

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/323260125>

Triterpenic acid content in the fruit peel of *Malus × domestica* Borkh. depends on the growing technology

Article in *Zemdirbyste* · February 2018

DOI: 10.13080/z-a.2018.105.010

CITATIONS

2

READS

152

8 authors, including:



Jonas Viškelis

Lithuanian Research Centre for Agriculture and Forestry

62 PUBLICATIONS 200 CITATIONS

[SEE PROFILE](#)



Nobertas Uselis

Sodininkystės ir daržininkystės institutas/Lietuvos agrarinių ir miškų mokslų cen...

52 PUBLICATIONS 196 CITATIONS

[SEE PROFILE](#)



Liaudanskas Mindaugas

Lithuanian University of Health Sciences

47 PUBLICATIONS 233 CITATIONS

[SEE PROFILE](#)



Valdimaras Janulis

Lithuanian University of Health Sciences

104 PUBLICATIONS 1,382 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Long-term program "Horticulture: agro-biological basics and technologies" implemented by Lithuanian Research Centre for Agriculture and Forestry [View project](#)



INTERREG project InnoFruit Nr. R004. "Advancement of non-technological innovation performance and innovation capacity in fruit growing and processing sector in selected Baltic Sea Region countries". 2016–2019. [View project](#)

ISSN 1392-3196 / e-ISSN 2335-8947

Zemdirbyste-Agriculture, vol. 105, No. 1 (2018), p. 71–78

DOI 10.13080/z-a.2018.105.010

Triterpenic acid content in the fruit peel of *Malus × domestica* Borkh. depends on the growing technology

Jonas VIŠKELIS¹, Nobertas USELIS^{1,3}, Mindaugas LIAUDANSKAS², Valdimaras JANULIS², Pawel BIELICKI⁴, Toivo UNIVER⁵, Janis LEPSIS⁶, Darius KVIKLYS¹

¹Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry
Kauno 30, Babtai, Kaunas distr., Lithuania
E-mail: j.viskelis@lsdi.lt

²Medicinal Academy, Lithuanian University of Health Sciences
Eivenių 4, Kaunas, Lithuania

³Aleksandras Stulginskis University
Studentų 11, Akademija, Kaunas distr., Lithuania

⁴Research Institute of Horticulture
Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

⁵Polli Horticultural Research Centre, Institute of Agricultural and Environmental Sciences,
Estonian University of Life Sciences
Polli 69108, Karksi-Nuia, Viljandi county, Estonia

⁶Institute of Horticulture, Latvia University of Agriculture
Pure 3124, Tukuma distr., Latvia

Abstract

Regardless of the numerous investigations of secondary metabolites, the impact of new growing technologies or individual technological means on fruit internal quality, particularly triterpene concentration in apple fruits has not been studied yet. Apple-tree (*Malus × domestica* Borkh.) growing technologies and geographical location of the growing site had clear effect on triterpene concentration in apple fruits. The effect of crop load, fruit location in apple-tree canopy, planting distance, apple-tree vegetative growth regulation and geographical location on triterpene content in apples was evaluated in 2015 and 2016. Four triterpenic compounds: corosolic, betulinic, oleonic and ursolic acids, were quantified in apple peel. Triterpene accumulation in apple fruits was directly correlated with crop load, where 17% more total triterpenes were found at the highest crop load (12 fruits cm⁻² trunk cross-sectional area (TCSA)) in comparison to the lowest crop load (4 fruits cm⁻² TCSA). Higher concentration of triterpenes was found in the fruits harvested from the inner canopy of the apple-tree. Apple-tree growth regulation affected the synthesis of triterpenes – trunk incision decreased the amount of total triterpenes by 16% compared to the control and by 26% compared to summer pruning. Also, a trend of higher accumulation of triterpenes in fruits was found along with decreasing apple-tree planting distances. Colder climate and shorter vegetation period resulted in significantly higher contents of triterpenes in the fruits of cultivar ‘Auksis’ in Lithuania and Estonia compared to the fruits grown in Poland. The same trend, yet insignificant, was noticed for the cultivar ‘Ligol’.

Key words: apple-tree, bioactive compounds, growing technologies.

Introduction

Apples are one of the most commonly consumed fruits in the world. Until now, development of apple growing and management technologies has been aimed at higher yields, fruit size and external quality (Hooge et al., 2017). In recent years, attention has

been focussed on fruit internal quality. Apples contain multiple nutrients and a variety of bioactive compounds, including phenols. Several studies have been conducted in Lithuania to explore the impact of rootstocks (Kviklys et al., 2014) and nutrition (Lanauskas et al., 2017) on

Please use the following format when citing the article:

Viškelis J., Uselis N., Liaudanskas M., Janulis V., Bielički P., Univer T., Lepsis J., Kviklys D. 2018. Triterpenic acid content in the fruit peel of *Malus × domestica* Borkh. depends on the growing technology. Zemdirbyste-Agriculture, 105 (1): 71–78
DOI 10.13080/z-a.2018.105.010

the accumulation of phenols. Fruit phenol content has been investigated in the main apple cultivars grown in Lithuanian commercial orchards too (Liaudanskas et al., 2015; Raudonė et al., 2017). Research into other important bioactive compounds including triterpenes in apples is performed to a much lesser extent.

