Proceedings of the Estonian Academy of Sciences,
2023, **72**, 2, 154–166
https://doi.org/10.3176/proc.2023.2.06
Available online at www.eap.ee/proceedings

PHYTOCHEMISTRY

Polyphenolic compounds in apple (*Malus domestica* Borkh.) cultivars grown in Estonia

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Received 21 November 2022, accepted 15 February 2023, available online 11 April 2023

Abstract. Several studies have shown that apples (*Malus domestica* Borkh.) in the daily diet are healthy due to their rich content of phytochemicals. The aim of this study was to compare the content of polyphenols in the peels, flesh, seeds and leaves of five apple cultivars (‘Antonovka’, ‘Åkerö’, ‘Cortland’, ‘Karksi renett’ and ‘Krista’) grown in Estonia. Of the 21 collected cultivars, these five were selected on the basis of their rich or distinct chemical composition according to the LC-DAD-MS/MS data. In addition, the weight of the fruit, the number of seeds in the fruit and the weight of the seeds were determined. A total of 33 compounds were detected in the peels, 23 in the flesh, 11 in the seeds, and 25 in the leaves. They belong to four groups: 1) flavon-3-ols (quercetin and its derivatives), 2) dihydrochalcones (phloretin and its derivatives), 3) flavan-3-ols (catechin, epicatechin and oligomers), and 4) esters formed between caffeic acid and L-quinic acid (chlorogenic acid). Based on the data presented in this article, the leaves contained the highest measured total polyphenol content (TP_{GA}). The peels contained high amounts of all the major polyphenolic groups mentioned. The apple flesh lacked flavon-3-ols and the seeds flavon-3-ols and flavan-3-ols. In the peels, the major polyphenols were quercetin galactoside (3–342 mg/100 g), procyanidin B1 (18–179 mg/100 g), and (epi)catechin trimer (28–200 mg/100 g); in the flesh chlorogenic acid (77–298 mg/100 g); in the seeds phloridzin (466 mg/100 g in ‘Cortland’); and in the leaves chlorogenic acid (147–446 mg/100 g) and quercetin glycosides, especially quercetin rhamnoside (242–350 mg/100 g), quercetin galactoside (39–334 mg/100 g) and quercetin glucoside (91–321 mg/100 g).

Keywords: apple, flesh, ‘Karksi renett’, ‘Krista’, leaves, peel, seeds.

INTRODUCTION

‘An apple a day keeps the doctor away’ is a well-known saying. Many studies have shown that apples (*Malus domestica* Borkh.) can be healthy when included in the daily diet (Boyer and Liu 2004; Hyson 2011). Moderate and regular apple consumption can help prevent obesity (Masumoto et al. 2016) or breast cancer (Sun and Liu 2008); it can lower bad cholesterol and reduce the risk of stroke (Ogino et al. 2007). Being rich in important antioxidants such as flavonoids, and also dietary fiber, apples are one of the healthiest foodstuffs (Boyer and Liu 2004; Hyson 2011).

The polyphenolic profile of different parts of apples has been investigated in several studies (Picinelli et al. 1995; Lee et al. 2003; Tsao et al. 2003; Wu et al. 2007; Drogoudi et al. 2008; Petkovšek et al. 2008; Lata et al. 2009; Mainla et al. 2011; Petkovšek et al. 2011; Fromm et al. 2012; Jakobek et al. 2013; Xu et al. 2016; Liaudanskas et al. 2018). The main classes of polyphenols in apples are

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flavonoids, procyanidins, anthocyanidins, dihydrochalcones, quinic acid derivatives such as chlorogenic acids, and other polyphenolic compounds. These compounds may provide the chemical basis for the health benefits of apple peel and flesh (Hyson et al. 2011). The apple peel has recently been demonstrated to be more abundant in polyphenols than the apple flesh, suggesting that it is the peel that provides a significant part of bioactive polyphenols in apples. The finding of an animal study carried out by Nie et al. (2015) demonstrates that the polyphenolic extract from an apple, particularly its peel, can be explored as a chemopreventive or chemotherapeutic agent against oxidative stress-related liver disorders. At least eight types of polyphenols have been found in the apple peel, while six types of polyphenols have been identified in the apple flesh. Apple seeds are a rich source of dihydrochalcones, hydroxycinnamic acids, flavan-3-ols and flavonols (Fromm et al. 2012). However, concentrations of dihydrochalcones, flavanols and quercetin derivatives are shown to be higher in leaves than in fruits (Wojdyło and Oszmiański 2020). Yet, the phenolic composition and bioactivity of apples are profoundly influenced by their variety and can be modified by post-harvest factors, including storage and processing (Napolitano et al. 2004). Also, the content of these substances can be affected by the age of the trees, the shape of the crown, the selection of apples for analysis and the analysis methodology (Kviklys et al. 2022).

The apple is also an economically important fruit crop and the fourth largest fruit crop in the world (Gharghani et al. 2009; Garkava-Gustavsson et al. 2013). Estonia belongs to the northern agricultural region of commercial apple cultivation, where low winter temperatures place high demands on apple cultivars' adaptation to the climate (Jaagus 1997; Toom et al. 2019). Cultivars suitable for cultivation should have winter-hardiness, frost tolerance and a short ripening period. Furthermore, due to high relative humidity and frequent precipitation, resistance to fungal diseases is important (Garkava-Gustavsson et al. 2013).

The collection of apple cultivars and scientific apple breeding started in 1945 at the Polli Horticultural Research Centre. The aim of the breeding has been to obtain apple cultivars with good flavour, high yields, and good resistance to winter damage and diseases of apple trees. Professor Aleksander Siimon (1900–1970) bred 16 cultivars and Kalju Kask (1927–2021) 19 cultivars (Kask 2010; Kask et al. 2010). Foreign and Estonian cultivars are well adapted and preserved in the collection orchard at Polli, Viljandi County, at an altitude of 86 m above sea level. The collection of fruit genetic resources of the Estonian University of Life Science contains 56 Estonian cultivars, 95 selections of Estonian origin and 237 introduced cultivars (Fruit Genetic Resources of Estonian University of Life Sciences 2022).

