Determination of phytochemical composition and antioxidant capacity of 22 old apple cultivars grown in Poland

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Abstract The basic chemical composition, polyphenols and antioxidant capacity in 22 old apple cultivars grown in Poland were determined. Fruits were analyzed for contents of individual polyphenolics with the ultra-performance liquid chromatography photodiode detector-quadrupole/time-of-flight mass spectrometry (UPLC-PDA-Q/TOF-MS) method, sugar with the high-performance liquid chromatography–evaporative light scattering detector (HPLC-ELSD) method, and antioxidant capacity with the ABTS and FRAP radical method. A total of 29 bioactive compounds, including 26 polyphenolic compounds (7 flavan-3-ols, 2 dihydrochalcones, 4 anthocyanins, 5 phenolic acids, 8 flavonols) and 3 triterpenoids (ursolic, betulinic and oleanolic acids), were identified in fruits. All the apple cultivars were found to be rich in polyphenols [average 2139.21 mg/100 g dry matter (dm)], especially phenolic acid (average 694.12 mg/kg dm), flavan-3-ols (average 1259.80 mg/kg dm), flavonols (average 106.78 mg/kg dm) and triterpenoids (average 2552.20 µg/g dm), particularly ursolic acid (average 2234.50 µg/g dm), with high ABTS and FRAP capacity (average 72.14 and 46.77 µmol Trolox/100 g dm). The apple

cultivars were also found to be a good source of pectins (average 1.19%), acids (average 0.67%) and sugars (average 9.11 g/100 g).

Keywords Old apple cultivars · Polyphenolic compounds · Antioxidant capacity · HPLC-ELSD · UPLC-PDA-Q/TOF-MS

Introduction

Fruit trees in orchards or home gardens are among the most important utilitarian elements of gardens. In the 19th century, they were also used for lining public roads and private properties. They fulfilled a number of functions, from provision of fruit, esthetic, landscaping to technical—protection against the sun, wind and snow. During this period, there were already several thousand cultivars. Different cultivars were cultivated in different parts of Poland. Especially in Western Poland, cultivated varieties came from Western Europe. There were also local cultivars, characteristic for a specific area. The development of modern fruit planting in the second half of the twentieth century contributed to the large changes in the available cultivars of apple trees [1, 2]. Cultivars grown up to that time (with different times of ripening, flavors and applications—from dessert to processed) were replaced by modern, annually yielding cultivar, of higher economic value [3, 4].

Protection of the important older cultivars is also carried out in the Drawa National Park. At sites of former human settlements located within the park, several dozen of old apple cultivars were found and identified. It is important to examine the quality of such fruit, to understand the metabolites and determine their potential beneficial health effects and processing properties of apple fruit. In addition, the

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chemodiversity (aroma and flavor of fruits) [5] and sensory and nutritional qualities were found to be higher in old cultivars compared to modern apple cultivars [6].

Phenolic compounds are a large group of secondary plant metabolites [3]. Because of the great health benefits of apples, their chemical composition and activity have been analyzed in recent years [7]. Apples are recognized as an excellent source of carbohydrates, vitamins, minerals, dietary fiber, pectin and different classes of phenolics [8–11]. A number of factors can influence the content of these compounds—agrotechnical as well as genetic [11, 12].

The presence of specific phenolic compounds can cause low susceptibility of apple fruit to the most important diseases. Phloridzin (a derivative of chalcone) is one such compound, and is the characteristic apple polyphenol. It is a phytoalexin that provides resistance to the pathogens—*Venturia inaequalis* and *Erwinia amylovora* [13, 14]. Phloridzin can inhibit lipid peroxidation [15, 16].

Therefore, the aim of this study was to identify and compare individual polyphenolic compounds by UPLC-PDA-Q/TOF-MS, and antioxidant capacity measured by different methods (ABTS and FRAP) in 22 old apple cultivars grown in Poland. An additional goal of this study was to compare chemical composition including sugars (fructose, glucose and sucrose), dry matter, pectins and titratable acidity in all old apple cultivars. Furthermore, an additional aim of this study was to select old apple cultivars as the richest source of bioactive substances.

Materials and methods

Chemicals

Acetonitrile, formic acid, methanol, ABTS (2,2'-azinobis(3-ethylbenzothiazoline -6-sulfonic acid), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), acetic acid, and phloroglucinol were purchased from Sigma-Aldrich (Steinheim, Germany). (–)-Epicatechin, (+)-catechin, chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, procyanidin B2, *p*-coumaric acid, quercetin-3-*O*-rutinoside and -3-*O*-glucoside, isorhamnetin -3-*O*-glucoside, caffeic acid, phloridzin, cyanidin-3-*O*-galactoside and cyanidin-3-*O*-glucoside were purchased from Extrasynthese (Lyon, France). Acetonitrile for ultra-phase liquid chromatography (UPLC; Gradient grade) and ascorbic acid were from Merck (Darmstadt, Germany).