Triterpenes are a large group belonging to the terpenoid compounds and are well known for their pharmacological effects (Fontanay et al., 2008; Ikeda et al., 2008; Jang et al., 2009). Corosolic, betulinic, oleanolic and ursolic acids are typical representatives of pentacyclic triterpenes and they are widely distributed in the plant kingdom and in food products (Jäger et al., 2009; Muffler et al., 2011). Oleanolic and ursolic acids are found in the waxes of fruit and leaves (Qi et al., 2006; Koch, Ensikat, 2008). Triterpene content of various apple varieties (Andre et al., 2012), effect of rootstock, harvest maturity and storage methods (Lv et al., 2015) have been investigated, but there are no data on growing technologies or growing site effects on triterpene accumulation in apple fruits.

New findings about apple nutritional value are especially important while facing overproduction of apples in the world market and decrease of fruit prices. New scientific results on the impact of modern technologies on fruit quality parameters could increase consumption of apples.

The aim of this study was to evaluate the influence of different orchard management technologies (crop load, planting distance between apple-trees and apple-tree growth regulation), biological factors (fruit location in apple-tree canopy) and growing site on triterpene concentrations in apple fruits.

Materials and methods

The effect of different growing and management technologies on the triterpene content in apple -tree (*Malus × domestica* Borkh.) fruit was investigated in the experimental orchards of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry in 2015 and 2016. Additionally, apple samples, grown at Research Institute of Horticulture in Skierniewice, Poland and Polli Horticultural Research Centre, Estonia, were investigated for the effect of the geographical location on the accumulation of triterpene compounds.

Growing and management technologies. Effect of crop load. Three crop load levels: 4, 8 and 12 fruits per unit of trunk cross-sectional area (fruits cm⁻² TCSA), were established by hand thinning before flowering in early May at the pink bud stage. Fruitlets remaining after the June drop were counted and thinned according to the crop load level. Apple-trees of cultivar ‘Ligol’ on rootstock P 60 were planted at the distance 3.5 × 1.25 m in 2011. The trial was established in four replicates with two apple-trees per replicate. The experiment was laid out in a fully randomized design. Ten fruits per each tree (20 fruits per replicate) from different canopy places were randomly selected for the analysis at optimum harvest time.

Effect of fruit location in apple-tree canopy. Investigations were carried out with an apple-tree cultivar ‘Ligol’ on rootstock P 60 planted at the distance 4 × 1.25 m in 2002. Orchard row direction was South-North. Four fruit locations in the apple-tree canopy were tested: 1) top of the apple-tree (above 2.3 m), 2) lower inside part of the canopy (bottom) and 3–4) east and west sides of apple-trees. Apples for the last two treatments were harvested at 1.2–1.8 m above the ground. The trial was established in four replicates with four apple-trees per replicate. The experiment was laid out in a fully randomized design. Five fruits per each apple-tree (20 fruits per replicate) from the tested fruit locations in the canopy were randomly selected for the analysis at optimum harvest time.

Effect of planting distance. Investigations were carried out with an apple-tree cultivar ‘Auksis’ on rootstock P 60 planted in 2001. Apple-trees were planted at three distances: 1) 3 × 1.50 m, 2) 3 × 1.00 m and 3) 3 × 0.50 m. According to the planting distance, apple-trees were trained as spindles, slender spindles and super spindles. The trial was established in four replicates with five apple-trees per replicate. Ten fruits per each apple-tree from different canopy places were randomly selected at optimum harvest time. From the bulk of 50 fruits from each replication 20 fruits were randomly selected for the analysis.

Effect of apple-tree vegetative growth regulation. Investigations were carried out with an apple-tree cultivar ‘Rubin’ on rootstock P 60 planted at the distance 4 × 1.5 m in 1999. Four treatments of apple-tree vegetative growth control were established: 1) control, where apple-trees were maintained according to intensive technologies; 2) apple-tree trunk incision before flowering by chain saw at the level of 20 cm above the ground from one side and 60 cm level from the other side; 3) application of growth regulator prohexadione-calcium at a rate of 2.5 kg ha⁻¹ immediately after flowering when new shoots had 5 leaves; 4) summer pruning performed in the middle of August.

Effect of geographical location. Two apple-tree cultivars ‘Auksis’ and ‘Ligol’ grown on rootstock B.396 were tested. Fruit samples of each cultivar were collected at the Research Institute of Horticulture located at Skierniewice (51°58′ N, 20°09′ E), Poland, Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry in Babtai (55°60′ N, 23°48′ E) and at Polli Research Centre (58°67′ N, 25°33′ E), Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences. The orchards are located ~400 km apart, at a similar distance from the Baltic Sea. The orchards were planted in the spring of 2005 under a uniform design in three replicates with five apple-trees per replicate. Planting distance was 4 × 1.5 m. Apple-trees were trained as slender spindle. Climate conditions and soil properties differed between the trial locations. In Poland: soil – deep sandy-loam, pH – 6.7, humus – 1.4%, P₂O₅ – 195 mg kg⁻¹, K₂O – 190 mg kg⁻¹, average annual

