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Qualitative and quantitative composition of triterpenic compounds in the fruit of apple old cultivars grown in Lithuania

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Abstract

The interest in old cultivars of apple trees, their fruit and processed products is growing worldwide. Studies on the qualitative and quantitative composition of biological compounds are important for the evaluation of the quality and nutritional properties of apple fruit. A variation in the qualitative and quantitative composition of triterpenic compounds was found in the fruit of apple cultivars included in the collection of National Plant Genetic Resources. A high-performance liquid chromatography (HPLC) analysis showed that the fruit of the cultivar 'Birutės pepinas' had the highest total amount (5.17 ± 0.86 mg g^{-1}) of triterpenic compounds. Higher total amounts of triterpenic compounds were also found in the fruit of apple cultivars 'Tabokinė' and 'Panemunės baltasis' (3.72 ± 0.57 and 4.25 ± 0.17 mg g^{-1}, respectively). By the quantitative composition, triterpenic compounds in apple fruit were ranked in the following order: ursolic acid > oleanolic acid > corosolic acid > betulinic acid.

The old apple cultivars 'Birutės pepinas', 'Panemunės baltasis' and 'Tabokinė' included in the collection of National Plant Genetic Resources have a potential for cultivation in industrial orchards and for the use of their apples, and processed apple products as natural functional foods rich in triterpenic compounds and adapted for medical purposes, including the prevention of various diseases.

Key words: apple, old cultivars, triterpenic compounds.

Introduction

Plants of the apple (*Malus* Mill.) genus are most common in moderate climate zones of Central Asia, Europe and Northern America (De Paepe et al., 2015). Domesticated apple trees (*Malus domestica* Borkh.) have been grown worldwide for at least 4000 years. The first written data on Lithuanian orchards, where apple trees were grown date back to 1387 (Kviklys et al., 2020). Part of the apple cultivars grown in Lithuania are thought to have been selected from naturally growing forest apple tree (*Malus sylvestris* (L.) Mill.) saplings (Blažytė, 2008; Bivilienė et al., 2010). Scientific data suggest that the old cultivars are more resistant to winter cold, fungal infections and other pathogenic conditions, are more adapted to the local climatic conditions and ripen fruit within a short vegetation period (Contessa, Botta, 2016). Apples of the old historically grown cultivars are nutritious and of high quality (Lo Piccolo et al., 2019), and they accumulate higher phenolic and triterpenic compounds compared to apple fruit of the cultivars grown in industrial orchards (Grigoras et al., 2013; Padua et al., 2014). Studies have shown that the amount of triterpenic compounds in the apples of the old cultivars is twice that found in the fruit of apple trees grown in industrial orchards (Lo Piccolo et al., 2019; Sut et al., 2019). The triterpenic acids (ursolic, oleanolic, corosolic and betulinic) detected in apple fruit are characterized by versatile biological effects, i.e. they reduce blood levels of low density lipoprotein cholesterol and glucose, suppress atherosclerotic plaque formation and inflammatory processes and have an antineoplastic, antibacterial and antiviral effect (Allouche et al., 2010; Jeong et al., 2015; Waldbauer et al., 2016).

The last decades have seen a highly intensive development of commercial horticulture (Kviklys et al., 2020). The apple cultivars grown in the USA industrial orchards: 'Golden Delicious', 'Delicious', 'Cox's Orange Pippin', 'Rome Beauty', 'Granny Smith', 'McIntosh',

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'Jonathan', 'Braeburn', 'Fuji', 'Gala' and 'Jonagold', have replaced the old ones (De Paepe et al., 2015). According to the data of the study, of the 7 098 apple cultivars cultivated between 1804 and 1904 in the USA, approximately 86% has been lost. In Europe, there still remains the heritage of the genetic resources of the old fruit-trees consisting of cultivars grown in private orchards.

As the interest in old cultivars of apple trees, their fruit and processed products is growing worldwide, studies of the qualitative and quantitative composition of biological compounds are important for the evaluation of the quality and nutritional properties of the apples. A search of scientific literature did not yield any studies on the composition of the active compounds in the fruit of old apple tree cultivars grown in Lithuania. Studies on the variations in the chemical composition of triterpenic compounds characterized by a versatile biological effect are important, when researching the genetic heritage of the old cultivars in order to increase the cultivation of such cultivars in orchards. The data of such studies will provide new scientifically important knowledge about the variations in the qualitative and quantitative chemical composition of biologically active compounds in apple fruit of the old cultivars. Target-oriented detailed studies of the phytochemical composition of triterpenic compounds will serve as a basis for the recommendation of the apple tree cultivars that would be most suitable for Lithuanian climatic conditions. Due to the content of biologically active compounds, apples of the old cultivars may be rationally used for food as well as for the manufacturing of high-quality food products. The data of such studies will provide new scientifically important knowledge about the variations in the qualitative and quantitative chemical composition of biologically active compounds in apple fruit of the old cultivars.

The aim of the study was to evaluate the variations in the qualitative and quantitative composition of triterpenic compounds in the apple fruit of the old apple cultivars grown in Lithuania.

Materials and methods

The experiment included 22 old apple cultivars, of which 21 (except for 'Golden Russet') are included in the collection of the National Plant Genetic Resources (Table).