Plant materials

Fruits of 22 old apple cultivars (Roter Delicious type Starkinson, Roter Herbstkalvill, Booskop (Piękna z Booskop), Wintergoldparman, Landsbergen Renette,

Riesenboiken, Gelber Richard, Kaiser Alexander, Altländer Pfannkuchenapfel, Roter Trier Weinapfel, Boikenapfel, Kaiser Wilhelm, Roter Eiserapfel, Rote Sternrenette, Geflam-mter Kardinal, Lausitzer Nelkenapfel, Weissner Winterkalvill, Dulmener Rosenapfel, Horneburger Pfannkuchenapfel, Wintergoldparmane, Charlamowsky, Parkers Pepping) were used in the study. Fruit samples (~ 2.0 kg each) were collected from trees growing in abandoned human settlements, and from the Palace orchard, which are now located in the Drawa National Park.

The area of the Drawa National Park (DNP) and its neighborhood lies in a plain called Równina Drawska, which is a fragment of the lake district Pojezierze Południowopomorskie, in the north-western part of Poland. It encompasses the central part of a forest complex called the Drawa Wilderness (Puszcza Drawska). The DNP represents a landscape of early glacial outwash plains. It lies entirely within the reception basin of the Drawa River, which—along with its tributary Płociczna—constitutes its main hydrographic axis. A significant element in the cultural landscape of the Park is the remains of old human settlements. The Drawa National Park is situated at longitude 15°45' to 16°45'E; latitude 53°00' to 53°15'N.

The raw materials were directly frozen in liquid nitrogen and freeze-dried (24 h; Christ Alpha 1–4 LSC; Germany). Homogeneous dry material was obtained by crushing the dried tissues using a closed laboratory mill (IKA A.11, Germany). The powders were kept in an ultrafreezer at – 80 °C until extract preparation

Identification and quantification of polyphenols

The powder samples of fruits (1 g) were extracted with 10 mL of mixture containing HPLC-grade methanol (30/100 mL), ascorbic acid (2.0 g/100 mL) and acetic acid in an amount of 1.0/100 mL of reagent. The extraction was performed twice by incubation for 20 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland) and with occasional shaking. Next, the slurry was centrifuged at 19,000g for 10 min, and the supernatant was filtered through a Hydrophilic PTFE 0.20 µm membrane (Millex Simplicity Filter, Merck, Darmstadt, Germany) and used for analysis. The content of polyphenols in individual extracts was determined by means of the ultra-performance liquid chromatography-photodiode array detector-mass spectrometry method. All extractions were carried out in triplicate.

Qualitative (LC/MS QTOF) and quantitative (UPLC-PDA-FL) analysis of polyphenols (anthocyanin, flavan-3-ol, flavonol, and phenolic acid) was performed as described previously by Lachowicz et al. [17]. All measurements were repeated three times. The results were expressed as mg per 100 g of dry matter (dm).

Analysis of proanthocyanidins by phloroglucinolysis method

Direct phloroglucinolysis of freeze-dried samples was performed as described by Lachowicz et al. [18]. Fruit lyophilisates were weighed in an amount of 5 mg into 2-mL Eppendorf vials. Subsequently, 0.8 mL of the methanolic solution of phloroglucinol (75 g/L) and ascorbic acid (15 g/L) were added to samples. After addition of 0.4 mL of methanolic HCl (0.3 M), the vials were incubated for 30 min at 50 °C with continuous vortexing in a thermo shaker (TS-100, BioSan, Riga, Latvia). The reaction was terminated by placing the vials in an ice bath, drawing 0.6 mL of the reaction medium and diluting with 1.0 mL of sodium acetate buffer (0.2 M). The samples were centrifuged immediately at 20,000 g for 10 min at 4 °C, and stored at 4 °C before reverse-phase HPLC (RP-HPLC) analysis. All incubations were done in triplicate. Phloroglucinolysis products were separated on a Cadenza CD C18 (75 mm × 4.6 mm, 3 µm) column (Imtakt, Japan). The liquid chromatograph was a Waters (Milford, MA) system equipped with diode array and scanning fluorescence detectors (Waters 474) and an autosampler (Waters 717 plus). Solvent A (25-mL aqueous acetic acid and 975-mL water) and solvent B (acetonitrile) were used in the following gradients: initial, 5% B; 0–15 min to 10% B linear; 15–25 min to 60% B linear; followed by washing and reconditioning of the column. Other parameters were as follows: a flow rate of 1 mL/min, an oven temperature of 15 °C, and volume of filtrate injected onto the HPLC system was 20 µL. All data were obtained in triplicate. The results were expressed as mg per 100 g dm.

