste of the fruits **(Esti et al., 1998**; **Wu et al., 2003; Ivanovaet al., 2021)**.

Citric and Quinic acids are indicated in the fruits of several actinidia species, and their content predominates over Apple acid **(Nishiyama et al., 2008)**.

Table 1 Antioxidant activity (AA) and total phenolic compounds (TPC) of *Actinidia* Lindl fruits. Values represent the mean of three replicate ± SE.

Species, sample name	AA of ethanol extraction, %	AA of water extraction, %	TPC, mg gallic acid (GEA) / 1g fruit weight
Actinidia kolomikta			
Vinogradnaya	95.15 ±0.174	95.39±0.192	7.747±0.166
V, %	0.45	0.49	5.23
Prazdnichnaya	94.99±0.214	95.76±0.290	9.727±1.102
V, %	0.55	0.74	27.76
Uslada	94.23±0.465	94.92±0.555	8.228±0.946
V, %	1.21	1.43	28.17
Sestra	94.06±0.395	94.06±0.450	8.166±0.160
V, %	1.03	1.17	4.79
Lakomka	93.19±0.080	92.17±0.929	10.253±0.520
V, %	0.21	2.47	12.41
Chempion	94.80±0.172	94.78±0.125	9.956±0.341
V, %	0.44	0.32	8.39
Actinidia polygama			
Ostropryanaya	25.35±1.924	8.33±0.723	0.836±0.122
V, %	18.59	21.26	35.68
Celebnaya	33.52±3.406	10.30±1.523	0.916±0.201
V, %	24.89	36.23	53.76
Osennyaya	25.58±1.214	7.57±0.674	0.733±0.096
V, %	11.63	21.82	32.02
Perchik	31.39±3.098	11.81±2.177	1.193±0.311
V, %	24.17	45.14	63.85
Uzorchataya	28.30±5.607	9.53±2.117	1.179±0.391
V, %	48.53	54.42	81.29
Krasna Devica	20.27±0.811	7.85±0.366	0.561±0.128
V, %	9.80	11.43	55.17

A low concentration of acids in *A. polygama* fruits in combination with a rather high content of Ascorbic acid and carotenoids make them unique and indispensable in dietetic nutrition in particularly for people with hyperpeptic stomach diseases **(Kozak et. al., 2018)**.

The content of titratable acids in the samples under study depends on the species and varies within 0.57 to 2.98% (Figure 4).

TTA of *A. kolomikta* fruits was 1.75% at average that notably higher in comparison to the average TTA value of *A. polygama* fruits (in 2.13 times). In the fruits of *A. kolomikta* samples, the maximum content of TTA was stated at Sestra and Prazdnichnaya cultivars, the minimal one – at Vinogradnaya cultivar. Among *A. polygama* fruits Ostropryanaya sample showed the high result on TTA (1.03%), the minimum value was stated at Uzorchataya genotype fruits (0.57%).

Antioxidant activity of water and ethanol extracts of *A. kolomikta* fruits is at a high level and does not show essential differences depending on extraction type. On average on *A. kolomikta* species in 2019-2020 AA of methanol extracts was 94.40%, AA of water ones – 94.51%. An essential difference in AA values depending on extraction type was stated at *A. polygama* fruits. On average AA of methanol extract was 2.5 – 3.5 times higher than water one that can be connected with quantitative differences of substances, extracted by water and methanol.

AA values of *A. polygama* fruits vary from 20.27% (Krasna Devica cultivar) to 33.52% (Celebnaya cultivar) at methanol extraction.