The apple trees were grown in the Collection of the Apple Tree Genetic Resources at the Institute of Horticulture (55°60′ N, 23°48′ E), a division of the Lithuanian Research Centre for Agriculture and Forestry. The research was conducted in 2019 and 2020.

Chemicals. All solvents, reagents and standards used were of analytical grade. Acetonitrile, acetone, ursolic acid, oleanolic acid, betulinic acid and corosolic acid were obtained from Sigma-Aldrich GmbH (Switzerland). Purified deionized water used in the tests was prepared with the Milli-Q® (Millipore, USA) water purification system.

Table. Origin and properties of the old apple cultivars of Lithuania

Cultivar	Year of introduction, discovery or description, country	Summer cultivar	Autumn cultivar	Winter cultivar	Other exclusive properties
Avenarijus	1886, Russia	+	–	–	sweet; flesh pink under the skin; susceptible to canker
Baltasis alyvinis	1848, Russia	+	–	–	white flesh, yellow skin; aromatic; susceptible to scab
Beržininkų ananasinis	1886, Lithuania	–	+	–	crispy flesh, yellow skin; aromatic; scab-resistant
Birutės pepinas	1941, Lithuania	–	+	–	white flesh, with suspicion of wine; susceptible to scab
Biržuvėnų žieminis	Lithuania	–	–	+	sweet; yellow skin; scab-resistant
Danų karalienė Luiza	1878, Denmark	–	–	+	creamy yellow flesh, skin covered with a rust grid; scab-resistant
Geltonasis arkadas	19th century, Russia	+	–	–	sweet, sometimes astringent; susceptible to scab
Golden Russet	1800–1849, USA	–	–	+	strong russet skin, creamy yellow flesh; scab-resistant
Jono pepinas	19th century, Lithuania	–	–	+	firm, yellow flesh; scab-resistant
Koštelė	19th century, Poland	–	+	–	sweet; firm and creamy flesh; scab-resistant
Lietuvos pepinas	18th century, Lithuania	–	–	+	vinous taste, white flesh; susceptible to scab
Montvilinis	1879, Lithuania	–	–	+	aromatic; scab-resistant
Paprastasis antaninis	18th century, Russia	–	+	–	acidic, very aromatic; moderately scab-resistant
Panemunės baltasis	1939, Lithuania	–	+	–	white flesh, greenish-yellow and waxed skin; scab-resistant
Pilkasis alyvinis	1653, Russia	+	–	–	white flesh; susceptible to scab
Popierinis	~1852, Lithuania or Latvia	+	–	–	white-yellow skin, white flesh; susceptible to scab
Raudonasis alyvinis	18th century, Russia	+	–	–	aromatic, susceptible to scab
Rudens dryžuotasis	~1870, Baltic countries	–	+	–	vinous taste, aromatic; flesh pinkish under skin; moderately scab-resistant
Sierinka	~1860, Baltic countries	–	+	–	fragrant with characteristic aroma; susceptible to canker, moderately scab-resistant
Tabokinė	19th century, Baltic countries	–	–	+	bitter-sweet, bitterness weakens by spring; scab-resistant
Virginijos rožinis	1816, Europe	+	–	–	vinous taste; susceptible to scab
Žemaičių grietininis	19th century, Lithuania or Latvia	+	–	–	white flesh; moderately scab-resistant

Preparation of samples. For the analysis, 20 apples were picked from different parts of the tree crown at the optimal maturity stage. Whole apples were immediately frozen in a freezer (at −35°C temperature) with air circulation. Subsequently, the frozen samples were lyophilised with a ZIRBUS sublimator 3 × 4 × 5/20 (ZIRBUS technology GmbH, Germany) at a pressure of 0.01 mbar (condenser temperature −85°C). The lyophilised samples were ground to fine powder by using a mill electric grinder Retsch 200 (Germany). Loss on drying before the analysis was determined by drying the apple lyophilisate in a laboratory drying oven to complete the evaporation of water and volatile compounds (at 105°C temperature; the difference in weight between measurements up to 0.01 g) and by calculating the difference in raw material weight before and after drying. The data were recalculated for the absolute dry lyophilisate weight. The prepared apple samples were stored in dark, tightly closed glass vessels.

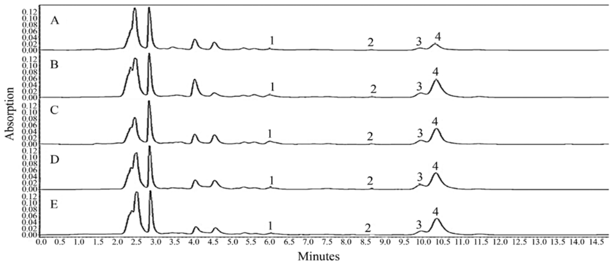
Extraction. During the analysis, 1 g of lyophilisate powder (exact weight) was weighed, added to 10 mL of acetone and extracted in a ultrasonic bath Sonorex Digital 10 P (Bandelin Electronic GmbH & Co. KG, Germany) at a room temperature for 10 min. The conditions of the extraction were chosen based on the results of the tests for setting the extraction conditions. The obtained extract was filtered through a paper filter, and the residue on the filter was washed with acetone in a 10 mL flask until the exact volume was reached.