Identification and quantification of triterpenoids

Fruit sample extraction was performed as described by Farneti et al. [19]. The powder samples (0.5 g) were extracted with 5 mL of ethyl acetate and 5 mL of hexane. The extraction was performed by incubation for 20 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland) with occasional shaking. After the first extraction, the samples were kept at 4 °C overnight. On the next day, the samples were re-extracted in the same conditions. Next, the slurry was centrifuged at 19,000g for 10 min, and the supernatant was evaporated to dryness. The pellet was re-extracted using 2 mL of 100% methanol, filtered through a hydrophilic PTFE 0.20 µm membrane (Millex Simplicity Filter, Merck, Darmstadt, Germany) and used for analysis. Identification and quantification of ursolic, oleanolic, and betulinic acids was done using the ACQUITY Ultra Performance LC system with a binary solvent manager (Waters Corp., Milford, MA, USA), a UPLC BEH C18 column (1.7 µm, 2.1 mm × 150 mm, Waters Corp., Milford, MA, USA), and a Q-TOF mass spectrometer (Waters, Manchester, UK) equipped with an electrospray

ionization (ESI) source, operating in negative mode. The elution solvents were 100% methanol (A) and 100% acetonitrile (B) (15:85, v/v). Ursolic, oleanolic, and betulinic acids were eluted isocratically at a flow rate of 0.1 mL/min for 10 min at 20 °C. The *m/z* for betulinic acid was 455.34, for oleanolic acid 455.34, and for ursolic acid 455.33, and the retention times were 6.80, 7.50, and 8.85 min, respectively. The compounds were monitored at 210 nm. All data were obtained in triplicate. The results were expressed as µg per g of dm.

Analysis of sugar by the HPLC-ELSD method

An analysis of sugar by the HPLC-ELSD method was performed according to the protocol described by Oszmiański and Lachowicz [20]. The samples of apple fruits (1–2 g) were diluted with redistilled water (50 mL). The extraction was performed by incubation for 15 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland) and with occasional shaking, and then incubation in 90 °C for 30 min. Next, the slurry was centrifuged at 19,000g for 10 min, and the supernatant was filtered through a Sep-Pak C-18 Cartridges (Waters Milipore), and through a Hydrophilic PTFE 0.20 mm membrane (Millex Simplicity Filter, Merck) and used for analysis. All extractions were carried out in triplicate.

Chromatographic analysis was carried out with a Merck-Hitachi L-7455 liquid chromatograph with an evaporative light scattering detector (ELSD; Polymer Laboratories PL-ELS 1000) and quaternary pump L-7100 equipped with D-7000 HSM Multisolvant Delivery System (Merck-Hitachi, Tokyo, Japan) and L-7200 autosampler. The separation was performed on a Prevail™ Carbohydrate ES HPLC Column-W 250 × 4.6 mm, 5 mm (Alltech, US) column. Oven temperature was set to 30 °C. The mobile phase was used with acetonitrile water (75:25) for isocratic elution, the flow rate was 1 mL/min and injection volume: 10 mL. The ELS detector was optimized for the analyses and following parameters were used: 80 °C for an evaporative temperature, 80 °C for a nebulizer and 1.2 mL/min for a nitrogen gas flow. Calibration curves ($R^2 = 0.9999$) were created for glucose, fructose, sorbitol and sucrose. All data were obtained in triplicate. The results were expressed as mg per 100 g dm.

Determination of antioxidant activity

The samples for analysis was prepared as described previously by Lachowicz et al. [21]. Freeze-dried fruits (0.35 g) were mixed with 5 mL of hexane:acetone:methanol (2:1:1), sonicated at 20 °C for 15 min and left for 24 h at 4 °C. Then, the extract was again sonicated for 15 min, and centrifuged at 19,000 g for 10 min.

The ABTS and the FRAP assays were determined according to Re et al. [22] and Benzie and Strain [23]. Briefly, 10 μL of the supernatant was mixed with 990 μL of ABTS or FRAP. After 10 min of reaction, the absorbance was measured at 734 nm for ABTS and 593 nm for FRAP. Determinations by the ABTS and FRAP methods were performed using the UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). The antioxidant activity was expressed as mmol of Trolox per 100 g.

Statistical analysis

Statistical analysis, principal component analysis (PCA) and hierarchical cluster (HA) were conducted using Statistica version 12.5 (StatSoft, Kraków, Poland) on mean values of three samples and 22 cultivars. Significant differences ($p \leq 0.05$) between mean values were evaluated by one-way ANOVA and Duncan's multiple range test.

Results and discussion

Major chemical compounds

The analytical results of all apple cultivars for fresh weight (fm), pectins, titratable acidity, pH and sugars are given in Table 1. Significant differences ($p < 0.05$) were revealed for the investigated basic chemical parameters among all old apple cultivars grown in Poland.