The content of the main polyphenols and the concentration of total polyphenols were determined previously in the peels of six apple cultivars: 'Talvenauding', 'Krista', 'Liivi Kuldrenett', 'Lobo', 'Cortland' and 'Antei', grown in Estonia (Mainla et al. 2011). However, there is limited information about the polyphenolic composition of other apple cultivars grown in Estonia.

The aim of this study was to determine and compare the composition and content of polyphenolic compounds in the peels, flesh and seeds of the fruits and leaves of five apple cultivars grown in Estonia. In addition, fruit weight, the number of seeds in apple fruit and seed weight were determined.

MATERIALS AND METHODS

Plant material

On 13 October 2011, fruits and leaves of 21 apple cultivars were collected at the Polli Horticultural Research Centre (58°07′16″N, 25°32′47″E) in Estonia and used for preliminary experiments. The apple cultivars selected were 'Antei' (country of cultivar origin: Belarus), 'Antonovka' (Russia), 'Cortland' (USA), 'Heta' (Finland), 'Hyslop' (USA), 'Kaimo' (Estonia), 'Karksi renett' (Estonia), 'Kastar' (Estonia), 'Kitaika institutskaja' (Russia), 'Krista' (Estonia), 'Liivika' (Estonia), 'Lobo' (Canada), 'Åkerö' (Sweden), 'Paide taliõun' (Estonia), 'Pamjat Issajeva' (Russia), 'Punane talvenauding' (Estonia), 'Sidrunkollane taliõun' (Estonia), 'Sügisjoonik' ('Streisling Herbst', Netherlands), 'Tellissaare' (Estonia), 'Teremok' (Ukraine) and 'Quercher Beauty' (Canada).

Before extraction, the plant material was stored in a freezer at 4 °C at the Institute of Pharmacy, University of Tartu, Estonia. Five cultivars with the most abundant or distinct chemical composition were selected for a more detailed study. The cultivars were selected according to the LC-DAD-MS/MS data (not presented here). These five cultivars were 'Antonovka', 'Åkerö', 'Cortland', 'Karksi renett' and 'Krista' (Fig. 1).

Two years later, in the autumn of 2013, fresh fruits and leaves of the cultivars 'Antonovka', 'Cortland', 'Karksi renett' and 'Krista' were collected from the orchard of the Polli Horticultural Research Centre. Four kilograms of apples per cultivar were collected at harvest maturity from the outer area of the canopy at a height of 1.5 to 2 m. The fruits of the autumn cultivars 'Antonovka', 'Åkerö', and 'Krista' were harvested on 20 September and the fruits of the winter cultivars 'Cortland' and 'Karksi renett' on 7 October. The fruits and leaves were stored for one month at a temperature of 2–4 °C and relative humidity (RH) of 80–95% in a storage depot at the Polli Horticultural Research Centre before extraction and LC-DAD-MS/MS runs.

'Antonovka'

'Åkero'

'Cortland'

'Karksi renett'

'Krista'

Fig. 1. Apple cultivars studied. Photos by A. Raal.

Characterization of the studied apple cultivars

'Antonovka' is a common local cultivar originating from Russia. The fruit of 'Antonovka' is large, the shape is spherical-conical to short-spherical-conical. The fruit is whitish-green to greenish-white, turning whitish-yellow when ripe. In sunny summers it may develop a slight reddish flush. The skin of 'Antonovka' is smooth, shiny, covered with a layer of cuticular wax. The flesh is whitish-yellow, coarse, juicy, with a sharp, sweet-tart and winey flavour. The fruit is rich in well-developed, brown, medium-sized and ovoid seeds with a sharp point. The fruits ripen in October and can be stored until January (Eslon et al. 1970; Fig. 1).

The cultivar 'Åkerö' is of Swedish origin. The fruit has an oval shape, with five indistinct ridges. Its main colour is yellowish-green with a pale red flush in patches or stripes on the side exposed to sunlight. The skin is thin, smooth, shiny and firm. The flesh of 'Åkerö' is greenish-white or whitish-yellow, with a pinkish hue under the skin. The flesh is juicy and has a sweet-tartish flavour and a weak aroma. The seeds are small to medium, thick and dark brown, egg-shaped. The fruits ripen in October and can be stored until March.

'Cortland' is a cultivar that was bred in the USA. The fruit is larger than the average apple fruit. Its shape is globose or oblate. Its main colour is greenish-yellow with a bright crimson blush. Its skin is thin, shiny and strong. Its flesh is snow-white, fine-grained, juicy and has a sweet-tartish flavour. The seeds are quite large and dark brown with a rounded tip. The fruits ripen in December and can be stored until May (Mägi 1975).

'Karksi renett' is a cultivar originating from Estonia. The cultivar became popular in the late 1930s (Mägi 1975). The fruit is medium to large in size, flat-globose. The main colour is greenish-yellow, with a slight pinkish flush on the sun-exposed side of the fruit. The skin is thin and shiny. The flesh is yellowish-white, firm, finely grained, juicy and sweet-tartish. The seeds are of medium size, brown, obovoid in shape. The fruits ripen in November to December and can be stored until May or June (Kask and Kivistik 2005; Kask 2010).

'Krista' is a cultivar bred by Kalju Kask at the Polli Horticultural Research Centre of the Estonian University of Life Sciences. The fruit size is medium to large, globose or flat-globose. Its main colour is whitish-yellow, with a bright red flush. The skin is shiny and thick. The flesh is white, firm and sweet-tart. The seeds are quite brown, large, oblong, and with a sharp point. The fruits ripen in October and can be stored until January (Kask 2010).