Instrumentation and chromatographic conditions. A chromatograph equipped with a photodiode array (PDA) detector Waters 2998 (Waters, USA) was used for high-performance liquid chromatography (HPLC) analysis. Chromatographic separations were carried out by using an ACE (advanced column engineering) column, 5 µm, C18, 250 × 4.6 mm inner diameter. The column was operated at a constant temperature of 25°C. The volume of the analysed extract was 10 µL. The flow rate was 1 mL min^{-1}. The mobile phase consisted of acetonitrile (solvent A) and water (solvent B); the isocratic elution, the eluent ratio being 88% (solvent A) and 12% (solvent B) was applied. For the quantitative analysis, the calibration curve was obtained by injecting the known concentrations of different standard compounds. All the identified triterpenic compounds were quantified at 205 nm wavelength (Butkevičiūtė et al., 2018).

Statistical analysis of the experimental data was performed by using software *Microsoft Office Excel* (Microsoft, USA) and *SPSS*, version 25.0 (SPSS Inc., USA). All the results obtained during the HPLC analysis were presented as means of three consecutive test results and standard deviations. To evaluate the variance in the quantitative composition, the coefficient of variation was calculated. Univariate analysis of variance (ANOVA) was applied to determine, whether the differences between the compared data were statistically significant. The hypothesis about the equality of variances was verified by Levine's test. If the variances of independent variables were found to be equal, Tukey's multiple comparison test was used. The differences were regarded as statistically significant at $p < 0.05$. The comparison of the chemical composition between the apple samples was carried out by applying the hierarchical cluster analysis using the squared Euclidean distance. Principal component analysis (PCA) was performed as well.

Results and discussion

The conducted research determined the variation in the qualitative and quantitative composition of triterpenic compounds in fruit samples of the old apple cultivars grown in Lithuania. In apple fruit, the following triterpenic compounds were detected and qualitatively evaluated: betulinic acid, corosolic acid, oleanolic acid and ursolic acid; retention times of these acids were 6.03, 8.71, 9.92 and 10.40 min, respectively (Fig. 1).

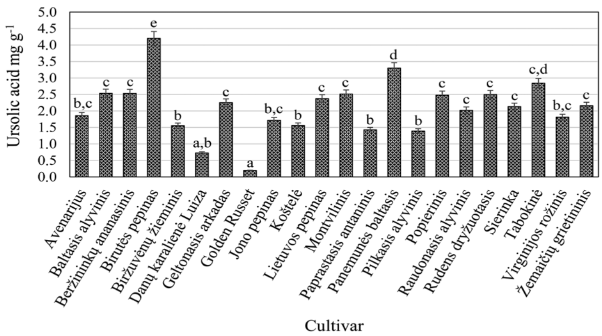


A – 'Danų karalienė Luiza', B – 'Panemunės baltasis', C – 'Paprastasis antaninis', D – 'Rudens dryžuotasis', E – 'Žemaičių grietininis'; analytes: 1 – corosolic acid, 2 – betulinic acid, 3 – oleanolic acid, 4 – ursolic acid

Figure 1. HPLC chromatograms of fruit of the old apple cultivars

Apple extracts are multi-component matrices of chemical compounds with complex structures, and they vary in the chemical composition (Contessa, Botta, 2016). In order to provide the consumers with high-quality apples and apple products with known chemical composition, the evaluation of the variations in the quantitative composition of triterpenic compounds is relevant.

The amount of ursolic acid in apple fruit ranged from 0.20 to 4.20 mg g^{-1} (Fig. 2). The highest amount of ursolic acid (4.20 ± 0.23 mg g^{-1}) was found in apple fruit of the 'Birutės pepinas', and this amount differed statistically significantly ($p < 0.05$) from that found in apple fruit of other cultivars. The lowest amount of ursolic acid (0.20 ± 0.06 mg g^{-1}) was found in apple fruit of the 'Golden Russet', and it did not differ statistically significantly ($p > 0.05$) from that detected in apple fruit of 'Danų karalienė Luiza'.



Note. The means followed by different letter are significantly different at $p < 0.05$; data described as mean ± standard deviation.

Figure 2. Variation in the quantitative amount of ursolic acid in fruit of the old apple cultivars

To evaluate the variation in the quantitative composition of triterpenic compounds between apple fruit of different cultivars, coefficients of variation indicating the range of variation of each compound were calculated. The amount of ursolic acid differed between apple fruit of the studied old Lithuanian cultivars. The coefficient of variation of the amount of ursolic acid (60.30%) in apple fruit was higher than that in the total amount of triterpenic compounds (CV = 57.63%).

In apple fruit of the studied cultivars, saponin (ursolic acid) was the predominant triterpene component.