The average dry matter concentration in the fruits of all analyzed old apple cultivars was 14.62/100 g and varied from 12.30/100 g in cv. Horneburger Pfannkuchenapfel to 17.12/100 g for cv. Roter Herbstkalvill. These results were comparable to those obtained in different apple cultivars [24]. The average value of pH of apple fruits was 3.27; the lowest value (2.97) was found for cv. Boikenapfel and the highest (4.34) for cv. Roter Herbstkalvill. The total titratable acidity in all old apple cultivars, expressed as g/100 g of malic acid, ranged from 0.17 for cv. Roter Herbstkalvill to 1.07 for cv. Gelber Richard. According to Hecke et al. [25], the total acid content of integrated cultivation and organically grown apple cultivars ranged from 0.83 to 1.78/100 g. The content range of total sugar determined in all old apple cultivars is presented in Table 1. Fructose, glucose and sucrose were the three main sugars analyzed in apple fruit. The average content of total sugar in all old apple cultivars grown in Poland was 9.11/100 g fm and ranged from 7.41 to 11.99/100 g for cvs. Kaiser Wilhelm and Altländer Pfannkuchenapfel, respectively. In new cultivars such as Gala, Elstar, Idared, Golden Delicious, Braeburn and Fuji, higher total sugar content was found (between 11.5 and 15.0/100 g), as was the case for Jonagold [18.3/100 g fresh matter (fm)] [24] and Boskoop (16.1/100 g) [26].

The dominant sugar in our analyzed old apple fruits was fructose, in the range 3.96–8.52/100 g (57–84% of total sugar), then glucose at 0.94–2.96/100 g (9–32% of total sugar) and sucrose at 0.06–2.72/100 g (1–28% of total sugar). The ratio of glucose to fructose ranged from 0.11 in cv. Charlamowsky to 0.56 in cv. Wintergoldparman. In experimental material of the new cultivar analyzed by Ticha et al. [26], respective sugars were estimated at comparable levels as follows: fructose 4.8–8.1/100 g, glucose 0.9–3/100 g and much more sucrose: 2.1–7.2/100 g. Previously, fructose was recommended as a sweetener for diabetic patients. Now fructose, in contrast to glucose, is known to potentially stimulate lipogenesis. Lipogenesis by fructose has negative effects in many diseases (diabetes mellitus or metabolic syndrome, including obesity) by passing through the phosphofructokinase pathway [27]. Moreover, the content of sugar and acid affected the tested quality of fruits [28].

Pectin is a mixture of hydrocarbons found in the cell walls of many plants that is effective in the prevention of cardiovascular disease, obesity and diabetes [29]. The average pectin volume in apple fruits of the tested cultivars was 1.19%; it ranged from 0.64 to 2.24% for cvs. Landsbergen Renette and Roter Eiserapfel, respectively. According to Rop et al. [29], differences were also noted in the contents of pectins in native apple cultivars from the Czech Republic. The lowest values were found in the Jeptiska cultivar (1.15% fm), while the highest one was found in the Strymka cultivar (3.26% fm).

Identification of phenolic compounds

Identification and quantification of 26 compounds belonging to phenolic acids, flavonols, anthocyanins, flavan-3-ols (oligomeric and polymeric) and dihydrochalcones was based on a comparison of their retention times, MS and MS/MS data with available standards and published data. The identification results are presented in Table 2.

In all old apple cultivars grown in Poland, five hydroxycinnamates belonging to phenolic acids were identified. Among them were neochlorogenic, chlorogenic, cryptochlorogenic, *p*-coumaroylquinic acids, identified by comparison with authentic standards. Three of them were characterized by the same $[\text{M}-\text{H}]^-$ at m/z as 353 but assigned by different compounds as: neochlorogenic acid ($R_t = 3.320$ min), chlorogenic acid ($R_t = 3.75$), cryptochlorogenic acid ($R_t = 4.05$). In addition, two *p*-coumaroylquinic acids were identified with $[\text{M}-\text{H}]^-$ at m/z 337 with $\lambda = 314$ at R_t as 4.53, 4.88 min. These compounds were found previously by Wojdyło et al. [10], Ceymann et al. [11], and Jakobek and Barron [30].

A total of four anthocyanins were detected in 22 old apple cultivars grown in Poland cyanidins as 3-*O*-galactoside and -3-*O*-glucoside (m/z 449), -3-*O*-arabinoside, and -3-*O*-xyloside (m/z 419). These results agreed quite well

Table 1 Chemical composition of all old apple cultivars (g/100 g fw)