Determination of fruit weight, the number and weight of seeds

Fruit weight, the number of seeds in each fruit and seed weight were determined for the cultivars 'Antonovka', 'Åkerö', 'Cortland', 'Karksi renett' and 'Krista'. The values were determined using ten apples of each cultivar. For determining the average weight of the seeds, ten seeds were weighed simultaneously (Table 1).

Preliminary tests for the extraction of polyphenols

In order to find the most exhaustive solvent for the extraction of polyphenols, extraction tests were carried out for the apple peels, flesh, seeds and leaves. For this purpose, Polish apples were purchased from an Estonian grocery store and leaves of 'Antonovka' were collected from the Polli Horticultural Research Centre. Four solvents [10%, 20% and 30% ethanol-water (v/v) and 20% methanol-water (v/v)] were tested for extraction. The extracts were prepared at a ratio of 1:20 (w/v) using 0.25 g of fresh plant material and 5 mL of the solvent. The samples were extracted for 24 hours at room temperature with occasional shaking. The resulting 16 extracts were then filtered through a filter paper, centrifuged at 4000 rpm at 20 °C for 15 min (Eppendorf TM Model 5804R, Hamburg, Germany) and analysed for polyphenols by LC-DAD-MS/MS. One sample of apple peel, flesh, seeds and leaves was tested with the solvent and analysed by LC-DAD-MS/MS.

Extraction of polyphenols from plant materials

Based on the results of the preliminary experiments (data not presented here), extracts of the five cultivars to be studied in detail were prepared from air-dried material. For this, the peel was removed from the fruit with a knife

Table 1. Weight of apple fruit (mean ± SD; n = 10), the number of seeds in one fruit (mean ± SD; n = 10) and the weight of 10 seeds (n = 1)

Cultivar	Fruit weight (g)	Number of seeds	Weight of 10 seeds (g)
'Antonovka'	147.9 ± 30.7	9.1 ± 3.9	2.4
'Åkerö'	131.5 ± 19.7	6.0 ± 2.0	2.8
'Cortland'	111.1 ± 12.9*	4.4 ± 4.0	3.2
'Karksi renett'	150.2 ± 28.9	4.9 ±3.2	2.7
'Krista'	146.9 ± 15.4	7.5 ± 2.7	3.2

* $p < 0.01$; t-test, compared to the common cultivar 'Antonovka'

and cut into 3–5 mm pieces. The leaves and flesh were equally cut into 3–5 mm pieces. The seeds were cut in half. The peels, flesh and seeds were dried using a food dehydrator (Bomann DR 435 CB, Kempen, Germany) for 12 hours at 63–65 °C. The leaves had dried up at 2–4 °C during the storage at the Polli Horticultural Research Centre. The extracts of the peels and leaves were prepared with 10% ethanol-water (v/v) and the extracts of the flesh and seeds with 30% ethanol-water (v/v). Subsequently, 0.25 g of the peel, leaves, flesh or seeds were weighed and 5 mL of the extractant was added. The samples were extracted for 24 hours at room temperature with occasional shaking. The extracts were fil- tered through a filter paper, centrifuged at 4000 rpm at 20 °C for 15 min and analysed by LC-DAD-MS/MS. One extract of the apple peel, flesh, seeds and leaves was prepared and analysed by LC-DAD-MS/MS.

LC-DAD-MS/MS analyses

Identification and quantification of individual phenolic compounds was carried out by LC-MS/MS on an Agilent 1100 Series LC/MSD Trap XCT equipped with an electrospray interface (ESI). The ion trap was connected to an Agilent 1100 Series HPLC instrument consisting of an autosampler, solvent membrane degasser, binary pump, column thermostat, and a UV-VIS diode array detector (DAD).

Compounds were separated using a reversed-phase Zorbax 300SB-C_{18} HPLC column (2.1×150 mm; 5 µm; Agilent Technologies) in a stepwise mobile phase gradient of 0.1% formic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.3 mL/min and a column temperature of 35 °C. MS/MS detection and quantitation were carried out in negative ion mode in the m/z interval of 50–1000 amu, targeted at 400 amu. The DAD was operated at 200–600 nm, and the absorbance of the eluate was continuously monitored at wavelengths of 250, 280, 330, 350, 370, and 590 nm. HPLC 2D ChemStation software with a ChemStation Spectral SW module was used for process guidance and data collection.

The TP_{GA} of the peel, flesh, seeds and leaves was estimated in gallic acid equivalents (mg GA/100 g of sample) by HPLC-UV at 280 nm. In order to convert the areas to milligrams per 1 g of sample, 1 mg/mL of gallic acid stock solution was prepared in water. For calibration, the areas under the curves (AUC280) of gallic acid were measured at different concentrations.

Polyphenols were identified by parent and daughter ion spectra, UV-spectra, peak retention times and comparison with standard compounds. The commercial standards of phloretin, phloridzin (phloretin-2-glucoside), catechin, chlorogenic (3-caffeoylquinic) acid, gallic acid, quercetin, quercitrin (quercetin-3-rhamnoside) and rutin (quercetin-3-rutinoside), all from Sigma-Aldrich, Buchs, Switzerland, with purity of 95–98% were used for the identification and quantitation of polyphenols.

Statistics

Basic statistics and comparison of groups by t-test for independent samples were carried out using IBM® SPSS® Statistics, version 29.0. The data was also processed with factor analysis through a Varimax rotation of the principal components (3 components, IBM® SPSS® Statistics, version 29.0).