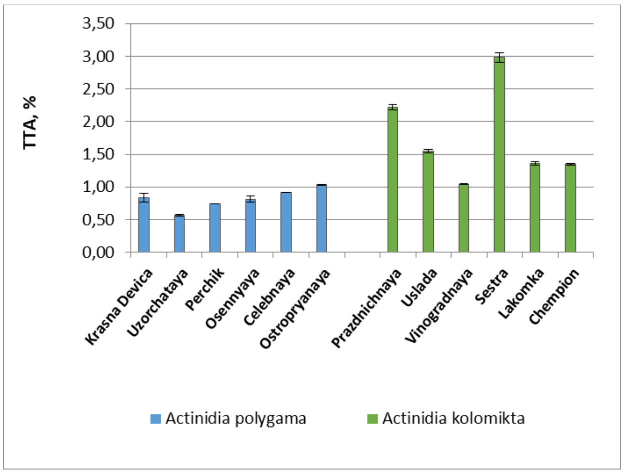

Figure 4 Titratable acidity content in actinidia fruits at average in 2019 – 2020; each value represents the mean of three independent experiments (±SD).

The AA values of water extraction – from minimal 7.57% at Osennyaya cultivar to maximum 11.81% at Perchik cultivar. On average on the species in 2019-2020 AA of methanol extract at *A. polygama* fruits was 27.40%, AA of water extract – 9.23%, that is 3.5 and 10 times lower than the values of *A. kolomikta* fruits relatively. On average the minimal values of AA were stated at *A. polygama* fruits of Krasna Devica cultivar (Table 1).

The results variability (V, %) during the studies of the fruits AA for *A. kolomikta* genotypes was insignificant, for *A. polygama* ones – for the methanol extracts of Krasna Devica sample and the methanol extracts of Osennyaya and Ostropryanaya samples fruits at an average level. For the rest genotypes, the variability of AA was essential (Table 1)

The majority of literary sources explain the essential difference of total antioxidant activity between species and samples by a high correlation of the vitamin C content and phenolic compounds thanks to the quick interaction of Ascorbic acid with DPPH.

Polyphenolic compounds, contained in fruits, vegetables, and grain crops, are secondary metabolites and are famous for their curative properties **(Biglari et al., 2008; Gan and Latiff, 2011; Gong et al., 2012)**. Despite polyphenol's wide-spreading in plants, researchers and manufacturers of food products have started to be interested in polyphenols only in recent years. This interest is due to a wide spectrum of pharmacological properties, antimicrobial and anti-inflammatory activity of polyphenols **(Cai et al., 2004; Scalbert et al., 2005)**, and their strong effect in preventive actions to reduce various diseases associated with oxidative stress **(Manach et al., 2004; Rasmussen et al., 2005; Darvesh et al., 2010)**.

In our studies, *A. kolomikta* fruit's TPC was almost 10 times (9.98) higher than the average *A. Polygama* species TPC. Among the studied *A. kolomikta* genotypes the highest TPC value in 2019-2020 was stated at Lakomka sample fruits (10.25 mg gallic acid (GEA)/1g fruit weight), the lowest one – at Vinogradnaya (7.75 mg gallic acids (GEA)/1g fruit weight). Among the studied *A. polygama* genotypes the best TPC results were registered at Perchik and Uzorchataya samples fruits. The essential variability of the results is characteristic for these samples as well (Tab. 1). Krasna Devica fruits showed the minimum TPC value – 0,56 mg gallic acid (GEA)/1g fruit weight.

The received results can be explained by the differences of bioactive compounds concentrations that vary depending on ecological factors such as climatic and soil conditions **(Pavarini et al., 2012)**, and on cultivar differences as well **(Kim et al., 2005; Usenik et al., 2008)** and the degree of fruit ripeness **(Manach et al., 2004; Pandey, Rizvi, 2009; Babou et al., 2016)**.

We conducted the metabolic profiles identification of fruits methanol extracts of 2 Actinidia species samples received via GC-MS analysis. In this paper, we do not give the full list of the identified secondary metabolites of the studied samples but describe three groups in detail, i.e. organic acids, fatty acids, and carbohydrates (sugars in particular).