	Dry weight (g/100 g)	Extracts Bx	Fructose	Glucose	Sucrose	Total sugar (g/100 g)	Ratio (glu/ fru)	Total acidity (g/100 g)	PH	Pectines (g/100 g)
Roter Delicious typ Starkinson	14.49 ± 0.12 ^{gh}	10.60 ± 0.08 ^l	5.56 ± 0.04 ^{ij}	2.21 ± 0.02 ^d	0.06 ± 0.01 ^m	7.83 ± 2.77 ^{mn}	0.40	0.32 ± 0.01 ^{ij}	3.62 ± 0.03 ^b	1.65 ± 0.01 ^{cd}
Roter Herbstkalvill	17.12 ± 0.14 ^a	14.70 ± 0.12 ^a	6.67 ± 0.05 ^{ef}	2.50 ± 0.02 ^c	0.46 ± 0.01 ^{ijkl}	9.63 ± 3.17 ^f	0.37	0.17 ± 0.01 ^j	4.34 ± 0.03 ^a	0.66 ± 0.02 ^{hi}
Booskop (Piękna z Booskop)	13.31 ± 0.11 ^k	11.40 ± 0.09 ⁱ	5.40 ± 0.04 ^{jk}	2.75 ± 0.01 ^b	0.67 ± 0.01 ^{ghi}	8.82 ± 2.37 ⁱ	0.51	0.78 ± 0.01 ^{cde}	3.15 ± 0.02 ^{ghi}	0.68 ± 0.02 ^{ghi}
Wintergoldparman	14.96 ± 0.12 ^e	12.30 ± 0.10 ^g	3.96 ± 0.06 ^p	2.22 ± 0.03 ^d	2.41 ± 0.02 ^b	8.59 ± 0.95 ^j	0.56	0.91 ± 0.01 ^{abcd}	3.11 ± 0.01 ^{hi}	1.12 ± 0.02 ^e
Landsbergen Renette	15.81 ± 0.13 ^d	14.00 ± 0.11 ^c	7.92 ± 0.03 ^c	2.43 ± 0.04 ^c	0.37 ± 0.02 ^{kl}	10.72 ± 3.90 ^c	0.31	0.79 ± 0.01 ^{cde}	3.38 ± 0.02 ^{cdef}	0.64 ± 0.04 ⁱ
Riesenboiken	14.08 ± 0.11 ⁱ	12.00 ± 0.10 ^h	6.13 ± 0.03 ^g	2.56 ± 0.02 ^c	0.61 ± 0.00 ^{hij}	9.29 ± 2.80 ^h	0.42	0.90 ± 0.02 ^{abcd}	3.13 ± 0.02 ^{ghi}	0.82 ± 0.03 ^{fghi}
Gelber Richard	15.69 ± 0.13 ^d	13.00 ± 0.10 ^e	8.52 ± 0.02 ^a	2.11 ± 0.02 ^{def}	0.46 ± 0.00 ^{ijkl}	11.09 ± 4.26 ^b	0.25	1.07 ± 0.00 ^a	3.09 ± 0.04 ^{hi}	1.11 ± 0.04 ^e
Kaiser Alexander	15.85 ± 0.13 ^d	13.80 ± 0.11 ^d	6.52 ± 0.05 ^f	2.26 ± 0.02 ^d	2.29 ± 0.00 ^b	11.07 ± 2.45 ^b	0.35	0.94 ± 0.00 ^{abc}	2.99 ± 0.03 ⁱ	1.19 ± 0.04 ^e
Altländer Pfannkuchenapfel	16.49 ± 0.13 ^b	14.40 ± 0.12 ^b	6.82 ± 0.03 ^{de}	2.45 ± 0.01 ^c	2.72 ± 0.01 ^a	11.99 ± 2.45 ^a	0.36	0.59 ± 0.00 ^{fgh}	3.54 ± 0.03 ^{bc}	0.83 ± 0.01 ^{fgh}
Roter Trier Wein- apfel	16.39 ± 0.13 ^b	13.70 ± 0.11 ^d	6.12 ± 0.05 ^g	2.60 ± 0.01 ^{bc}	0.53 ± 0.01 ^{ijk}	9.25 ± 2.83 ^h	0.42	0.91 ± 0.01 ^{abcd}	3.21 ± 0.02 ^{efgh}	0.94 ± 0.01 ^f
Boikenapfel	14.67 ± 0.12 ^f	12.00 ± 0.10 ^h	5.69 ± 0.04 ⁱ	2.16 ± 0.03 ^{de}	0.71 ± 0.01 ^{fghi}	8.56 ± 2.56 ^j	0.38	0.99 ± 0.01 ^{ab}	2.97 ± 0.02 ⁱ	1.70 ± 0.01 ^{cd}
Kaiser Wilhelm	13.41 ± 0.11 ^{jk}	11.10 ± 0.09 ^k	4.39 ± 0.06 ^o	2.23 ± 0.02 ^d	0.79 ± 0.01 ^{fgh}	7.41 ± 1.81 ^p	0.51	0.69 ± 0.02 ^{efg}	3.22 ± 0.01 ^{efgh}	1.74 ± 0.02 ^c
Roter Eiserapfel	15.07 ± 0.12 ^e	11.20 ± 0.09 ^{jk}	5.17 ± 0.07 ^{lm}	1.65 ± 0.02 ^g	0.86 ± 0.01 ^f	7.68 ± 2.29 ^{no}	0.32	0.42 ± 0.03 ^{hi}	3.21 ± 0.01 ^{efgh}	2.24 ± 0.02 ^a
Rote Sternrenette	14.95 ± 0.13 ^e	12.60 ± 0.10 ^f	6.14 ± 0.03 ^g	2.09 ± 0.04 ^{def}	0.64 ± 0.02 ^{hi}	8.87 ± 2.85 ⁱ	0.34	0.93 ± 0.02 ^{abc}	3.09 ± 0.01 ^{hi}	1.57 ± 0.04 ^{cd}
Geflammt Kar- dinal	13.27 ± 0.10 ^k	10.60 ± 0.08 ^l	4.71 ± 0.02 ⁿ	1.68 ± 0.02 ^g	1.53 ± 0.02 ^c	7.93 ± 1.79 ^{lm}	0.36	0.70 ± 0.02 ^{efg}	3.31 ± 0.03 ^{defg}	1.19 ± 0.03 ^e
Lausitzer Nelk- enapfel	16.20 ± 0.11 ^c	12.90 ± 0.01 ^e	5.28 ± 0.03 ^{kl}	1.97 ± 0.02 ^f	0.82 ± 0.01 ^{fg}	8.07 ± 2.32 ^{kl}	0.37	0.73 ± 0.01 ^{def}	3.43 ± 0.04 ^{cd}	2.00 ± 0.03 ^b
Weisser Winterkal- vill	12.62 ± 0.10 ^l	10.50 ± 0.08 ^l	5.89 ± 0.02 ^h	1.27 ± 0.01 ^h	0.38 ± 0.01 ^{kl}	7.53 ± 2.96 ^{op}	0.22	0.71 ± 0.02 ^{efg}	3.09 ± 0.03 ^{hi}	1.56 ± 0.01 ^{cd}
Dulmener Rosenapfel	13.54 ± 0.11 ^j	11.90 ± 0.09 ^h	5.02 ± 0.02 ^m	1.99 ± 0.02 ^{ef}	1.12 ± 0.01 ^e	8.13 ± 2.05 ^k	0.40	0.58 ± 0.03 ^{fgh}	3.31 ± 0.02 ^{defg}	0.75 ± 0.01 ^{fghi}
Horneburger Pfannkuchenapfel	12.30 ± 0.10 ^m	11.20 ± 0.09 ^{jk}	6.02 ± 0.02 ^{gh}	2.96 ± 0.01 ^a	0.28 ± 0.01 ^l	9.26 ± 2.87 ^h	0.49	0.78 ± 0.01 ^{cde}	2.99 ± 0.03 ⁱ	0.77 ± 0.01 ^{fghi}
Wintergoldpar- mane	14.35 ± 0.12 ^h	11.30 ± 0.10 ^{ij}	6.56 ± 0.05 ^f	1.62 ± 0.01 ^g	0.42 ± 0.00 ^{kl}	8.60 ± 3.25 ^j	0.25	0.83 ± 0.01 ^{bcd}	3.19 ± 0.01 ^{fgh}	1.56 ± 0.01 ^{cd}
Charlamowsky	12.51 ± 0.11 ^l	11.10 ± 0.11 ^k	8.31 ± 0.04 ^b	0.94 ± 0.04 ⁱ	0.68 ± 0.00 ^{ghi}	9.94 ± 4.33 ^e	0.11	0.79 ± 0.01 ^{cde}	3.25 ± 0.01 ^{efgh}	0.65 ± 0.01 ^{hi}
Parkers Pepping	14.61 ± 0.10 ^{fg}	12.70 ± 0.06 ^f	6.93 ± 0.07 ^d	1.99 ± 0.03 ^{ef}	1.34 ± 0.01 ^d	10.26 ± 3.06 ^d	0.29	0.53 ± 0.01 ^{gh}	3.38 ± 0.03 ^{cde}	0.86 ± 0.02 ^{fg}

