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Lower nitrogen nutrition determines higher phenolic content of organic apples

Juozas Lanauskas[1], Darius Kviklys[1], Mindaugas Liaudanskas[2], Valdimaras Janulis[2], Nobertas Uselis[1], Jonas Viškelis[1], Pranas Viškelis[1]

[1]*Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai, Lithuania*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania*

Corresponding author: j.lanauskas@lsdi.lt

Abstract

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Phenolic content of apples from an integrated and organic apple tree orchard was analysed at the Institute of Horticulture of Lithuanian Research Centre for Agriculture and Forestry in 2012–2013. Apples of 'Lodel' and 'Aldas' cultivars on rootstock B.396 in 8–9th leaf were tested. Nitrogen and potassium fertilizers were applied annually at the rates of 50 kg/ha N and 66.4 kg/ha K in an integrated fruit orchard. A mixture of perennial grasses with 25% of white clover was sown in interrows in the second year after tree establishment in the organic production system. Every two years sward in alternate interrows was tilled for natural organic matter mineralization. Fertilizers were not used in the organic apple orchard. Organic apples of both cultivars had a higher content of procyanidins B1 and B2, phloridzin, chlorogenic acid, (+)-catechin, and (–)-epicatechin. Organic 'Aldas' apples also contained more hyperoside, avicularin and quercitrin than non-organic apples. On average, the total phenolic content of organic apples of both cultivars was 43% higher than that of apples from the integrated orchard. These differences could be explained by nitrogen nutrition: the N content in organically grown apple leaves was significantly lower than that in leaves from the integrated production system. Tree trunk diameter and yield of organic apple were less, and fruits were better coloured.

Keywords: procyanidins; phloridzin; chlorogenic acid; epicatechin

People who are concerned about healthy lifestyle prefer functional and healthy food products. Fruits that are rich in secondary metabolites can provide adequate nutrition. Polyphenols are some of the most important bioactive components; a direct positive correlation between the phenol content and antioxidative activity has been reported (Matthes, Schmitz-Eiberger 2012). Apples show intermediate values of phenol content (Fu et al. 2011) and are one of the most commonly consumed fruits in the world (Francini, Sebastiani 2013). Fruits of different apple cultivars show significant variation in the content of phenols (Rop et al. 2011; Liaudanskas et al. 2015). According to Kolodziejczyk et al. (2010), the total polyphenol content of apples ranges from 161.9 to 882.4 mg/kg fresh weight. Kviklys et al. (2014) reported the effect of rootstocks on the phenolic content of apples: super-dwarf P 61 and P 22 determined the highest phenolics.

Environmental and orchard management practices also affect the phenolic content of apples. It was found that exposure to the sun significantly increased the content of anthocyanins, quercetin-glycosides, epicatechin, procyanidins, phloridzin and chlorogenic acid in the peel of fruits (Hagen

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et al. 2007). The accumulation of phenolic compounds in plants can be negatively affected by excessive N-fertilization (Treutter 2010).

Organically grown products have recently increased in popularity. Organic farming systems offer advantages for nature and society, for example, by preventing environmental and product contamination with synthetic products. Information concerning the fruit phenolic content from organically and conventionally grown apples differs: some experiments have demonstrated an increased amount of phenolic compounds in organically grown apples (Veberic et al. 2005; Mikulic Petkovsek et al. 2010), but other studies have shown no differences (Peck et al. 2009). The aim of this research was to evaluate the phenolic content of apples from integrated and organic apple tree orchards.

MATERIAL AND METHODS

The trial was conducted in the experimental orchard of the Institute of Horticulture (55°60'N; 23°48'E), at the Lithuanian Research Centre for Agriculture and Forestry in 2012–2013. The experiment was designed in two adjacent blocks (integrated and organic) with randomly arranged plots (cultivars). Each experimental plot contained four apple trees planted at the distance of 4 × 2 m. Cvs 'Lodel' and 'Aldas' on rootstock B.396 in 8–9th leaf were tested. The experiment was performed in three replicates. The apple trees within blocks were maintained according to integrated or organic production specifications. Sward in interrows and herbicide-applied strips in the rows were maintained in the integrated production system (IPS). Nitrogen (N) and potassium (K) fertilizers were applied annually at the rate of 50 kg N/ha and 66.4 kg K/ha in the IPS. Organic block was certified in accordance with the Council Regulation (EC) No. 834/2007 (No. of Ekoagros certificates SER–K–12–01085 and SER–K–13–01301). In the organic production system (OPS) mixture of perennial grasses with 25% of white clover was sown in interrows in the second year after tree establishment. After two years, sward in every second interrows was tilled for natural organic matter mineralization. Every two years, the same procedures were repeated in alternate interrows. Soil in the strips was cultivated mechanically. Fertilizers were not used in the OPS.