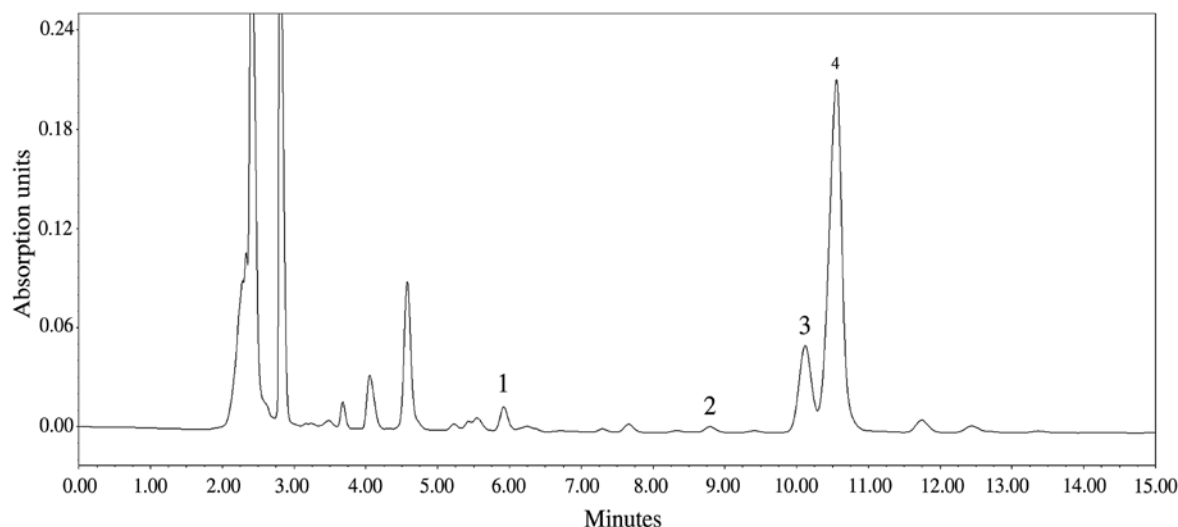
precipitation: 550 mm, average sum of active temperatures: 2550°. In Lithuania: soil – clay loam, pH – 7.3, humus – 2.8%, P_2O_5 – 255 mg kg⁻¹, K_2O – 230 mg kg⁻¹, average annual precipitation – 630 mm, average sum of active temperatures (>10°C) – 2300°. In Estonia: soil – clay loam, pH – 5.3, humus – 2.0%, P_2O_5 – 130 mg kg⁻¹, K_2O – 223 mg kg⁻¹, average annual precipitation – 455 mm, average sum of active temperatures – 1930°. 50 randomly selected apples were harvested at 1.2–1.8 m above the ground from 5 apple-trees from each experimental plot at the optimal harvest time individually established for every cultivar and country.

Chemical analysis. Sample preparation. Each apple was peeled with a semi-automatic peeling machine AS 4 (Kronen GmbH, Germany). The peels were immediately frozen in a freezer (at –35°C) with air circulation and then lyophilized with a sublimator Zirbus 3 × 4 × 5 (ZIRBUS technology GmbH, Germany) at a pressure of 0.01 mbar (condenser temperature –85°C). The lyophilized apple peels were ground to fine powder by using a knife mill GM (Retsch GmbH, Germany). Loss on drying before analysis was determined by drying the apple peel lyophilizate in a laboratory drying oven to complete evaporation of water and volatile compounds (temperature – 105°C, 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 absolute dry weight (DW).

Extraction. An amount of 1 g of lyophilized apple peel powder (exact weight) was weighed, added to 10 mL of acetone (100%, v/v), and extracted in a Sonorex Digital 10 P ultrasonic bath (Bandelin Electronic GmbH & Co. KG, Germany) for 10 minutes. The conditions of extraction (type of extraction, duration, temperature, solvent and its concentration) were chosen considering the results of extraction optimization. The extract

obtained was filtered through a paper filter; the apple peel lyophilizate on the filter was washed with acetone (100%, v/v) in a 10-mL flask. The extract was filtered through a membrane filter with a pore size of 0.22 µm (Carl Roth GmbH, Germany).

Instrumentation and chromatographic conditions. A chromatograph Waters 2695 equipped with a detector Waters 2998 PDA (“Waters”, USA) was used for high-performance liquid chromatography (HPLC) analysis. Chromatographic separation was managed, chromatograms were recorded, and data were processed with the software *Empower*® v.3.0 (“Waters”). Chromatographic separations were carried out by using an ACE® (5 µm, C₁₈, 250 × 4.6 mm) column. The column was operated at a constant temperature of 25°C. The volume of the extract being investigated was 10 µL. The flow rate was 1 mL min⁻¹, and isocratic elution was used. The mobile phase consisted of 88% acetonitrile (solvent A) and 12% water (solvent B). The duration of the analysis was 15 minutes. The identification of the chromatographic peaks was achieved by comparing the retention times and spectral characteristics (λ = 200–400 nm) of the eluting peaks with those of reference compounds. The compounds identified were confirmed by spiking the sample with the standard compound and monitoring the changes in the peak shape and spectral characteristics. For quantitative analysis, a calibration curve was obtained by injection of known concentrations of different standard compounds. The concentrations of triterpenic compounds, identified in the apple peel extracts, were within the limits of calibration curves. Triterpenic compounds were quantified at 205 nm. The concentration of triterpene compounds was calculated based on the peak areas by using standard compounds (Fig.). All trials were repeated three times (biological replicates) each year.