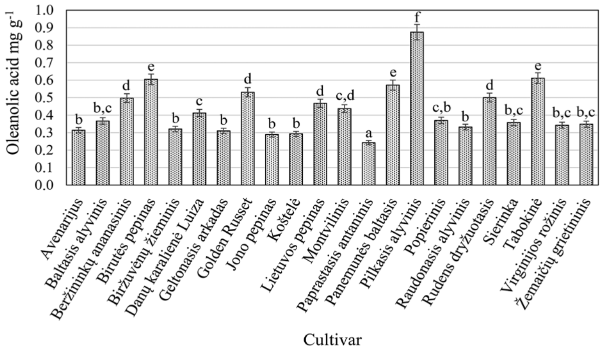
The results of the study conducted by Sut et al. (2019) confirm those obtained in our experiment, indicating that the ursolic acid was the most abundant compound. It has been documented that ursolic acid may comprise 70% or more of the total amount of triterpenic compounds detected in extracts of apple fruit (Poirier et al., 2018). The data of other studies (Ikeda et al., 2008; Brendolise et al., 2011; Waldbauer et al., 2016) have shown that ursolic acid protects hepatocytes and mucous membranes and has a strong anti-inflammatory, antibacterial, antiviral and glycemia-lowering effect. The amount of ursolic acid in apple fruit of the cultivars 'Aldas', 'Auksis', 'Connel Red', 'Ligol', 'Lodel' and 'Rajka' grown in Lithuanian industrial orchards ranges from 1.18 ± 0.06 to 2.58 ± 0.14 mg g^{-1}. The highest amount of ursolic acid (2.58 ± 0.14 mg g^{-1}) was found in apple fruit of the 'Lodel' and the lowest (1.18 ± 0.06 mg g^{-1}) – in the 'Aldas' (Butkevičiūtė et al., 2018). Apple fruit of old cultivars included in the collection of National Plant Genetic Resources were found to have higher amount of ursolic acid compared to apple fruit of the cultivars grown in Lithuanian industrial orchards.

Bars-Cortina et al. (2017) conducted a study, where they evaluated the phytochemical composition of the fruit of red-fleshed and white-fleshed apple cultivars grown in industrial orchards of Spain and the phytochemical composition of the fruit of old white-fleshed apple cultivars. The results of this study showed that the amount of ursolic acid in fruit of red-fleshed apple cultivars ranged from 1.32 ± 0.24 to 3.09 ± 0.23 mg g^{-1}, in fruit of white-fleshed apple cultivars – from 1.40 ± 0.14 to 3.26 ± 0.42 mg g^{-1} and in fruit of old white-fleshed apple cultivars – from 1.84 ± 0.33 to 2.68 ± 0.99 mg g^{-1}. The results of this study showed that the amount of ursolic acid found in fruit of apple cultivars grown in industrial and private orchards of Spain was lower than that found in apples of the cultivars included into the collection of National Plant Genetic Resources.

Nour et al. (2010) and Yanrong (2016) have found that the quantitative composition of biologically active compounds varied depending on the following factors: climatic conditions, geographic latitude, soil, orchard management technologies, harvesting time and storage conditions. The results of study of Viškelis et al. (2018) established a dependence of triterpene accumulation on orchard management technologies and fruit position in tree canopy. Triterpene accumulation in apple fruit was directly correlated with crop load: a higher amount of triterpenes was found in fruit harvested from the inner canopy of the apple tree. Tree growth regulation affected the synthesis of triterpenes – trunk incision decreased the amount of total triterpenes.

A comparative analysis of the quantitative composition of apple fruit of cultivars grown in industrial orchards of Spain and Lithuania showed that in apple fruit of the old cultivars included into the collection of National Plant Genetic Resources the amount of ursolic acid was higher than that found in apple fruit of cultivars grown in Spain (Bars-Cortina et al., 2017; Butkevičiūtė et al., 2018).

The quantitative composition of oleanolic acid in apple fruit of the studied old Lithuanian cultivars ranged from 0.24 ± 0.05 to 0.87 ± 0.96 mg g^{-1} (Fig. 3). The highest amount of oleanolic acid (0.87 ± 0.96 mg g^{-1}) was found in apple fruit of the 'Pilkasis alyvinis' and the lowest (0.24 ± 0.05 mg g^{-1}) – in the 'Paprastasis antaninis'. The highest amount of oleanolic acid did differ statistically significantly ($p < 0.05$), and this amount differed statistically significantly ($p < 0.05$) from that found in apple fruit of other cultivars. The detected amount of oleanolic acid varied in apple fruit of the old cultivars. The coefficient of variation of the amount of oleanolic acid in apple fruit ranged from CV = 0.75% to CV = 80.82%.



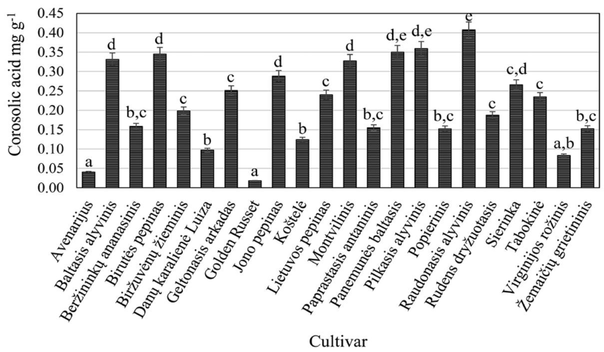
Note. The means followed by different letter are significantly different at $p < 0.05$; data described as mean ± standard deviation.