Values are mean ± standard deviation. *n* = 3a–e Mean ± SD followed by different letters within the same line represent significant differences (*p* < 0.05)

Table 2 Identification of polyphenolic compounds in old apple cultivars using LC–MS Q-TOF

Tentative identification	R_t (min)	$\lambda_{\max}$ (nm)	MS (H–M) [–] / [H–M] ⁺	MS/MS fragments (m/z)
B-type procyanidin trimer	3.17	275	856	577
Neochlorogenic acid	3.32	323	353	191
Chlorogenic acid	3.75	323	353	191
(+)-Catechin	3.62	280	289	245
Cryptochlorogenic acid	4.05	320	353	191
Cyanidin-3-glucoside	4.14	520	449 ⁺	287
B-type procyanidin dimer	4.27	275	577	289
<i>p</i> -Coumarylquinic acid	4.53	314	337	191
Cyanidin-3-galactoside	4.64	525	449 ⁺	287
(–)-Epicatechin	4.70	280	289	245
<i>p</i> -Coumarylquinic isomer acid	4.88	314	337	191
B-type procyanidin trimer	4.98	280	865	577/289
B-type procyanidin tetramer	5.19	280	1153	289
Cyanidin-3-arabinoside	5.21	520	419 ⁺	287
Cyanidin-3-xyloside	5.27	520	419 ⁺	287
Quercetin-3- <i>O</i> -rutinoside	6.27	350	609	301
Quercetin-3- <i>O</i> -galactoside	6.33	355	463	301
Quercetin-3- <i>O</i> -glucoside	6.49	350	463	301
Quercetin-3- <i>O</i> -arabinoside	6.76	355	433	301
Quercetin-3- <i>O</i> -xyloside	7.10	350	433	301
Phloretin-2'- <i>O</i> -xyloglucoside	7.23	285	567	273
Quercetin-3- <i>O</i> -rhamnoside	7.30	345	447	301
Isorhamnetin-3- <i>O</i> -galactose	7.42	350	477	315
Isorhamnetin-3- <i>O</i> -glucose	8.14	350	477	315
Phloretin-2'- <i>O</i> -glucoside	8.21	285	435	273

with the recently published data [10, 11, 30]. The different old apple cultivars had the different profile of anthocyanins and different levels were evaluated. The exception was cv. Lausitzer Nelkenapfel, in which anthocyanins were not identified (Table 2).