Note. Numbers indicate the peaks of analytes: 1 – corosolic acid, 2 – betulinic acid, 3 – oleonolic acid, 4 – ursolic acid.

Figure. Chromatogram of acetone extracts of apple peel sample

Statistical analysis. The data on the main traits were subjected to the analysis of variance (ANOVA). Results in tables are presented as means, since there were no year / treatment interactions. Significance of differences between treatments was estimated by Tukey's HSD (honest significant difference) test at $P < 0.05$.

Results

Effect of crop load on triterpene content.

Apples harvested from the trees, that had higher number of fruits, accumulated larger quantities of all investigated triterpenic compounds (Table 1). With the highest crop load (12 fruits cm⁻² TC SA) this difference was statistically significant for all investigated triterpene compounds.

Table 1. The effect of crop load on the content (mg g⁻¹ dry weight) of triterpenic acids in the fruit of cultivar 'Ligol' (2015–2016)

Compounds	Crop load, fruits cm ⁻² trunk cross-sectional area		
	4	8	12
	Corosolic acid	1.48 b	1.48 b
Betulinic acid	0.10 b	0.11 b	0.17 a
Oleonolic acid	1.81 b	1.97 b	2.29 a
Ursolic acid	8.78 b	8.93 b	10.13 a
Total	12.17 b	12.48 b	14.28 a

Note. The different letters in the same line indicate statistically significant differences between the individual compounds in the apple peel samples ($p < 0.05$).

Effect of fruit location in apple-tree canopy on triterpene content. Apples, harvested from the bottom of an apple-tree, accumulated the highest amount of total triterpene compounds (Table 2). Betulinic acid concentration was significantly higher in these apples. A lower amount of the investigated triterpenes was found in fruits, picked from the top position of an apple-tree. Fruits from bottom and east side accumulated more triterpenes than fruits from top and west side.

Table 2. The effect of fruit location in the apple-tree canopy on the content (mg g⁻¹ dry weight) of triterpenic acids in the fruit of cultivar 'Ligol' (2015–2016)

Compounds	Fruit location in apple-tree canopy			
	bottom	top	east	west
Corosolic acid	1.44 a	1.25 b	1.34 ab	1.41 ab
Betulinic acid	0.14 a	0.10 c	0.12 b	0.09 c
Oleonolic acid	2.13 a	1.89 ab	1.95 ab	1.77 b
Ursolic acid	10.05 a	8.65 b	8.90 ab	8.65 b
Total	13.76 a	11.89 b	12.31 ab	11.92 b

Explanation under Table 1

Effect of apple-tree growth regulation on triterpene content. Of all the treatments applied, only summer pruning had a positive effect on the accumulation of all triterpenic compounds in apple fruits (Table 3). Trunk incision by chain saw from both sides of the trunk had a negative effect on triterpene concentration in apples – it was significantly lower in comparison to the control samples. Spraying apple-trees with a growth regulator prohexadione-calcium had no effect on triterpene concentration, except for betulinic acid, which increased in comparison with the control samples.

Table 3. The effect of the apple-tree growth regulation method on the content (mg g⁻¹ dry weight) of triterpenic acids in the fruit of cultivar 'Rubin' (2015–2016)

Compounds	Apple-tree growth regulation method			
	control	trunk incision	prohexadione-calcium	summer pruning
Corosolic acid	1.33 a	1.15 b	1.29 ab	1.42 a
Betulinic acid	0.19 c	0.15 d	0.24 b	0.32 a
Oleonolic acid	2.10 ab	1.85 b	2.02 b	2.35 a
Ursolic acid	9.06 a	7.70 b	8.81 ab	9.63 a
Total	12.68 a	10.85 b	12.35 ab	13.73 a

Explanation under Table 1

Effect of apple-tree planting distance on triterpene content. More triterpenes were accumulated when the trees were planted at high densities (Table 4). The amount of total triterpenes depended on the planting distances between trees. Lower triterpene concentrations were found in the fruits from trees that had been planted at the widest distance. Only betulinic acid content was higher in these samples, but it had a small influence on the total concentration of triterpenes.

Table 4. The effect of apple-tree planting distance on the content (mg g⁻¹ dry weight) of triterpenic acids in the fruit of cultivar 'Auksis' (2015–2016)

Compounds	Apple-tree planting distance m		
	3 × 1.50	3 × 1.00	3 × 0.50
Corosolic acid	0.40 a	0.42 a	0.43 a
Betulinic acid	0.22 a	0.16 b	0.17 b
Oleonolic acid	2.05 b	2.34 ab	2.57 a
Ursolic acid	9.71 a	10.77 a	10.86 a
Total	12.38 a	13.69 a	14.04 a

Explanation under Table 1

Effect of apple-tree geographical location on triterpene content. Significantly lower amount of triterpenic compounds was accumulated in the apples of the cultivar 'Auksis' from Poland (Table 5). This trend was observed in both years.