The soil was analysed in the first year of the experiment, in aggregated samples taken from different blocks in tree rows at a depth 0–60 cm (Table 1). Soil pH was determined using 1N KCl potentiometric method, plant available phosphorus, potassium calcium and magnesium by the Egner-Riehm-Domingo A-L method (soil extraction with buffer solution pH 3.7 from lactic and acetic acids and ammonium acetate). Phosphorus was determined with spectrometer Shimadzu UV 1800 and potassium with flame photometer Jenway PFP7. Calcium and magnesium were determined by atomic absorption spectrometer Analyst 200 (Perkin Elmer, USA). Total organic carbon was measured by a dry combustion method by Total Organic Carbon analyzer Liqui TOC II (Elementar Analysen systeme GmbH, Germany). Soil humus was calculated by multiplying the value of total organic carbon by coefficient 1.72.

The soil in the experiment field was Epicalcari-Endohypogleyic Cambisol, heavy clay loam. The orchard was not irrigated. During the vegetation period soil moisture was measured at the depth of 30 cm with irrometers (Irrometer Comp., Inc., USA) in tree row strips. Soil moisture status during vegetation period was the same in both blocks of the experiment and ranged from 16 to 38 centibars.

Each year in late July, random samples of 50 leaves were taken for leaf chemical analyses from the middle part of the shoots. Ten randomly selected fruits from each experimental plot were taken for phenolic analyses.

Leaf nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) concentration (% dry weight (d.w.)) was established. The content of leaf nitrogen was measured by the Kjeldahl method using Tecator Digestion System DK 20 (VelP Scientifica, Usmate, Italy) and Semi-automatic Distillation Unit UDK139 (VelP Scien-

Table 1. Agrochemical properties of the soil (0–60 cm layer) in integrated and organic apple tree production systems

Sampling place	Nutrient content (mg/kg)				Humus content (%)	pH
	P	K	Ca	Mg		
Integrated orchard	88.9	106.2	6,947	2,564	2.29	7.4
Organic orchard	96.8	102.9	8,197	1,729	1.54	7.4

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Table 2. Leaf nutrient content (%) in integrated and organic production systems

Production system		N	P	K	Ca	Mg
'Aldas'						
2012	Integrated	2.21[b]	0.20[a]	0.96[a]	1.28[b]	0.24[a]
	Organic	1.96[a]	0.22[a]	1.32[b]	1.00[a]	0.19[a]
2013	Integrated	2.16[b]	0.29[a]	1.17[a]	1.24[a]	0.24[a]
	Organic	1.98[a]	0.27[a]	1.03[a]	1.25[a]	0.26[a]
'Lodel'						
2012	Integrated	2.13[b]	0.23[a]	1.17[a]	1.05[a]	0.20[a]
	Organic	1.79[a]	0.24[a]	1.34[b]	0.99[a]	1.79[a]
2013	Integrated	2.18[b]	0.20[a]	1.10[a]	1.22[b]	0.22[a]
	Organic	1.65[a]	0.32[b]	1.55[b]	0.79[a]	0.17[a]

different letters in columns indicate significant differences between values for cultivars ($P < 0.05$)

tifica, Usmate, Italy). Phosphorus was determined by spectrometer ICP Optima 2100 (Perkin Elmer, USA), potassium by flame photometry with Jenway PFP7 (Bibby Scien-tific Limited, Staffordshire, UK), and the contents of calcium and magnesium by atomic absorption spectrophotometry (AAnalyst 200; Perkin Elmer precisely, USA).

Preparation of samples. Each apple with the peel was cut into slices and was frozen and lyophilised with a ZIRBUS sublimator 3 × 4 × 5/20 (ZIRBUS technology, Germany) and ground to a fine powder using a Retsch 200 mill (Haan, Germany).