RESULTS AND DISCUSSION

Fruit weight, the number and weight of seeds of the studied apple cultivars

'Karksi renett' had the highest fruit weight and 'Cortland' the lowest fruit weight of the studied apples, 150.2 ± 28.9 g and 111.1 ± 12.9 g, respectively (Table 1). The commercially optimum size of apple fruit in France is over 200 g (Herregods 1999). Also in Canada, the most preferred size of apple fruit was found to be nearly 200 g (Hampson and Quamme 2000). In a New Zealand study, the average harvested size of the common cultivars 'Delicious', 'Golden Delicious' and 'Fuji' was 224 g, 171 g and 194 g, respectively (Warrington et al. 1999). Thus, the studied Estonian apple fruits do not reach the size of the abovementioned fruits. However, the fruit size of apples grown in Estonia tends to be larger than in Finland, where the typical weight of domestic apple fruit is often below 120 g (Seppä 2014). Also in Sweden, the size of the well-known cultivar 'Aroma' has been proved to be less than 130 g (Tahir et al. 2007). As regards comparison with the other Baltic countries, a study conducted on the cultivar 'Auksis' established the largest fruit weights to be in Lithuania, followed by fairly equal weights in Latvia and Estonia (Kviklys et al. 2012).

The mean number of seeds varied among the five cultivars. 'Antonovka' was the richest in seeds, containing an average of nine seeds per fruit (Table 1). The cultivar 'Cortland' had the smallest number of seeds, with an average value of nine seeds per fruit. In a study by Buccheri and Di Vaio (2004), the cultivar 'Annurca Tradizionale' had mostly two or three seeds, while 'Red Delicious' and 'Golden Delicious' had an average of 5.25 and 5.05 seeds, respectively. By comparison, the fruit of the studied Estonian apple 'Annurca Tradizionale' weighed 110–160 g and contained 1–6 seeds. Furthermore, Buccheri and Di Vaio (2004) indicated that a higher number of seeds can stimulate better growth due to greater attraction of nutrients, since growing seeds are the site of

the production of hormones such as auxin, gibberellins and cytokinin (Luckwill et al. 1969). Interestingly, regarding Estonian apples, this trend of higher fruit weight and seed number can be seen only for the cultivars 'Antonovka' and 'Krista'.

The lowest seed weight was found in the cultivar 'Antonovka', with ten seeds weighing 2.4 g. 'Cortland' and 'Krista' excelled in the highest weight of ten seeds, both 3.2 g.

Qualitative polyphenolic composition

In the qualitative analyses of the present study, a total of 33 compounds were identified in the peel, 23 in the flesh and 11 in the seeds. Twenty-five compounds were found in the leaves (Table 2). The MS base peak chromatogram (BPC) and the UV chromatogram of the peel sample of the cultivar 'Krista' at 280 and 360 nm are shown in Fig. 2.

In addition to the polyphenols listed in Table 2, the peels contained five compounds with pseudomolecular (parent) ions with m/z values of 423, 409, 430, 505 and 469, which could not be identified (Table 2). Additionally, a compound with a m/z value of 449 was detected in the flesh, tentatively identified as 3'-*O*-methyl-dihydroxy-quercetin or myricetin pentoside. The leaves additionally contained compounds with a m/z value of 579 and 417, which were tentatively identified as a catechin derivative and hydroxyphloretin, respectively.

In general, the main polyphenols found in apple fruits or leaves can be divided into four major groups: 1) flavon-3-ols (quercetin and its derivatives), which are generally widespread in the plant kingdom; 2) dihydrochalcones, quite specific to apple trees (phloretin and its derivatives); 3) flavan-3-ols (catechin, epicatechin and oligomers); and 4) esters formed between caffeic acid and L-quinic acid (represented by chlorogenic acids; Monfoulet et al. 2020). All these groups were also detected in the five analysed cultivars. Based on the qualitative data, the peel had the highest number of compounds, containing all the major polyphenolic groups found in apples. All these groups were also present in the leaves, which, however, contained a smaller variety of compounds (Table 2).

Flavon-3-ols were absent in the apple flesh, and the seeds lacked flavon-3-ols and flavan-3-ols. Quercetin, which belongs to flavon-3-ols, was detected in eight glycosidic forms in the peels and in six forms in the leaves. The