Table 5. The effect of apple-tree geographical location on the content (mg g⁻¹ dry weight) of triterpenic acids in the fruit of cultivar ‘Auksis’

Year	Compounds	Apple-tree geographical location		
		Skierniewice, Poland	Babtai, Lithuania	Polli, Estonia
2015	corosolic acid	0.18 c	0.28 b	0.45 a
	betulinic acid	0.07 b	0.08 ab	0.09 a
	oleonolic acid	1.06 c	1.55 b	1.88 a
	ursolic acid	4.83 c	7.17 b	9.29 a
	Total	6.14 c	9.08 b	11.71 a
2016	corosolic acid	0.41 c	0.68 a	0.53 b
	betulinic acid	0.14 b	0.18 a	0.11 c
	oleonolic acid	1.75 c	2.65 a	2.04 b
	ursolic acid	9.33 b	12.18 a	9.78 b
	Total	11.62 b	15.69 a	12.45 b
Average	corosolic acid	0.29 b	0.48 a	0.49 a
	betulinic acid	0.11 a	0.13 b	0.10 a
	oleonolic acid	1.41 b	2.10 a	1.96 a
	ursolic acid	7.08 b	9.68 a	9.54 a
	Total	8.88 b	12.39 a	12.08 a

Explanation under Table 1

The apples of the cultivar ‘Ligol’ from Polish, Lithuanian and Estonian trial orchards did not significantly differ in the total triterpenes content (Table 6).

Table 6. The effect of apple-tree geographical location on the content (mg g⁻¹ dry weight) of triterpenic acids in the fruit of cultivar ‘Ligol’

Year	Compounds	Apple-tree geographical location		
		Skierniewice, Poland	Babtai, Lithuania	Polli, Estonia
2015	corosolic acid	1.06 b	1.35 a	0.98 b
	betulinic acid	0.12 b	0.15 a	0.12 b
	oleonolic acid	1.76 b	2.05 a	1.96 ab
	ursolic acid	8.46 a	9.29 a	8.93 a
	Total	11.40 a	12.82 a	11.99 a
2016	corosolic acid	1.28 b	1.76 a	0.85 c
	betulinic acid	0.12 b	0.16 a	0.14 b
	oleonolic acid	2.03 b	2.44 a	2.66 a
	ursolic acid	10.80 ab	9.85 b	11.28 a
	Total	14.23 a	14.21 a	14.93 a
Average	corosolic acid	1.17 c	1.55 a	0.91 b
	betulinic acid	0.12 b	0.15 a	0.13 b
	oleonolic acid	1.89 b	2.24 a	2.31 a
	ursolic acid	9.63 a	9.57 a	10.10 a
	Total	12.81 a	13.52 a	13.46 a

Explanation under Table 1

Also, it was noticed that cultivar ‘Ligol’ accumulated more triterpenes than ‘Auksis’ apples, and mainly it was due to the corosolic acid, which was found at much higher concentrations in ‘Ligol’ apples.

Discussion

Our study determined the quantities of the four triterpenic compounds: corosolic, betulinic, oleonolic and ursolic acids, which are the most common triterpenes found in apple peel. However, a total of thirteen triterpenoids have been identified in apple peel (He, Liu, 2007). The highest concentrations, found in apple peel, was of oleonolic and ursolic acids (Cefarelli et al., 2006; Jäger et al., 2009), which agrees with the findings of our study. Compared with apple flesh, apple peel shows more potent antioxidant activity and antiproliferative activity (Wolfe et al., 2003), and this can be linked to triterpenes, because they are found mostly in peel.

Regardless of the numerous investigations of secondary metabolites, the impact of new growing technologies or separate technological means on fruit internal quality has not been explored yet. Until now, investigations of bioactive compounds and especially phenolics in apples have been limited to determination of differences between cultivars or growing systems (organic and integrated) or to the impact of light, apple-tree nutrition and some stress factors as drought, pathogens or soil salinity (Awad et al., 2001; Vieira et al., 2009; Lanauskas et al., 2017).

In previous investigations, it has been found that crop load has a significant effect on plant nutritional status and phytohormones (Samuolienė et al., 2016 a, b), it also influences the concentration of anthocyanins in fruits (Jakopic et al., 2009). Very contrasting data on crop load influence on apple fruit quality have been obtained by various researchers (Viškelis, Kviklys, 2017). This confirms the idea that fruit quality is determined by many different growing technologies and biological aspects, including cultivar, growing site, and other factors.

Apple-tree growth regulation, such as spraying with a growth regulator prohexadione-calcium or summer pruning, is a common practise in commercial orchards, but fruit quality is mostly determined by the external quality parameters, fruit colour, size and firmness, and basic internal quality parameters, such as soluble solids, sugar content and acidity (Zadavec et al., 2008; Ashraf, Ashraf, 2014). There are few research data available on the relationship between bioactive compounds, such as phenolics or triterpenes, and apple-tree growth regulation. Our data suggest that summer pruning increases triterpene accumulation in apples, while trunk incision has a negative effect.