Six compounds belonging to flavan-3-ols as monomers, dimer, trimers and tetramers were detected in all old apple cultivars. (+)-Catechin and (–)-epicatechin (with R_t = 3.62 and 4.70 min, respectively, and $\lambda_{\max}$ = 280 nm) had an [M–H][–] at m/z 289 and an MS/MS fragment at m/z 245. The B-type procyanidin dimer (R_t = 4.27 min) and trimers (R_t = 3.17 and 4.98 min) had a pseudomolecular ion [M–H][–] at m/z 577 and 865 and fragmentation ions at m/z 289, respectively. The B-type procyanidin tetramer had a pseudomolecular ion [M–H][–] at m/z 1153 and fragmentation ions at m/z 289. These results agreed quite well with the recently published data [10, 11, 30]. Additionally, the sum of polymeric proanthocyanidins was presented in these results carried out using the phloroglucinol method by HPLC-FL. This method provides more detailed information on the proanthocyanidin fraction of these berries,

especially when these compounds have low detection in analysis of UPLC-PDA.

Two types of flavonol derivatives with a fragment at m/z 301 and 315, characteristic for quercetin and isorhamnetin derivatives, respectively, were found in all the old apple cultivars (Table 2). Quercetin derivatives are mainly flavonols found in apple fruits. In these fruits, six quercetin derivatives having the MS/MS fragment at m/z 301 characteristic for quercetin were represented by: -3-*O*-rhamnoside (MS ion at m/z 447 and R_t = 7.30), -3-*O*-rutinoside (MS ion at m/z 609 and R_t = 6.27), -3-*O*-galactoside (MS ion at m/z 463 and R_t = 6.33), -3-*O*-glucoside (MS ion at m/z 463 and R_t = 6.49), -3-*O*-arabinoside (MS ion at m/z 433 and R_t = 6.76) and -3-*O*-xyloside (MS ion at m/z 433 and R_t = 7.10). Two isorhamnetins were represented by: -3-*O*-galactoside (R_t = 7.42) and -3-*O*-glucose (R_t = 8.14). These compounds had a [M–H][–] at m/z 477 and the typical isorhamnetin ion fragments at m/z 315. Some of these quercetin and isorhamnetin derivatives were qualitatively consistent with previous reports on the flavonols occurring in different cultivars of apple fruits

by Wojdyło et al. [10], Ceymann et al. [11], Jakobek and Barron [30].

Additionally, dihydrochalcones were identified as phloretin derivatives, according to their UV spectrum and MS fragmentation. These compounds were identified as phloretin-2'-*O*-xyloglucoside (m/z 567 and $R_t = 7.23$ min) and phloretin-2'-*O*-glucoside (m/z 435 and $R_t = 8.21$ min). All of these compounds possess characteristic MS/MS as $[M-H]^-$ m/z 273. The presence of some of these compounds was suggested previously by Wojdyło et al. [10], Ceymann et al. [11].

Quantification of polyphenols

The concentration results are presented in Fig. 2. Phenolic compounds mainly determine quality parameters of fruits such as flavor, appearance and health-promoting properties (anti-inflammatory, anti-allergic, anti-diabetic, anti-tumor, antiviral and chemo-protective effects) [20]. The average contents of main groups of phenolic compounds in all old apple cultivars were as follows: phenolic acids (~ 59%) > flavan-3-ols (~ 33%) ≥ flavonols (~ 5%) > anthocyanins (~ 2%) > dihydrochalcone (~ 1%). The average content of phenols in all old apple cultivars grown in Poland was 2139.21 mg/100 g dm, with significant differences among the cultivars. It ranged from 1348.40 to 4310.52 mg/100 g dm for cvs. Parkers Pepping and Roter Delicious type Starkinson, respectively. The lowest levels of phenolic compounds, below 1500 mg/100 g dm, were found in old apple fruits of cvs. Charlamowsky, Parkers Pepping, Wintergoldparmane, Horneburger Pfannkuchenapfel and Dulmener Rosenapfel, but the highest contents of polyphenols, above 3000 mg/100 g dm, were observed in cvs. Booskop (Piękna z Booskop), Roter Herbstkalvill and Roter Delicious type Starkinson. According to Wojdyło et al. [10], the content of polyphenolic compounds in 69 old and new apple varieties grown in Western Europe ranged from 523.02 to 2011.30 mg/100 g for cvs. Topez and Kosztela, respectively. The average content was 2.0 times lower than old cultivars grown in Poland. In the studies of Valavanidis et al. [31], Vieira et al. [32] and Podsędek et al. [33], the average contents of polyphenols in apple cultivars grown in Greece, Brazil and Poland were 3.0, 2.4 and 1.4 times lower than in old apple cultivars grown in Poland. Furthermore, in research by Panzella et al. [34], Faramarzi et al. [35] and Feliciano et al. [36] all the old apple cultivars from Southern Italy, Iran and Portugal showed higher polyphenols than the commercial fruits, exotic apple cultivars. However, in apple fruits both the range and abundance of polyphenols may vary depending on the year of harvest, growth period, storage conditions, geographic location and genetic variation, the effect of the region, agricultural practices, and cultivation method. Additionally, the extraction process of fruits,