Figure 3. Variation in the quantitative amount of oleanolic acid in fruit of the old apple cultivars

The results of our experiment showed that in apple fruit the amount of oleanolic acid was by 5.20 times lower than that of ursolic acid. Triterpenes are one of the dominant groups of compounds in cuticular wax (Buschhaus, Jetter, 2011). Oleanolic and ursolic acids are the main compounds of the wax layer of fruit and leaves (Qi et al., 2006; Koch, Ensikat, 2008), and they can be found in various fruit and berries (Peschel et al., 2007; Neto, 2011) as well as in apples (Cefarelli et al., 2006; Jäger et al., 2009). Sut et al. (2019) have found that in apple fruit of cultivars grown in industrial orchards ursolic and oleanolic acids may comprise 79–95% of the total amount of triterpenic compounds. According to the data of the study conducted by Szakiel et al. (2012), the amount of oleanolic acid in apple fruit may comprise 7–15% of the total amount of triterpenic compounds. Butkevičiūtė et al. (2018) found that in fruit of apple cultivars grown in industrial orchards of Lithuania the amount of oleanolic acid ranged from 0.32 ± 0.02 to 0.47 ± 0.11 mg g^{-1}. The amount of oleanolic acid found in apple fruit of old cultivars was higher than that detected in apple fruit grown in Lithuanian industrial orchards. The amount of oleanolic acid in fruit of apple cultivars grown in industrial orchards of France and Germany ranged from 0.16 to 1.0 g 100 g^{-1} (Jäger et al., 2009; Jemmali et al., 2016). Meanwhile, the amount of oleanolic acid in fruit of old apple cultivars grown in private orchards of Spain ranged from 0.71 ± 0.13 to 0.93 ± 0.38 mg g^{-1} (Bars-Cortina et al., 2017). The amount of oleanolic acid detected in apple fruit of old cultivars grown in Lithuania is in line with that reported in the studies published by French, German and Spanish researchers.

The quantitative composition of corosolic acid in apple fruit of studied old Lithuanian cultivars ranged from 0.02 ± 0.004 to 0.41 ± 0.04 mg g^{-1} (Fig. 4). The highest amount of corosolic acid (0.41 ± 0.04 mg g^{-1}) was detected in apple fruit of the 'Raudonasis alyvinis', but no statistically significant difference ($p > 0.05$) was found in the amount of this acid among the 'Panemunės baltasis' or 'Pilkasis alyvinis'. The lowest amount of corosolic acid (0.02 ± 0.004 mg g^{-1}) was detected in apple fruit of the 'Golden Russet', and this amount did not differ statistically significantly ($p > 0.05$) from that found in 'Avenarijus' or 'Virginijos rožinis'. The coefficient of variation of the amount of corosolic acid (34.48%) in apple fruit was lower than that in the total amount of triterpenic compounds (CV = 57.63%).

The results of our experiment showed that the amount of corosolic acid was by 12.12 times lower



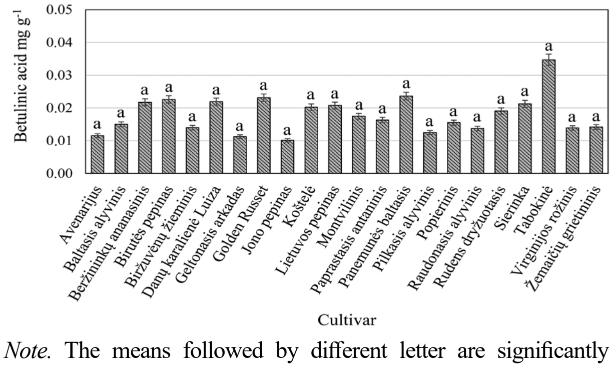
Note. The means followed by different letter are significantly different at $p < 0.05$; data described as mean ± standard deviation.

Figure 4. Variation in the quantitative amount of corosolic acid in fruit of the old apple cultivars

than that of ursolic acid and by 3.60 times lower than that of oleanolic acid. The amount of corosolic acid in fruit of apple cultivars grown in industrial orchards under Lithuanian climatic conditions ranges from 0.06 ± 0.01 to 0.27 ± 0.08 mg g^{-1} (Butkevičiūtė et al., 2018). The amount of corosolic acid found in apple fruit of old cultivars was higher than that found in apple fruit grown in industrial orchards of Lithuania. Jäger et al. (2009) published results, indicating that the highest amount of corosolic acid in apple fruit was 0.51 g 100 g^{-1}. The amount of corosolic acid detected in our experiment confirm the results published by the afore mentioned German researchers.

The amount of betulinic acid detected in fruit of old apple cultivars grown in Lithuania was the lowest of all the identified and quantitatively evaluated triterpenic compounds. The results of our experiment did not reveal any statistically significant difference ($p > 0.05$) in the amount of betulinic acid between apple fruit of the studied old Lithuanian cultivars (Fig. 5). The highest amount of betulinic acid (0.03 ± 0.008 mg g^{-1}) was found in apple fruit of the 'Tabokinė', and the lowest amounts (0.01 ± 0.001 mg g^{-1}) were detected in 'Avenarijus', 'Biržuvėnų žieminis', 'Geltonasis arkadas', 'Jono pepinas', 'Pilkasis alyvinis', 'Raudonasis alyvinis', 'Virginijos rožinis' and 'Žemaičių grietininis'. The coefficient of variation of the amount of betulinic acid (58.03%) in apple fruit was higher than that in the total amount of triterpenic compounds (CV = 57.63%).