The effect of fruit location in apple-tree canopy on fruit quality has been researched in much more detail: fruits from outer-canopy had higher phenolic content, higher fresh weight and higher soluble solids content. Research on the effects of location within the canopy

on both primary and secondary metabolites showed the importance of light exposure on apple fruit quality (Nilsson, Gustavsson, 2007; Drogoudi, Pantelidis, 2011; Unuk et al., 2012; Feng et al., 2014). In the case of triterpenes, our study indicated higher accumulation in fruits harvested from the bottom of the apple-tree – in the shaded zone of the apple-tree canopy.

Our research data show that the concentration of triterpene compounds in most cases could be attributed to solar irradiation and is negatively related to the amount of received sunlight. In Lv et al. (2015) research, apple cultivars with obvious sun-exposed and shaded sides were investigated for triterpene concentration in both sides of the fruit. The results showed that oleanolic and ursolic acid concentrations were higher in the peel of the shaded side than of the sun-exposed side. This trend was also observed in grapefruits (McDonald et al., 1993). The data of some researchers show that differences between shaded and sun-exposed fruits cause different wax formation and its structure, which differs both qualitatively and quantitatively (Curry, 2008; Tahir et al., 2009). So, this fruit wax formation can be linked to triterpene concentration and explains higher triterpene concentration in cultivar ‘Ligol’ apples which has waxier peel than cultivars ‘Auksis’ and ‘Rubin’.

Conclusions

1. The concentration of triterpenes in apple-tree (*Malus × domestica* Borkh.) fruits was directly correlated with the crop load: 17% more triterpenes were found at the highest crop load of 12 fruits cm⁻² trunk cross-sectional area (TCSA) compared with the lowest crop load of 4 fruits cm⁻² TCSA.

2. Significantly the highest amount of triterpenes was found in the fruits harvested from the bottom and inside of the apple-tree canopy.

3. Apple-tree growth regulation by technological means affected synthesis of triterpenes. Trunk incision decreased the amount of total triterpenes by 16% compared to the control and by 26% compared to summer pruning.

4. A trend towards higher accumulation of triterpenes in fruits was found along with decreasing planting distances between the apple-trees.

5. Colder climate and a shorter vegetation period resulted in significantly higher triterpene concentrations in the fruits of the cultivar ‘Auksis’ grown in Lithuania and Estonia compared with those grown in Poland. The same trend, yet insignificant, was noticed for the cultivar ‘Ligol’.

Received 03 08 2017

Accepted 15 01 2018

References

1. Andre C. M., Greenwood J. M., Walker E. G., Rassam M., Sullivan M., Evers D., Perry N. B., Laing W. A. 2012. Anti-inflammatory procyanidins and triterpenes in 109 apple varieties. *Journal of Agricultural and Food Chemistry*, 60 (42): 10546–10554.
<https://doi.org/10.1021/jf302809k>
2. Ashraf N., Ashraf M. Summer pruning in fruit trees. 2014. *African Journal of Agricultural Research*, 9 (2): 206–210.
<https://doi.org/10.5897/AJAR2013.7916>
3. Awad M., Wagenmakers P., de Jager A. 2001. Effects of light on flavonoids and chlorogenic acid levels in the skin of ‘Jonagold’ apples. *Scientia Horticulturae*, 88: 289–298.
[https://doi.org/10.1016/S0304-4238\(00\)00215-6](https://doi.org/10.1016/S0304-4238(00)00215-6)
4. Cefarelli G., D’Abrosca B., Fiorentino A., Izzo A., Mastellone C., Pacifico S., Piscopo V. 2006. Free-radical-scavenging and antioxidant activities of secondary metabolites from reddened cv. Annurca apple fruits. *Journal of Agricultural and Food Chemistry*, 54 (3): 803–809.
<https://doi.org/10.1021/jf052632g>
5. Curry E. 2008. Effects of 1-MCP applied postharvest on epicuticular wax of apples (*Malus domestica* Borkh.) during storage. *Journal of the Science of Food and Agriculture*, 88 (6): 996–1006.
<https://doi.org/10.1002/jsfa.3180>
6. Drogoudi P. D., Pantelidis G. 2011. Effects of position on canopy and harvest time on fruit physico-chemical and antioxidant properties in different apple cultivars. *Scientia Horticulturae*, 129 (4): 752–760.
<https://doi.org/10.1016/j.scienta.2011.05.036>
7. Feng F., Li M., Ma F., Cheng L. 2014. Effects of location within the tree canopy on carbohydrates, organic acids, amino acids and phenolic compounds in the fruit peel and flesh from three apple (*Malus × domestica*) cultivars. *Horticulture Research*, 1: 14019.
<https://doi.org/10.1038/hortres.2014.19>
8. Fontanay S., Grare M., Mayer J., Finance C., Duval R. E. 2008. Ursolic, oleanolic and betulinic acids: antibacterial spectra and selectivity indexes. *Journal of Ethnopharmacology*, 120 (2): 272–276.
<https://doi.org/10.1016/j.jep.2008.09.001>
9. He X., Liu R. H. 2007. Triterpenoids isolated from apple peels have potent antiproliferative activity and may be partially responsible for apple’s anticancer activity. *Journal of Agricultural and Food Chemistry*, 55 (11): 4366–4370.
<https://doi.org/10.1021/jf063563o>
10. Hooge de I. E., Oostindjer M., Aschemann-Witzel J., Normann A., Loose S. M., Almlı V. L. 2017. This apple is too ugly for me!: consumer preferences for suboptimal food products in the supermarket and at home. *Food Quality and Preference*, 56 (A): 80–92.
<https://doi.org/10.1016/j.foodqual.2016.09.012>
11. Ikeda Y., Murakami A., Ohigashi H. 2008. Ursolic acid: an anti- and pro-inflammatory triterpenoid. *Molecular Nutrition and Food Research*, 52 (1): 26–42.
<https://doi.org/10.1002/mnfr.200700389>
12. Jäger S., Trojan H., Kopp T., Laszczyk M., Scheffler A. 2009. Pentacyclic triterpene distribution in various plants – rich sources for a new group of multi-potent plant extracts. *Molecules*, 14 (6): 2016–2031.
<https://doi.org/10.3390/molecules14062016>