Extraction. Lyophilised apple powder (2.5 g) was mixed with 30 mL ethanol (70%, v/v) and was extracted in a Sonorex Digital 10 P ultrasonic bath (Bandelin Electronic, Germany) for 20 min at 40°C.

Instrumentation and chromatographic conditions. A Waters 2695 (Waters Co., USA) chromatograph equipped with a Waters 2998 photodiode array (PDA) detector was used for HPLC analysis, which was controlled with Empower® v.2.0. software (Waters, Czech Republic). Chromatographic separations were carried out using a YMC-Pack ODS-A (5 µm, C_{18}, 250 × 4.6 mm i.d.) column equipped with a YMC-Triart (3 µm, C_{18}, 10 × 3.0 mm i.d.) precolumn (YMC Europe GmbH, Germany). The volume of the extract under investigation was 10 µl. The flow rate was 1 ml/min, and a gradient elution was performed. The mobile phase consisted of 2% (v/v) acetic acid in water (solvent A) and 100% (v/v) acetonitrile (solvent B). The following conditions of elution were applied: 0–30 min, 3–15% B; 30–45 min, 15–25% B; 45–50 min, 25–50% B; and 50–55 min, 50–95% B.

Detection was simultaneously performed at three wavelengths: 280 nm (dihydrochalcones, catechins, procyanidins), 320 nm (phenolic acids), and 360 nm (quercetin glycosides). All the phenolic compounds were identified by comparing their retention times and spectra (from 200 to 600 nm) with those of standard compounds.

The results were elaborated statistically by one-way analysis of variance for each cultivar and the significance of differences between the means were evaluated using the Duncan's multiple range test at $P < 0.05$.

RESULTS AND DISCUSSION

Different fertilization and orchard floor management revealed differences in apple tree nutrition. N content in organically grown apple leaves was lower than that in leaves from the integrated production system (IPS). The mean decrease in N content of cv. 'Aldas' leaves was about 10% and in 'Lodel' leaves it was 20% (Table 2). Leaf K content for cv. 'Lodel' was higher in the OPS. The soil K content was similar for both production systems. The increased K content in organically grown tree leaves might be due to N deficiency or lower yield (Fiedler 1970). A higher calcium (Ca) content in cv. 'Aldas' leaves was observed in 2012 and in cv. 'Lodel' in 2013 in the IPS. The content of Ca in the soil was high in both production systems (Table 1).

Trunk diameter indicates that apple trees of both cultivars were less vigorous in OPS (Table 3). The yield of organic cv. 'Aldas' was lower by 30% and cv. 'Lodel' by 43%. Fruit weight of organic 'Lodel' apples was about 15% less than in IPS. Organic 'Lodel' apples were better coloured in both years, 'Aldas' in one year of the experiment.

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Table 3. Growth and yield parameters of integrated and organic 'Aldas' and 'Lodel' apples

Production system		Trunk diameter (cm)	Yield (kg/tree)	Fruit weight (g)	Red blush (%)
'Aldas'					
2012	Integrated	–	11.2[b]	185[a]	60[a]
	Organic	–	7.4[a]	172[a]	74[b]
2013	Integrated	9.6[b]	13.8[b]	180[a]	70[a]
	Organic	8.7[a]	10.1[a]	169[a]	78[a]
'Lodel'					
2012	Integrated	–	18.3[b]	130[b]	78[a]
	Organic	–	11.2[a]	112[a]	90[b]
2013	Integrated	7.5b	23.1[b]	124[b]	82[a]
	Organic	6.4[a]	12.3[a]	105[a]	100[b]

different letters in columns indicate significant differences between the values from separate years for cultivars ($P < 0.05$)

The content of hyperoside in organically grown 'Aldas' apples was 25–62% higher than that in the IPS (Table 4). For cv. 'Lodel', no significant differences were observed. In 2012, the content of isoquercitrin was higher in organic 'Aldas' apples than in fruits from the integrated orchard. In 'Lodel' fruits the trend was opposite. The content of rutin was higher in 'Lodel' apples in the integrated system in one year of the experiment. Organic 'Aldas' fruit had a higher content of avicularin and quercitrin than apples from the integrated orchard 37–48% and 30–40%, respectively. Production system had no effect on the content of latter glycosides in 'Lodel' apples. The total quercetin glycoside content in organic 'Aldas' fruit was 31–46% higher than in non-organic apples. 'Lodel' apples from both production systems contained a similar amount of quercetin glycosides.