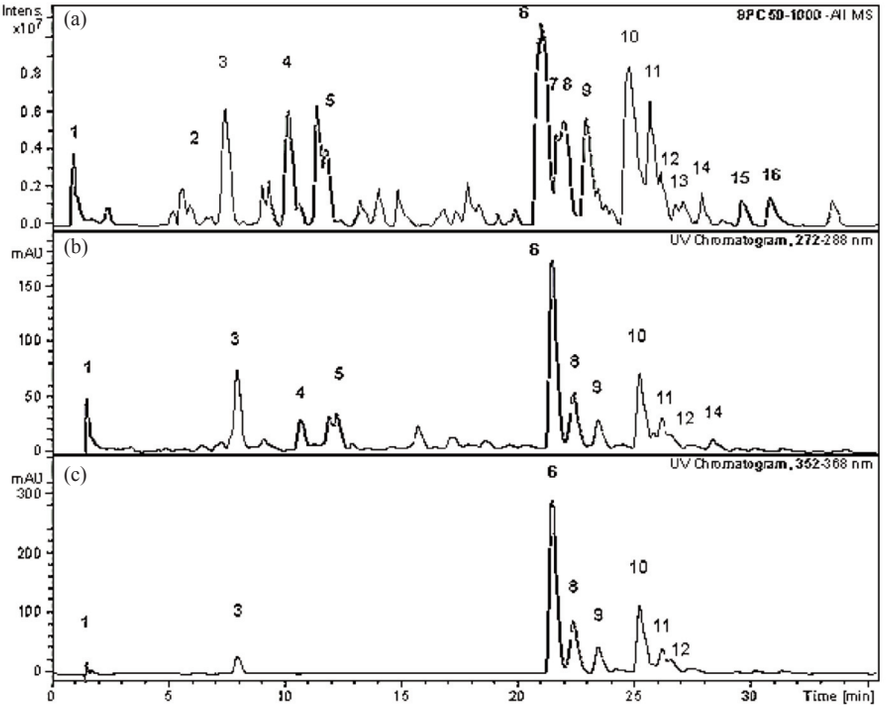

Fig. 2. Base peak chromatogram (a) and UV-chromatograms (b) – 280 nm, (c) – 360 nm of the 'Krista' peel extract. 280 nm is considered the most representative wavelength for the estimation of total polyphenols, 360 nm is specific and characteristic of the flavon-3-ol group of polyphenols. 1 – quinic acid glucoside, 2 – procyanidin B1, 3 – chlorogenic acid, 4 – procyanidin B2, 5 – epicatechin, 6 – quercetin galactoside, 7 – quercetin rutinoside, 8 – quercetin glucoside, 9 – quercetin pentoside 1, 10 – quercetin pentoside 2, 11 – quercetin rhamnoside, 12 – isorhamnetin hexoside, 13 – isorhamnetin rutinoside, 14 – phloridzin, 15 – isorhamnetin pentoside 1, 16 – isorhamnetin pentoside 2.

Table 2. Compounds detected in the apple peels, flesh, seeds and leaves by LC-DAD-MS/MS (n = 1)

Peels	Flesh	Seeds	Leaves
Catechin	Catechin	Apigenin glucuronide	Chlorogenic acid
Chlorogenic acid	Caffeic acid hexoside	Chlorogenic acid	Chlorogenic acid isomer
Chlorogenic acid isomer	Caffeic acid dihexoside	Hydroxyphloretin glucoside	Chlorogenic acid glucoside
Coumarylquinic acid	Chlorogenic acid isomer	Phloretin	Coumarylquinic acid
Epicatechin	Coumarylquinic acid isomer 1	Phloretin diglucoside	Epicatechin dimer A-type
(Epi)catechin dimer	Coumarylquinic acid isomer 2	Phloretin xyloglucoside isomer 1	(Epi)catechin trimer
(Epi)catechin trimer A-type	Epicatechin	Phloretin xyloglucoside isomer 2	(Epi)gallocatechin
Isorhamnetin hexoside	(Epi)catechin trimer	Phloretin pentoside	(Epi)gallocatechin glycoside
Isorhamnetin rutinoside	Hydroxyphloretin glucoside	Phloridzin	(Epi)gallocatechin galloylglycoside
Isorhamnetin pentoside 1	Isorhamnetin pentoside		Eriodictyol
Isorhamnetin pentoside 2	Malic acid		Hydroxyphloretin
Kaempferol pentoside	Phloretin glycoside		Kaempferol rhamnoside
Kaempferol rhamnoside	Phloretin xyloglucoside		Phloretin
Phloretin xyloglucoside	Phloridzin		Phloridzin
Phloridzin	Procyanidin B1		Procyanidin B1
Procyanidin B1			Protocatechinic acid glycoside
Procyanidin B1 isomer			Quercetin
Quercetin acetyl-glycoside			Quercetin galactoside
Quercetin dideoxy-methoxy-hexoside			Quercetin hexoside
Quercetin galactoside			Quercetin pentoside
Quercetin glycoside			Quercetin rutinoside
Quercetin pentoside 1			Quercetin rhamnoside
Quercetin pentoside 2			
Quercetin rutinoside			
Quercetin rhamnoside			
Quinic acid glycoside			

quercetin glycosides in the peels and leaves were the same, except that the peels contained quercetin in the form of acetylglycoside and dideoxy-methoxy-hexoside. The other flavon-3-ols identified were isorhamnetin and kaempferol derivatives, which were the most abundant in the peels. Among the polyphenols of the dihydrochalcone group, all the studied apple parts contained phloretin glucoside phloridzin. The apple seeds had six derivatives of phloretin, followed by four derivatives in the leaves and flesh and two derivatives in the peels (Table 2).

Regarding flavan-3-ols, the peel had the highest number (seven) of catechin derivatives, the leaves contained six and the flesh contained four flavan-3-ols. No flavan-ols were found in the seeds. Flavan-3-ol derivatives were represented as procyanidin B1, catechin and epicatechin, as well as their dimers or trimers (Table 2).

Chlorogenic acid as an ester formed between caffeic acid and L-quinic acid was detected in all parts of the apples studied. In addition, apigenin glucuronide as a flavone was found in the apple seeds and flavanone erio-

dictyol was detected in the apple leaves. Among the phenolic acids, quinic acid glycoside was detected in the peels and coumarylquinic acid in the peels, flesh and leaves. Caffeic acid glycosides and malic acid were found only in the apple flesh (Table 2).

Procyanidin B2 and aglyconic quercetin found by Mainla et al. (2011) were not detected in the peels of the present study. Moreover, in this study, catechin was only found in the peel of 'Karksi renett', whereas Mainla et al. reported the presence of catechin in all Estonian apple peels.

Quantitative polyphenolic composition

Among the generally edible parts of apple fruit, i.e. the peel, flesh and seeds, the highest TP_{GA} was established in the peel of 'Krista' (1158 mg/100 g as GA equivalents; Table 3). The TP_{GA} of 'Krista' was estimated in 2006–2007 by Mainla et al. (2011). Also in 2006, 'Krista' had the highest TP_{GA} among the studied cultivars.

The lowest TP_{GA} was found in the peel of Estonian-grown 'Cortland' (183 mg/100 g; Table 3). The TP_{GA} of 'Cortland' grown in Ontario, Canada, was shown to contain 388.5 mg/100 g of polyphenols (Tsao et al. 2003). By comparison, the TP_{GA} in the peels of the apple cultivars grown in Greece was 840–1990 mg/100 g (Drogoudi et al. 2008).