13. Jakopic J., Stampar F., Veberic R. 2009. The influence of exposure to light on the phenolic content of 'Fuji' apple. *Scientia Horticulturae*, 123 (2): 234–239.
<https://doi.org/10.1016/j.scienta.2009.09.004>
14. Jang S.-M., Yee S.-T., Choi J., Choi M.-S., Do G.-M., Jeon S.-M., Yeo J., Kim M.-J., Seo K.-I., Lee M.-K. 2009. Ursolic acid enhances the cellular immune system and pancreatic β -cell function in streptozotocin-induced diabetic mice fed a high-fat diet. *International Immunopharmacology*, 9 (1): 113–119.
<https://doi.org/10.1016/j.intimp.2008.10.013>
15. Koch K., Ensikat H.-J. 2008. The hydrophobic coatings of plant surfaces: epicuticular wax crystals and their morphologies, crystallinity and molecular self-assembly. *Micron*, 39 (7): 759–772.
<https://doi.org/10.1016/j.micron.2007.11.010>
16. Kviklys D., Liaudanskas M., Janulis V., Viškelis P., Rubinskienė M., Lanauskas J., Uselis N. 2014. Rootstock genotype determines phenol content in apple fruits. *Plant, Soil and Environment*, 60 (5): 234–240.
17. Lanauskas J., Kviklys D., Liaudanskas M., Janulis V., Uselis N., Viškelis J., Viškelis P. 2017. Lower nitrogen nutrition determined higher phenolic content of organic apples. *Horticultural Science*, 44 (3): 113–119.
<https://doi.org/10.17221/50/2016-HORTSCI>
18. Liaudanskas M., Viškelis P., Kviklys D., Raudonis R., Janulis V. 2015. A comparative study of phenolic content in apple fruits. *International Journal of Food Properties*, 18 (5): 945–953.
<https://doi.org/10.1080/10942912.2014.911311>
19. Lv Y., Tahir I. I., Olsson M. E. 2015. Factors affecting the content of the ursolic and oleanolic acid in apple peel: influence of cultivars, sun exposure, storage conditions, bruising and *Penicillium expansum* infection. *Journal of the Science of Food and Agriculture*, 96 (6): 2161–2169.
<https://doi.org/10.1002/jsfa.7332>
20. McDonald R. E., Nordby H. E., McCollum T. G. 1993. Epicuticular wax morphology and composition are related to grapefruit chilling injury. *HortScience*, 28 (4): 311–312.
21. Muffler K., Leipold D., Scheller M. C., Haas C., Steingroewer J., Bley T., Neuhaus H. E., Mirata M. A., Schrader J., Ulber R. 2011. Biotransformation of triterpenes. *Process Biochemistry*, 46 (1): 1–15.
<https://doi.org/10.1016/j.procbio.2010.07.015>
22. Nilsson T., Gustavsson K.-E. 2007. Postharvest physiology of 'Aroma' apples in relation to position on the tree. *Postharvest Biology and Technology*, 43 (1): 36–46.
<https://doi.org/10.1016/j.postharvbio.2006.07.011>
23. Qi S., Ding L., Tian K., Chen X., Hu Z. 2006. Novel and simple nonaqueous capillary electrophoresis separation and determination bioactive triterpenes in Chinese herbs. *Journal of Pharmaceutical and Biomedical Analysis*, 40 (1): 35–41.
<https://doi.org/10.1016/j.jpba.2005.06.003>
24. Raudonė L., Raudonis R., Liaudanskas M., Janulis V., Viškelis P. 2017. Phenolic antioxidant profiles in the whole fruit, flesh and peel of apple cultivars grown in Lithuania. *Scientia Horticulturae*, 216: 186–192.
<https://doi.org/10.1016/j.scienta.2017.01.005>
25. Samuolienė G., Viškelienė A., Sirtautas R., Kviklys D. 2016 (a). Relationships between apple tree rootstock, crop-load, plant nutritional status and yield. *Scientia Horticulturae*, 211: 167–173.
<https://doi.org/10.1016/j.scienta.2016.08.027>
26. Samuolienė G., Čeidaitė A., Sirtautas R., Duchovskis P., Kviklys D. 2016 (b). Effect of crop load on phytohormones, sugars, and biennial bearing in apple trees. *Biologia Plantarum*, 60 (2): 394–400.
<https://doi.org/10.1007/s10535-015-0581-3>
27. Tahir I. I., Johansson E., Olsson M. E. 2009. Improvement of apple quality and storability by a combination of heat treatment and controlled atmosphere storage. *HortScience*, 44 (6): 1648–1654.
28. Unuk T., Tijkskens L. M., Germšek B., Zadavec P., Vogrin A., Hribar J., Simčič M., Tojnko S. 2012. Effect of location in the canopy on the colour development of three apple cultivars during growth. *Journal of the Science of Food and Agriculture*, 92 (12): 2450–2458.
<https://doi.org/10.1002/jsfa.5651>
29. Vieira F. G., Borges G. D., Copetti C., Amboni R. D., Denardi F., Fett R. 2009. Physicochemical and antioxidant properties of six apple cultivars (*Malus domestica* Borkh.) grown in southern Brazil. *Scientia Horticulturae*, 122 (1): 421–425.
<https://doi.org/10.1016/j.scienta.2009.06.012>
30. Viškelis J., Kviklys D. 2017. Crop load management in fruit-trees (review). *Sodininkystė ir daržininkystė*, 36 (1–2): 30–42 (in Lithuanian).
31. Wolfe K., Wu X., Liu R. H. 2003. Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry*, 51 (3): 609–614.
<https://doi.org/10.1021/jf020782a>
32. Zadavec P., Cmelik Z., Tojnko S., Unuk T., Schlauer B. 2008. Vegetative growth, yield and fruit quality of 'Gala' apple treated with Regalis (prohexadione-ca). *Acta Horticulturae*, 774: 287–290.
<https://doi.org/10.17660/ActaHortic.2008.774.38>