No differences were observed in the content of (+)-catechin between tested production systems in the first year (Table 5). In the second year of the experiment, the content of (+)-catechin in apples of both cultivars was 52–58% higher in organic apples than in non-organic apples. The content of (–)-epicatechin was higher in organic apples in both years and for cv. 'Aldas', the increase was 18–35%. Extremely large differences were observed in 'Lodel' apples in the first year of the experiment. Organic apples contained 2.8-fold more (–)-epicatechin than apples from the integrated orchard. (–)-Epicatechin was predominant and determined differences of total content of catechins. In organic 'Aldas' apples, the content of total catechins increased by 16–38%, and in 'Lodel' – by 49–149%.

In organic 'Aldas' apples, the content of procyanidin B1 was 39–49% higher than that in the IPS (Ta-

Table 4. Content of quercetin glycosides (μg/g of dry weight) in 'Aldas' and 'Lodel' apples from integrated and organic production systems

Production system		Hyperoside	Isoquercitrin	Rutin	Avicularin	Quercitrin
'Aldas'						
2012	Integrated	138.2[a]	23.0[a]	15.4[a]	153.3[a]	108.9[a]
	Organic	224.4[b]	32.6[b]	15.8[a]	226.3[b]	141.6[b]
2013	Integrated	190.0[a]	33.6[a]	15.9[a]	164.0[a]	149.9[a]
	Organic	237.2[b]	40.4[a]	14.8[a]	224.5[b]	209.2[b]
'Lodel'						
2012	Integrated	148.1[a]	28.6[b]	50.8[b]	100.0[a]	98.8[a]
	Organic	151.8[a]	12.6[a]	16.0[a]	114.4[a]	97.8[a]
2013	Integrated	147.3[a]	27.1[a]	21.6[a]	114.3[a]	114.7[a]
	Organic	153.4[a]	22.4[a]	15.1[a]	111.8[a]	108.3[a]

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Table 5. Content of catechins (μg/g of dry weight) in 'Aldas' and 'Lodel' apples from integrated and organic production systems

Production system		(+)-Catechin	(–)-Epicatechin
'Aldas'			
2012	Integrated	90.5[a]	611.2[a]
	Organic	92.1[a]	720.3[b]
2013	Integrated	144.4[a]	545.5[a]
	Organic	218.8[b]	736.7[b]
'Lodel'			
2012	Integrated	54.3[a]	335.0[a]
	Organic	42.3[a]	928.4[b]
2013	Integrated	99.1[a]	659.1[a]
	Organic	156.3[b]	974.1[b]

different letters in columns indicate significant differences between the values from separate years for cultivars ($P < 0.05$)

ble 6) and the content of procyanidin B1 in organic 'Lodel' apples was 2.4-fold higher. The content of procyanidin B2 in organic apples was 32–54% and 22–182% higher, respectively, for 'Aldas' and 'Lodel' cultivars. Because procyanidin B2 was predominant, the differences in the total content of procyanidins were similar to those in procyanidin B2. The mean phloridzin content was 25 and 54% higher for organic 'Aldas' and 'Lodel' apples, respectively. Chlorogenic acid was the predominant phenolic compound and its content was on average 24 and 76% higher for organic 'Aldas' and 'Lodel' apples.

The mean total phenolic content of organic 'Aldas' apples increased by 30% and 'Lodel' by 63% compared to apples from the integrated orchard (Fig. 1).

The results demonstrate that the farming system had a significant effect on the phenolic content of apples. The contents of (–)-epicatechin and procyanidin B2 in organic 'Lodel' fruits harvested in 2012 was 2.8-fold higher than in fruits produced in the integrated system and the mean content of procyanidin B1 in organic 'Lodel' apples was 2.4-fold higher. In a similar experiment, Vanzo et al. (2013) observed a higher total phenolic content in organic Golden Delicious apples, whereas differences in other three cultivars were not significant. In our study, the mean total phenolic content increased significantly in both tested cultivars. The differences were observed in the accumulation of quercetin glycosides between 'Aldas' and 'Lodel' cvs. Organic 'Aldas' apples contained more hyperoside, avicularin and quercitrin than apples in the integrated system, but the content of the latter constituents was similar in 'Lodel' fruits from both production systems. The content of isoquercitrin and rutin was higher in 'Lodel' apples from the integrated system than in organic apples.