In the comparison of apple flesh, the highest TP_{GA} (699 mg/100 g) was detected in the cultivar of 'Karksi renett' and the lowest in 'Krista' (363 mg/100 g). The seeds of 'Cortland' were the richest in terms of polyphenol content (690 mg/100 g). Among the leaves, 'Karksi renett' had the highest TP_{GA} (3977 mg/100 g; Table 3).

Previous studies have shown that the peel has a significantly higher TP_{GA} value than the flesh (McGhie et al. 2005; Veberic et al. 2005; Petkovšek et al. 2007; Drogoudi et al. 2008; Lata et al. 2009). It has also been reported that the phenol content at all stages of fruit ripening is higher in the peels than in the flesh (Treutter 2001; Renard et al. 2007). In the studied apples grown in Estonia, this regularity was clearly visible in the cultivar 'Krista', where the TP_{GA} was 3.1 times higher in the peel than in the flesh (Table 3). This difference was rather similar to the results of the study by Drogoudi et al. (2008), where the TP_{GA} was 1.2–3.3 times higher in the peels of Greek apples. However, in a study of Slovenian apples, the TP_{GA} in the peels was up to 9.7 times higher (Petkovšek et al. 2007). The cultivar 'Antonovka' had quite similar content of polyphenols in the peel and flesh – 704 mg/100 g and 691 mg/100 g, respectively. For the remaining three cultivars, the TP_{GA} was lower in the peels than in the flesh (Table 3).

In comparing the TP_{GA} in the studied cultivars and plant parts, the principal component analysis showed that the plant part was the statistically determining factor ($p < 0.001$). The cultivars did not show statistically significant difference ($p = 0.496$) in the TP_{GA}. Based on the data presented in this article, the leaves contained the highest TP_{GA}.

The high content of catechin, epicatechin and procyanidin B1 in apples has been shown to be responsible for the cholesterol-lowering ability of apples (Serra et al. 2012). Thus, the consumption of the studied apple peels may reduce the risk of cardiovascular diseases. The

Table 3. TP_{GA} in the peels, flesh, seeds and leaves as gallic acid equivalents (mg GA/100 g of sample) by HPLC-UV (280 nm) (n = 1)

	Cultivar	TP_{GA} mg/100 g
Peel	'Antonovka'	704
	'Åkerö'	465
	'Cortland'	183
	'Karksi renett'	596
	'Krista'	1158
Flesh	'Antonovka'	691
	'Åkerö'	612
	'Cortland'	678
	'Karksi renett'	699
	'Krista'	363
Seeds	'Antonovka'	527
	'Åkerö'	678
	'Cortland'	690
	'Karksi renett'	362
	'Krista'	593
Leaves	'Antonovka'	3318*
	'Cortland'	2133*
	'Karksi renett'	3977*
	'Krista'	3033*

* $p < 0.01$; t-test, leaves compared to the corresponding peel, flesh, seeds

main polyphenols in the peels were quercetin galactoside (3–342 mg/100 g), procyanidin B1 (18–179 mg/100 g), chlorogenic acid (5–153 mg/100 g) and (epi)catechin trimer (28–200 mg/100 g; Table 4). Among the quercetin glycosides in the peels, the dominant compound was found to be quercetin galactoside, which was similarly found to be the major compound in the peels of apples grown in Canada (Tsao et al. 2003). In our study, the peel was the only part of the whole apple fruit that contained quercetin glycosides, similarly to the study by Petkovšek et al. (2007), which indicated that the peel was the main source of quercetin. As in Slovenian apples (Petkovšek et al. 2007), quercetin rutinoside was present in Estonian apples only in the peels. However, the content of quercetin rutinoside in the peels was variable (1–59 mg/100 g) and sig- nificantly lower than in Polish apples (average 534 mg/ 100 g; Lata et al. 2009). Petkovšek et al. (2007) and Lata et al. (2009) reported the presence of catechin in all the apple peels studied. In apples grown in Estonia, catechin was present only in the peel of 'Karksi renett' at a concentration of 26 mg/100 g. By comparison, the concentra- tion of catechin in Polish apple peels was 9.9–44.2 mg/ 100 g (Lata et al. 2009). The levels of chlorogenic acid in the peels of the current study were high, similarly to the results of other authors (Kondo et al. 2002; Veberic et al. 2005). The only exception was 'Cortland', the peel of which had 17.2 times lower chlorogenic acid content than that of 'Krista' (Table 4).

Chlorogenic acid was the polyphenol with the highest content (77–298 mg/100 g) in the apple flesh. Other dominant phenolic compounds were (epi)catechin trimer (31–99 mg/100 g) and procyanidin B1 (5–78 mg/100 g; Table 4). The high content of chlorogenic acid in the flesh has also been shown in Polish apples, but the content of chlorogenic acid in 'Karksi renett' (298 mg/100 g) was significantly higher than in the Polish 'Red Rome', which had the highest chlorogenic acid content (231 mg/100 g). The flesh of apples grown in Poland contained on average 82 mg/100 g of quercetin rutinoside (Lata et al. 2009). However, aglyconic quercetin was not detected in apples grown in Estonia, similarly to apples grown in Croatia (Jakobek et al. 2013), Poland (Duda-Chodak et al. 2011) and Slovenia (Petkovšek et al. 2007). Several studies have shown that the content of phloridzin is notably higher in the apple peel than in the flesh (Veberic et al. 2005; Petkovšek et al. 2007; Lata et al. 2009). Interestingly, this tendency was not observed in Estonian cultivars. Also, Veberec et al. (2005) reported catechin to be the second most abundant phenol in the flesh of apples grown in Austria and Slovenia. In Estonian apples, catechin was detected only in 'Karksi renett' and at a rather low concentration (8 mg/100 g; Table 4).