The amount of betulinic acid detected in fruit of apple cultivars grown in industrial orchards of Lithuania ranged from 0.02 ± 0.005 to 0.03 ± 0.005 mg g^{-1} (Butkevičiūtė et al., 2018). The amount of betulinic acid found in apple fruit of cultivars grown in industrial orchards under Lithuanian climatic conditions did not differ from that detected in apple fruit of the cultivars included in the collection of National Plant Genetic Resources. Bars-Cortina et al. (2017) found that the variation in the amount of betulinic acid in apple fruit of old cultivars reached 0.24 ± 0.08 mg g^{-1}. The evaluation of the qualitative and quantitative composition of apple fruit of cultivars included in the collection of National Plant Genetic Resources showed that the amount of betulinic acid did not differ from that found in apple fruit of cultivars grown in industrial orchards in Lithuania. However, it was higher than that detected in apple fruit of old cultivars grown in orchards in Spain.

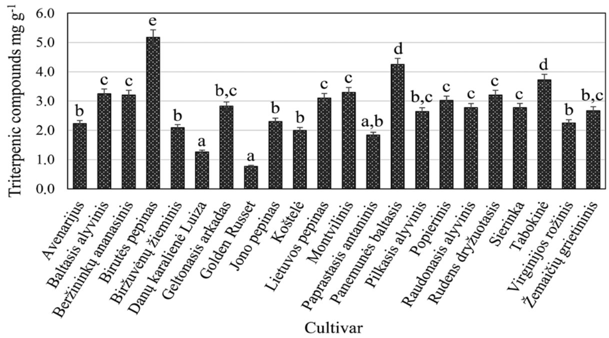


Note. The means followed by different letter are significantly different at $p < 0.05$; data described as mean ± standard deviation.

Figure 5. Variation in the quantitative amount of betulinic acid in fruit of the old apple cultivars

Currently, there are ongoing discussions on the application of health-enhancing food in medical practice, i.e. the prevention of various diseases. The nutritional value of the fruit of apple cultivars included in the collection of National Plant Genetic Resources is equal to that of the fruit of cultivars grown in industrial orchards. For this reason, detailed studies of the chemical composition of biologically active compounds in apples of the old cultivars are relevant. Such studies would allow for offering the consumers high-quality apples and apple products with a known composition of triterpenic compounds, which could be potentially valuable for medical purposes and creation and manufacturing of functional food.

The results of our experiment showed that the total amount of triterpenic compounds in apple fruit of old Lithuanian cultivars ranged from 0.77 ± 0.38 to 5.17 ± 0.86 mg g^{-1} (Fig. 6). The highest total amount of triterpenic compounds (5.17 ± 0.86 mg g^{-1}) was detected in apple fruit of the 'Birutės pepinas', and this amount differed statistically significantly ($p < 0.05$) from that found in apple fruit of other cultivars. The lowest total amount of triterpenic compounds (0.77 ± 0.38 mg g^{-1}) was detected in apple fruit of the 'Golden Russet', and it did not differ statistically significantly ($p > 0.05$) from that found in 'Danų karalienė Luiza' or 'Paprastasis antaninis'.



Note. The means followed by different letter are significantly different at $p < 0.05$; data described as mean ± standard deviation.

Figure 6. Variation in the total amount of triterpenic compounds in fruit of the old apple cultivars

The genetic background of apple cultivars is the main factor in the accumulation of triterpenes in fruit. Numerous studies have reported cultivar differences in triterpene concentration. He and Liu (2007) identified 13 triterpenoids in 'Red Delicious' apples, and McGhie et al. (2011) identified 43 compounds in the fruit of 7 tested apple cultivars and found that the 'Fuji' had the highest total amount, while the 'Granny Smith' had the lowest.

The total amount of triterpenic compounds in 'Aldas', 'Auksis', 'Connel Red', 'Ligol', 'Lodel' and 'Rajka' apples grown in Lithuanian industrial orchards ranged from 1.64 ± 0.07 to 3.17 ± 0.14 mg g^{-1}. The highest total amount of triterpenic compounds (3.17 ± 0.14 mg g^{-1}) was found in apple fruit of the 'Lodel' and the lowest (1.64 ± 0.07 mg g^{-1}) – in the 'Aldas' (Butkevičiūtė et al., 2018). The amount of triterpenic compounds found in fruit of apple trees grown in Lithuanian industrial orchards was lower than that detected in apple fruit of old cultivars included in the collection of National Plant Genetic Resources. The total amount of triterpenic compounds found in apple fruit of old cultivars grown in Polish orchards ranged from 0.47 to 3.75 mg g^{-1} (Oszmiański et al., 2018) and was lower than that found during our experiment.