Organic acids together with sugars are the basic soluble components of ripe fruits cause an essential influence on sourness-sugariness redounding the aroma formation **(Neri, Pratella and Brigati, 2003)**. A lot of fruits on certain stages of ripening accumulate organic acids in their flesh. The main part of this content is one or two acids. Even though the most widely spread organic acids in fruits are Citric and Malic acids, the variety depends on the studied crop**.** The

Table 2 Comparative composition of acids and carbohydrates *Actinidia* Lindl. fruits.

Species, sample name	Organic acids	Fatty acids	Ketoses and their derivates	Aldoses and their derivates	Disaccharides	Substances quantity in all the studied groups, total
Actinidia kolomikta						
Vinogradnaya	5	1	5	4	1	16
Prazdnichnaya	6	-	5	4	1	16
Uslada	6	-	5	5	1	17
Sestra	5	-	5	5	1	16
Lakomka	7	-	5	6	1	19
Chempion	7	1	6	5	2	21
Actinidia polygama						
Ostropryanaya	8	-	5	4	2	19
Celebnaya	14	4	6	11	7	42
Osennyaya	-	1	3	3	1	8
Perchik	10	1	4	8	3	26
Uzorchataya	17	3	7	9	7	43
Krasna Devica	8	1	5	7	2	23

main part of the present acids in fruits flesh is formed from the synthesized sugars **(Sweetman et al., 2009; Etienne et al., 2013)**. The basic acids are formed and take part in breathing processes, gluconeogenesis, fermentation till ethanol, amino acids synthesis/interconversion, and pigments synthesis **(Famianiet al., 2000; Famiani et al., 2005; Famiani et al., 2007; Famiani et al.,2014; Sweetman et al., 2009; Etienne et al., 2013)**.

The total and an individual number of identified components in methanol extracts of the actinidia studied samples fruits is presented in Table 2. According to the data of **Heatherbell (1975)** *Actinidia chinensis* fruits contain the essential amount of Chinic acid in ripe fruits. Small amounts of Phosphoric, Ascorbic, Glucuronic, Galactouronic, Oxalic, Amber, and p-Coumaric acids can be determined as well. **Wojdyło et al., (2017)** identified 24 polyphenolic compounds in *A. arguta* fruits. **Lim et al. (2006)** wrote about the presence of several compounds in *A. arguta* fruits, i.e. Protocatechic and Caffeic acids, β-D-glucopyranoside, Esculin, Coumarin, and three flavonoids. In the paper of **Ren, Han and Chung (2007)** it is said about the extraction and identification of polyunsaturated fatty α-Linolic acid in *A. polygama* fruits. **Motyleva et al., (2018)** identified ten organics and three fatty acids in water extracts of actinidia fruits.

However, the number of papers on metabolomic profiling of actinidia fruits is not enough, the main problem of many authors is orientation on local cultivars. Also, there is not much data on metabolomic sugar content in actinidia fruits. In the papers of **Heatherbell (1975)** and **Latocha (2017)** it is mentioned about only several sugars, such as Glucose, Fructose, and Sucrose.

In our paper, the basic common components of actinidia fruits metabolomic profiles are: acids – Pyruvic, Lactic, Itaconic, Fumaric, Malic, Erythronic, Citric, and Erythro-Pentonic; one fatty acid – Acrylic acid, thirteen monosaccharides (four of which belong to ketoses, and nine ones – to aldose) and two disaccharides (Turanose, Sucrose). Various secondary metabolites identified in the samples of this paper are found individually and can serve as cultivar markers. Thus, for example, Erythrose is found only in *Actinidia polygama* fruits of Celebnaya sample*,* as well as Lyxoses are found only in *Actinidia polygama* fruits.

CONCLUSION

The fruits component composition and biochemical parameters depend not only on actinidia species, but on the sample as well. As a result of the studies it can be concluded that Chempion and Lakomka genotypes of *Actinidia kolomikta*, as well as Celebnaya and Uzorchataya ones of *Actinidia polygama* are the most perspective genotypes for breeding from the point of view of fruits qualitative properties (SSC, TAA and TPC, metabolomics analysis of secondary metabolites). These samples are also worth noticing from the point of view of using in functional nutrition.

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