Phloridzin predominated in the apple seeds, with the highest phloridzin concentration in the seeds of 'Cortland' (466 mg/100 g) and the lowest in the seeds of 'Karksi renett' (218 mg/100 g; Table 4). Also in apples grown in Germany (Fromm et al. 2012), China (Xu et al. 2016) and Poland (Duda-Chodak et al. 2011), phloridzin was the main phenolic compound in the seeds. The other two major polyphenols in the seeds of Estonian apples were chlorogenic acid (15–98 mg/100 g) and phloretin xyloglucoside (28–62 mg/100 g). Previous studies (Duda-Chodak et al. 2011; Fromm et al. 2012) have also shown that the latter two compounds are dominant in seeds. In a study by Xu et al. (2016), hyperin (quercetin-3-*O*-galactoside) was the second most abundant phenolic compound in the seeds. It is noteworthy that hyperin was not detected in the seeds of cultivars grown in Estonia.

Regarding the polyphenols in apple leaves, dihydrochalcones, especially phloridzin and its aglycone phloretin, have been identified as the dominant polyphenols (Picinelli et al. 1995; Petkovšek et al. 2011). Other major phenolics in apple leaves are quercetin glycosides, chlorogenic acid (Picinelli et al. 1995; Petkovšek et al. 2011) and procyanidin B2 (Picinelli et al. 1995). All these polyphenols were also present in the leaves of apple trees grown in Estonia. However, the dominating compounds were controversially chlorogenic acid (147–446 mg/100 g) and quercetin glycosides, in particular quercetin rhamnoside (242–350 mg/100 g), quercetin galactoside (39–334 mg/100 g) and quercetin glucoside (91–321 mg/100 g). In the studied leaves, phloretin and phloridzin were present at a concentration of 25–56 and 35–157 mg/100 g, respectively. However, their content was quite low compared to the leaves of apple trees grown in Slovenia, where phloretin and phloridzin concentrations were 20–90 mg/100 g and 7–11 g/100 g, respectively. Procyanidins were detected in the leaves of the cultivars 'Antonovka' and 'Karksi renett' in the form of procyanidin B1 (Table 4).

Potential health benefits of the detected polyphenols

There is *in vitro* data that phloretin, a rather unique polyphenol in apple, is a fairly efficient inhibitor of a number of cytochrome 450 (CYP450) isoenzymes in phase 1 of the metabolism of both endogenous substances and xenobiotics, such as CYP3A4 and CYP2C9 (Kimura et al. 2010), and CYP2C19 (Nguyen et al. 2020) which metabolizes phloretin to 3-hydroxy phloretin. It also inhibits the catalytic activity of the most important CYP450s, such as CYP1A1 (Pohl et al. 2006), CYP1A2 and CYP3A4 (Gao et al. 2012). Phloretin also has strong inhibitory ability against a number of UDP-glucuronosyltransferase UGT1A and 1B enzymes, which catalyse the synthesis of glucuronide derivatives in the second phase of metabolism (Chen et al. 2022). All of these inhibitions can lead to *in vivo* food-drug interactions that undesirably prolong the half-life of a drug or its potentially toxic metabolites in

Table 4. Polyphenols and their content in the peels, flesh, seeds and leaves (mg/100 g dry weight; n = 1)

Identification	m/z [M-H]⁻	Peels					Flesh					Seeds					Leaves*			
		A	Å	C	KA	KR	A	Å	C	KA	KR	A	Å	C	KA	KR	A	C	KA	KR
Phloretin	273	–	–	–	–	–	–	–	–	–	–	1	4	5	1	3	56	29	47	25
Phloretin diglucoside	597	–	–	–	–	–	–	–	–	–	–	11	18	3	2	7	–	–	–	–
Phloretin xyloglucoside	567	19	16	1	13	8	7	1	–	5	5	62	31	36	35	28	–	–	–	–
Phloretin pentoside	405	–	–	–	–	–	–	–	–	–	–	1	1	–	1	1	–	–	–	–
Hydroxyphloretin glucoside	451	–	–	–	–	–	1	1	–	1	–	–	–	–	–	–	–	–	–	–
Phloridzin	435	8	6	1	7	14	6	6	4	3	2	358	386	466	218	370	48	35	157	51
Catechin	289	–	–	–	26	–	–	–	–	8	–	–	–	–	–	–	–	–	–	–
Procyanidin B1	577	117	54	18	121	179	24	26	78	35	5	–	–	–	–	–	138	–	141	–
(Epi)catechin trimer	577	130	91	28	123	200	31	83	99	69	33	–	–	–	–	–	–	–	–	–
Chlorogenic acid	353	115	110	5	153	86	173	274	77	298	94	30	98	49	32	15	446	147	412	301
Quercetin rutinoside	609	36	1	2	6	59	–	–	–	–	–	–	–	–	–	–	40	22	165	6
Quercetin galactoside	463	61	15	5	12	342	–	–	–	–	–	–	–	–	–	–	240	39	334	133
Quercetin glucoside	463	22	3	5	8	72	–	–	–	–	–	–	–	–	–	–	186	91	321	120
Quercetin pentoside 1	433	13	4	4	7	50	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Quercetin pentoside 2	433	39	9	9	20	127	–	–	–	–	–	–	–	–	–	–	27	41	37	36
Quercetin rhamnoside	447	37	3	6	145	43	–	–	–	–	–	–	–	–	–	–	350	242	320	257

A = ‘Antonovka’, Å = ‘Åkerö’, C = ‘Cortland’, KA = ‘Karksi renett’, KR = ‘Krista’.
* Leaves of ‘Åkerö’ were not studied

the organism. Phloretin has a strong dual chemopreventive effect against mycotoxin aflatoxin B1 through its inhibitory effect on CYP1A2, CYP3A4 (1st phase of xenobiotic metabolism) and its inductive effect on the glutathione S-transferase activity (2nd phase of xenobiotic metabolism; Gao et al. 2012). Whether the concentration of phloretin and its derivatives that can be hydrolysed to produce aglycone phloretin in apple seeds is sufficiently high to cause different *in vivo* health effects needs to be investigated in the future.