Some studies (Smania et al., 2003; Kumar et al., 2007; Yuan et al., 2009) showed that triterpene compounds have antifungal activity and can play a role in plant defence against diseases. Poirier et al. (2018) established that triterpene accumulation in apple peal can be related to superficial scald, a postharvest apple browning disorder. Triterpenes accumulated in the wax layer act as a protection against biotic stresses and can enhance post-harvest quality of the fruit (Lara et al., 2014). Studies with persimmon fruit (Tsubaki et al., 2013) demonstrated that triterpenoids might increase the mechanical resistance of the cuticle and protect against water loss. The difference in triterpene concentration among the tested cultivars can be associated with the resistance of fruit to various diseases.

To evaluate the variation in the quantitative composition of triterpenic compounds between apple fruit of the studied old Lithuanian cultivars, coefficients of variation, which showed the range of the variation in the total amount of triterpenic compounds, were calculated. The coefficient of variation of the total amount of triterpenic compounds ranged from 3.90% to 57.63% (Table).

Following the hierarchical cluster analysis, the apple fruit of the studied old Lithuanian cultivars were distributed into four clusters (Fig. 7A). Apple fruit assigned to cluster I contained moderate total amount of triterpenic compounds (from 1.84 ± 0.41 to 3.30 ± 0.69 mg g^{-1}). Cluster II included the 'Birutės pepinas', whose fruit was found to contain the highest total amount of triterpenic compounds (5.17 ± 0.86 mg g^{-1}). Apple fruit of 'Golden Russet' and 'Danų karalienė Luiza' assigned to cluster III had lower than moderate total amount of triterpenic compounds (from 0.77 ± 0.38 to 1.26 ± 0.49 mg g^{-1}). Apple fruit of 'Panemunės baltasis' and 'Tabokinė' assigned to cluster IV was found to have higher than moderate total amount of triterpenic compounds (from 3.72 ± 0.57 to 4.25 ± 0.17 mg g^{-1}) (Fig. 7A).

The PCA of triterpenic compounds found in apple fruit included in the collection of National Plant Genetic Resources was conducted. Two principal components that explain 75.89% of the overall dispersion of the studied data were used for the detailed analysis (Fig. 7B). The amount of ursolic acid very strongly positively (0.875) correlated with the first component, which described 54.60% of the overall data dispersion. The correlation of the amount of corosolic acid with this component was very strong as well (0.846). A very strong positive (0.922) correlation was found between the amount of betulinic acid and the second component, which described 21.29% of the overall data dispersion, while the correlation of the amount of oleanolic acid with this component was moderately strongly positive (0.578).

Around the world, a selection of local cultivars is performed with the aim of creating new or improving the existing fruit tree cultivars. The appearance of the apples of the old cultivars does not meet the commercial standards set for apples grown in industrial orchards. Besides, the fruit is usually smaller, its shape is irregular and has thick leathery skin. Despite the aforementioned differences, the interest in the fruit of old apple tree cultivars is continually growing (Lo Piccolo et al., 2019). Apple trees of old cultivars growing or grown in local neighbourhoods are gaining popularity, their fruit contains higher amount of biologically active compounds, and the trees are more resistant to diseases and better adapted to climatic conditions, thus capable of ripening fruit within a short vegetation period (Sut et al., 2019). Apple fruit of cultivars included in the collection of National Plant Genetic Resources were found to have higher total amount of triterpenic compounds compared to apples grown in Lithuanian industrial orchards. According to experimental data, apple fruit grown in private orchards of Poland and Italy had lower total amount of triterpenic compounds, compared to apple fruit of old cultivars grown under Lithuanian climatic conditions.

The obtained results provided new knowledge about the apple cultivars included in the collection of National Plant Genetic Resources and about the variation in the qualitative and quantitative composition of triterpenic compounds in their fruit.

The old apple cultivars 'Golden Russet', 'Beržininkų ananasinis', 'Biržuvėnų žieminis', 'Danų karalienė Luiza', 'Jono pepinas', 'Koštelė', 'Montvilinis' and 'Panemunės baltasis' grown in Lithuania are scab-resistant (Table). The highest total amount of triterpenic compounds were found in apple fruit of scab-resistant cultivars 'Panemunės baltasis' and 'Tabokinė' and in the 'Birutės pepinas', which is susceptible to scab. The fruit of the 'Panemunės baltasis' have a waxy skin. Apple fruit of the scab-resistant cultivars 'Golden Russet' and 'Danų karalienė Luiza' was found to have the lowest amount of triterpenic compounds. The fruit skin is covered with a rust grid.

The results of the current study suggest that the resistance of the 'Panemunės baltasis' and 'Tabokinė' to scab is due to a combination of factors, including the