Organic apples also contained a higher level of total phenolics than integrated or conventional apples in other trials (Hecke et al. 2006; Bogs et al. 2012). The cultivation type had an important influence on the polyphenolic level and antioxidant capacity of the apple fruits and leaves. Organic apples contained higher concentrations of hydroxycinnamic acids, flavanols, dihydrochalcones, querce-

Table 6. Content of the procyanidins, phloridzin and chlorogenic acid (μg/g of dry weight) in 'Aldas' and 'Lodel' apples from integrated and organic production systems

Production system		Procyanidin B1	Procyanidin B2	Phloridzin	Chlorogenic acid
'Aldas'					
2012	Integrated	113.2[a]	645.7[a]	151.0[a]	2,745.1[a]
	Organic	156.9[b]	854.3[b]	187.9[b]	3,274.4[b]
2013	Integrated	111.3[a]	791.0[a]	150.2[a]	2314.7[a]
	Organic	165.4[b]	1219.4[b]	189.5[b]	3018.9[b]
'Lodel'					
2012	Integrated	37.6[a]	453.3[a]	131.2[a]	709.2[a]
	Organic	94.0[b]	1,278.6[b]	268.2[b]	1629.5[b]
2013	Integrated	54.6[a]	1,316.2[a]	213.0[a]	1270.8[a]
	Organic	125.6[b]	1,606.9[b]	260.4[b]	1859.1[b]

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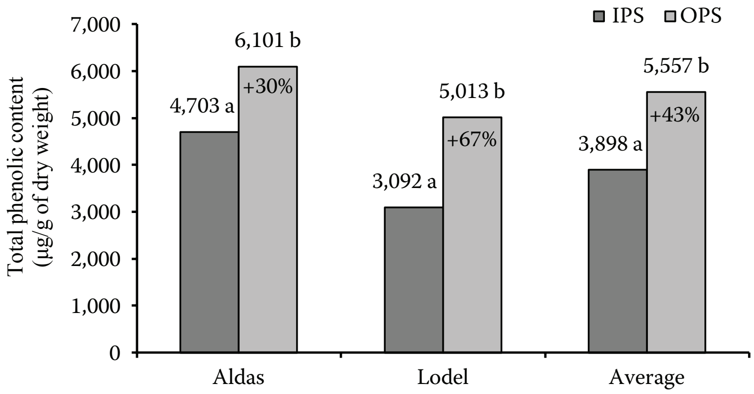

Fig. 1. Total phenolic content in 'Aldas' and 'Lodel' apples from integrated (IPS) and organic (OPS) production systems
different letters in column pairs indicate significant differences between the values ($P < 0.05$)

tins and total phenolics than apples from integrated cultivation (Mikulic Petkovsek et al. 2010).

Some studies have demonstrated no differences in the total phenolic content or in the classes of polyphenols between apples produced by organic and conventional/integrated methods (Briviba et al. 2007; Peck et al. 2009; Valavanidis et al. 2009).

In terms of why the results differ, the organic farming method itself should Wnot cause phenolic accumulation in fruits, which is instead, probably due to the impact of a distinctive aspect of the OPS. According to Veberic et al. (2005) the higher concentration of phenolic compounds in organically grown cultivars might represent a response of the plant to stress. In our experiment, such a stress factor was apparently N nutrition, because apple trees in the OPS were not supplied with additional N. Furthermore, the soil in the organic orchard had lower humus content. This led to deficient N nutrition of fruit trees. An inadequate apple tree supply with the nitrogen in OPS was confirmed by weaker tree growth, lower yield, fruit weight and better fruit colour. According to Stracke et al. (2009), organic fertilizers used in organic farming contain less-available forms of N; therefore, depending on the orchard, soil properties and fertilization practices, organically grown fruit trees might suffer from N deficiency.

Apple trees respond to a high N-supply by increased shoot growth and the reduced accumulation of total phenolic compounds in their leaves (Leser, Treutter 2005). The application of N might cause a decrease in the content of some phenolics and in the percentage of blush skin of apples (Awad, De Jager 2002).

Organic apples that contain a high phenolic content offer additional advantages to consumers, but this might not be beneficial for the fruit grower, as N deficiency usually results in smaller fruits and lower yields.

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