On the other hand, we have tentatively shown that, due to the inhibition of specific CYP450 isoenzymes, phloretin can to some extent shift the enzymatic (per)oxidation of linoleic acid from the CYP450-controlled pathway to the lipoxygenase (LOX)-controlled pathway (Gabbs et al. 2015), thereby reducing the concentration of potentially toxic oxy-metabolites of linoleic acid, such as leukotoxins (9- and 13-EpoME) and their hydrolysis products leukotoxin diols (9- and 13-diHOME; Markaverich et al. 2007; Bergmann et al. 2022) in an oxidizing food matrix such as meat. Another dihydrochalcone phloridzin, glucoside of phloretin, could hypothetically be used for body weight reduction and prevention of type 2 diabetes (Liaudanskas et al. 2018). As the peel is a very important source of phenolic compounds in addition to the flesh, it is recommended that apples should be eaten unpeeled. On the other hand, when buying commercial apples, peeling the apples can be an important tool to remove the preservatives on the surface along with the peel. Therefore, it is worth eating with peels apples that are grown in one's own garden or organic products from a trusted apple grower.

Another practical aspect is the solubility and bioavailability of phenolic compounds in apples. In our studies, ethanol was used as a solvent, but the phenolic compounds contained in apples can be assimilated by the human body in a completely different ratio, depending on how the apples were processed. Since the peel of apples is also very important as a source of these substances, the question arises whether apple juice should be preferred to eating apples? Technologically, preparation of juice is preceded by apple crushing, which is expected to significantly contribute to a higher extractability of the biologically active substances found in apples juice. Therefore, it would also be important to study the composition of bioactive substances in apple juice.

Marcotte et al. (2022) concluded in their review of human intervention studies that apple juice consumption is associated with several markers of cardiovascular health that may ultimately be important for cancer and neurodegenerative diseases. In 20 studies, apple juice consumed in moderation exerted positive effects on the markers of cardiovascular disease risk, especially on oxidative stress.

The health benefits of apples could also depend on the content of vitamins, including vitamin C, which would be useful to study separately in the future.

CONCLUSIONS

The apple peel has been considered a valuable part of apple fruit due to its polyphenol content. In general, the peel of 'Krista' is a good source of the high TP_{GA}. The results of the present study show that the apple flesh can also be a good source of polyphenols. 'Karksi renett' had the highest fruit weight and 'Antonovka' the highest number of seeds in the fruit. 'Cortland' and 'Krista' excelled in the highest weight of ten seeds.

It is thus recommendable that the whole apple fruit be used as part of a healthy diet, since the valuable substances are stored not only in the flesh but also in the seeds and skin. In the future, it would be useful to study antioxidant and other health related biological activities of apples originating from Estonia, taking into account different methods of apple processing.

ACKNOWLEDGEMENT

The publication costs of this article were partially covered by the Estonian Academy of Sciences.

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Polüfenoolsed ühendid Eestis kasvatatavates õunapuu (*Malus domestica* Borkh.) sortides

Karmen Kapp, Kaisa Kalder, Ave Kikas, Toivo Univer, Tõnu Püssa ja Ain Raal

Mitmed uuringud näitavad, et õunad on oma rikkaliku bioloogiliselt aktiivsete ainete sisalduse tõttu tervislik toiduaine. Töö eesmärk oli võrrelda polüfenoolide sisaldust viie Eestis kasvatatava õunasordi viljalihas ja -koores, seemnetes ning lehtedes. 21 sordi hulgast valiti LC-DAD-MS/MS andmete põhjal välja viis rikkalikuma koostisega õunasorti: 'Antonovka', 'Åkerö', 'Cortland', 'Karksi renett' ja 'Krista'. Määrati viljade mass, seemnete hulk viljas ja nendegi mass. Kokku tuvastati õunakoortes 33 ühendit, viljalihas 23, seemnetes 11 ja lehtedes 25 ühendit. Need ained kuuluvad nelja rühma: 1 – flavoon-3-oolid (kvertsetiin ja selle derivaadid), 2 – dihüdrohalkoonid (floretiin ja selle derivaadid), 3 – flavaan-3-oolid (katehiin, epikatehiin ja oligomeerid) ning 4 – kofeiini ja L-kiinhappe (klorogeeni) vahel moodustunud estrid.

Artiklis esitatud andmete põhjal on suurim polüfenoolide sisaldus õunapuu lehtedes. Viljade koor sisaldas suures koguses kõiki mainitud peamisi polüfenoolide rühmi. Õuna viljalihas puudusid flavoon-3-oolid ning seemnetes flavoon-3-oolid ja flavaan-3-oolid. Koortes olid peamised polüfenoolid kvertsetiin-galaktosiid (3–342 mg/100 g), protsüanidiin B1 (18–179 mg/100 g) ja (epi)katehiini trimeer (28−200 mg/100 g), viljalihas klorogeenhape (77–298 mg/100 g), seemnetes floridsiin (466 mg/100 g) ning lehtedes klorogeenhape (147–446 mg/100 g) ja kvertsetiinglükosiidid, sealhulgas peamiselt kvertsetiinramnosiid (242–350 mg/100 g), kvertsetiingalaktosiid (39–334 mg/100 g) ning kvertsetiinglükosiid (91–321 mg/100 g).

Uuring tõestab, et õunad sisaldavad tervisele kasulikke polüfenoole. Igati mõistlik on alati ära süüa õun tervikuna, sest väärtuslikud ained on lisaks viljalihale talletunud ka seemneis ning õunakoores.