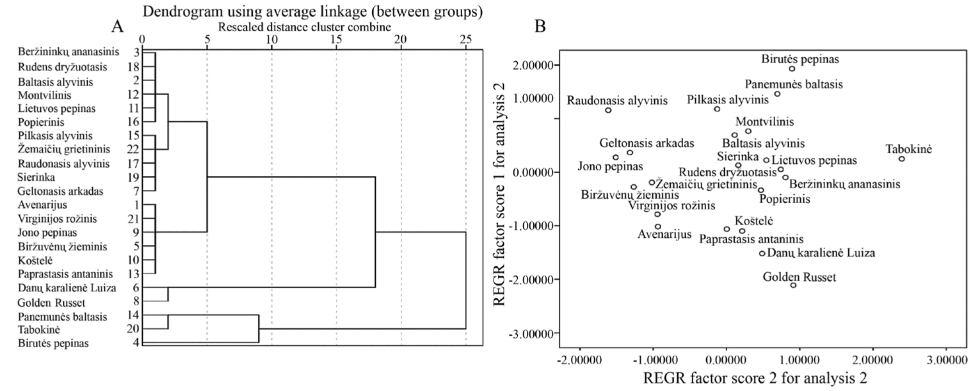


Figure 7. A dendrogram of similarities between apple fruit with respect to the total amount of triterpenic compounds (A) and PCA of triterpenic compounds in apple fruit (B)

effect of polygenes on scab resistance and the antifungal effect of triterpenic compounds, which depends on the composition of triterpenic compounds, their combinations and amount ratios. The resistance of the 'Golden Russet' and 'Danų karalienė Luiza' is due to genetic factors, i.e. scab resistance genes. A high total amount of the studied triterpenic compounds in the fruit of the 'Birutės pepinas' does not protect it from scab. This supports the finding that resistance genes are the main factor in apple resistance to scab (Annu et al., 2019). The fruit of the old apple cultivars 'Panemunės baltasis' and 'Tabokinė' grown in collection of National Plant Genetic Resources is a promising option for ecological harvesting and isolation of triterpenic compounds with a versatile and significant effect on human health.

Conclusions

1. A variation in the qualitative and quantitative composition of triterpenic compounds was found in the apple fruit of old Lithuanian cultivars included in the collection of National Plant Genetic Resources. The highest total amount of triterpenic compounds (5.17 ± 0.86 mg g^{-1}) was found in the 'Birutės pepinas'; higher total amounts were also found in 'Tabokinė' and 'Panemunės baltasis' (3.72 ± 0.57 and 4.25 ± 0.17 mg g^{-1}, respectively).

2. The following triterpenic compounds: betulinic acid, corosolic acid, oleanolic acid and ursolic acid, were identified and quantified in apple fruit of old cultivars. By their quantitative composition triterpenic compounds in apple fruit may be listed in the following order: ursolic acid > oleanolic acid > corosolic acid > betulinic acid. Among the apple fruit of the studied cultivars, ursolic acid was the predominant triterpenic compound. Its highest amount (4.20 ± 0.23 mg g^{-1}) was found in apple fruit of the 'Birutės pepinas'. The results of our experiment showed that the amount of oleanolic acid in apple fruit was by 5.20 times, and the amount of corosolic acid – by as many as 12.12 times lower than that of ursolic acid. The highest amount of oleanolic acid (0.87 $\pm$ 0.96 mg g^{-1}) was found in apple fruit of the 'Pilkasis alyvinis', and the highest amount of corosolic acid (0.41 $\pm$ 0.04 mg g^{-1}) – in the 'Raudonasis alyvinis'. The amount of betulinic acid was the lowest of all the qualitatively and quantitatively evaluated triterpenic compounds.

3. The old apple cultivars 'Birutės pepinas', 'Panemunės baltasis' and 'Tabokinė' included in the collection of National Plant Genetic Resources have a potential for cultivation in industrial orchards and for the use of their apples and processed apple products as natural functional foods rich in triterpenic compounds and adapted for medical purposes, including the prevention of various diseases.

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Lietuvos senųjų obels veislių vaisių triterpeninių junginių kokybinė ir kiekybinė sudėtis

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Santrauka

Pasaulyje didėja susidomėjimas senųjų veislių obelimis, jų vaisiais ir perdirbtais produktais. Biologiškai aktyvių junginių kokybinės ir kiekinės sudėties tyrimai yra svarbūs vertinant obuolių kokybę ir jų mitybines savybes. Taikant efektyviosios skysčių chromatografijos metodą Augalų nacionalinių genetinių išteklių kolekcijai priskirtų obels veislių obuolių ėminiuose nustatyta triterpeninių junginių kokybinė ir kiekinė sudėtis. Didžiausias suminis triterpeninių junginių kiekis (5,17 ± 0,86 mg g^{-1}) nustatytas veislės ‘Birutės pepinas’ obuolių ėminiuose. Didesni suminiai triterpeninių junginių kiekiai (3,72 ± 0,57 ir 4,25 ± 0,17 mg g^{-1}) nustatyti veislių ‘Tabokinė’ ir ‘Panemunės baltasis’ obuolių ėminiuose. Pagal kiekinę sudėtį obuolių ėminiuose triterpeniniai junginiai pasiskirstė taip: ursolio rūgštis > oleanolio rūgštis > korosolio rūgštis > betulino rūgštis.
Augalų nacionalinių genetinių išteklių kolekcijai priskirtos senosios veislės ‘Birutės pepinas’, ‘Panemunės baltasis’ ir ‘Tabokinė’ obelys yra perspektyvios auginti pramoniniuose soduose, obuolius bei jų perdirbimo produktus vartoti kaip natūralų, daug triterpeninių junginių turintį funkcinį maistą, taikomą medicinos srityje ligų prevencijai.

Reikšminiai žodžiai: obuoliai, senosios veislės, triterpeniniai junginiai.