ISSN 1392-3196 / e-ISSN 2335-8947

Zemdirbyste-Agriculture, vol. 105, No. 1 (2018), p. 71–78

DOI 10.13080/z-a.2018.105.010

Triterpeninių rūgščių kiekį obuolių luobelėse lemia vaismedžių auginimo technologijos

J. Viškelis¹, N. Uselis^{1,3}, M. Liaudanskas², V. Janulis², P. Bielicki⁴, T. Univer⁵, J. Lepsis⁶,
D. Kviklys¹

¹Lietuvos agrarinių ir miškų mokslų centro Sodininkystės ir daržininkystės institutas

²Lietuvos sveikatos mokslų universiteto Medicinos akademija

³Aleksandro Stulginskio universitetas

⁴Lenkijos sodininkystės ir daržininkystės institutas

⁵Estijos gyvybės mokslų universiteto Žemės ūkio ir aplinkos mokslų instituto

Polli sodininkystės tyrimų centras

⁶Latvijos žemės ūkio universiteto Sodininkystės institutas

Santrauka

Nors atlikta daug antrinių metabolitų tyrimų, naujų auginimo technologijų ar atskirų technologinių priemonių įtaka vaisių vidinei kokybei, ypač triterpenų koncentracijai obuoliuose, dar nėra tyrinėta. Naminės obelės (*Malus × domestica* Borkh.) auginimo technologijos ir auginimo vieta turi įtakos triterpenų kaupimuisi obuoliuose. Vaisių krūvio, vaisių vietos vaismedžių vainike, vaismedžių sodinimo atstumo, jų vegetatyvinio augimo reguliavimo ir geografinės augimo vietos įtaka triterpenų kiekiui obuoliuose 2015–2016 m. buvo tirta Lietuvos agrarinių ir miškų mokslų centro Sodininkystės ir daržininkystės institute. Obuolių žievelėse buvo nustatyti keturi triterpeniniai junginiai: korosolo, betulino, oleonolo ir ursolo rūgštys. Triterpenų kaupimasis obuoliuose tiesiogiai susijęs su vaisių krūviu. Obelyse esant dideliame vaisių krūviui (12 vaisių cm⁻² kamieno skerspjūvio ploto (KSP)), obuoliai sukauptė 17 % daugiau triterpenų nei vaismedžiai su mažu vaisių krūviu (4 vaisiai cm⁻² KSP). Esmingai daugiau triterpenų sukauptė obuoliai, augę vaismedžio vainiko apačioje ir vidinėje dalyje. Triterpenų sintezei vaisiuose taip pat turėjo įtakos vaismedžių vegetatyvinio augimo reguliavimo priemonės. Kamieno įpjovimas triterpenų kiekį obuoliuose sumažino 16 %, lyginant su kontroliniu variantu, ir 26 %, lyginant su vasariniu genėjimu. Nustatyta tendencija, jog mažinant obelių sodinimo atstumą triterpenų kiekis obuoliuose didėja. Šaltesnis klimatas ir trumpesnė vegetacijos trukmė reikšmingai padidino triterpenų kiekį veislės 'Aukasis' obuoliuose Lietuvoje bei Estijoje, lyginant su Lenkija. Panaši tendencija nustatyta ir tiriant veislės 'Ligol' obuolius.

Reikšminiai žodžiai: auginimo technologijos, bioaktyvūs junginiai, naminė obelis.