the solvent and the methodology used to identify phenolic compounds have been investigated [10, 31, 32].

Phenolic acids

Phenolic acids are one of the major groups of phenolic compounds in apple fruits. The content of phenolic acids in all the tested old cultivars ranged from 126.62 in cv. Dulmener Rosenapfel to 2557.23 mg/100 g dm in cv. Roter Delicious type Starkinson; the average content was 694.12 mg/100 g dm (Fig. 1). The lowest levels of phenolic acids, below 500 mg/100 g dm, were found in old apple fruits of cv. Altländer Pfannkuchenapfel, Roter Trier Weinapfel, Boikenapfel, Geflammter Kardinal, Lausitzer Nelkenapfel, Dulmener Rosenapfel, Horneburger Pfannkuchenapfel, Wintergoldparmane, and Charlamowsky, while Parkers Pepping, cvs. Roter Delicious type Starkinson, Riesenboiken and Gelber Richard had the highest, above 1000 mg/100 g dm. Total hydroxycinnamic concentrations in this study were comparable to those reported in different apple cultivars [10, 31, 32]. In all old apple cultivars grown in Poland, chlorogenic acid was the major component (62–94% of total phenolic acids). Our results were in agreement with those reported by Wojdyło et al. [10] and Veberic et al. [37]. It is known that phenolic acids, especially chlorogenic acid, are precursors of flavor in fruits and vegetables and they exhibit carcinogenic, antimutagenic and antioxidant properties in vitro, and scavenge reactive oxygen species. Unfortunately, chlorogenic acid is metabolized mainly by colonic microflora, because it is very poorly absorbed in the human body [38].

Flavan-3-ols

Flavan-3-ols consisting of oligomers and polymeric procyanidins were the second group of polyphenolic compounds in the 22 old apple cultivars (Table 3). Flavan-3-ols regulate the level of glycogen and glucose accumulation and represent compounds influencing lipid metabolism. The average concentration of total flavan-3-ols in apple fruits of the tested old cultivars grown in Poland was 1259.80 mg/100 g dm. The highest (2067.81 mg/100 g dm) content of total flavan-3-ols was determined in fruits of cv. Roter Herbstkalvill, and the lowest (709.70 mg/100 g dm) was in cv. Weisser Winterkalvill. The lowest levels of flavan-3-ols, below 800 mg/100 g dm, were found in fruits of cvs. Weisser Winterkalvill and Wintergoldparmane; the highest, above 2000 mg/100 g dm, was observed in apple fruits of cvs. Roter Herbstkalvill and Booskop (Piękna z Booskop). The dominant group of total flavan-3-ols in all old apple cultivars grown in Poland was polymeric procyanidins (7–95% of total flavan-3-ols) because in cvs. Riesenboiken, Gelber Richard, Kaiser Wilhelm, Geflammter Kardinal,

Fig. 1 Content of polyphenolic compounds (mg/100 g dm) of all old apple cultivars

Horneburger Pfannkuchenapfel and Wintergoldparmane the dominating compounds belong to the oligomeric group (4–92%). The total polymeric procyanidin contents ranged from 76.48 to 1814.46 mg/100 g dm for cvs. Kaiser Wilhelm and Boskop (Piękna z Booskop), respectively. The lowest concentration of flavan-3-ols oligomers was found in cv. Dulmener Rosenapfel (51.55 mg/100 g dm) and the highest content was found in cv. Wintergoldparman (809.29 mg/100 g dm). Our results are concordant with those reported by Podsędek et al. [33]. As reported

by Podsędek et al. [33], the level of flavan-3-ols in the different cultivars of apple depends on growing location, cultivar, environmental factors and genetic traits.

The high concentration of procyanidins in different cultivars of apple can explain their slight bitterness and astringency, typical of apple fruits. The degree of polymerization (DP) determines the chemical and physical properties of procyanidins [39]. The average degree of polymerization of all old apple cultivars was 8.61 (ranging from 5.35 in cv. Altländer Pfannkuchenapfel to 27.79 in cv. ‘Kaiser

Table 3 Concentration of flavan-3-ols, DPn and dihydrochalcone (mg/100 g dm) of all old apple cultivars

Apple variety	Flavan-3-ols			DPn	Dihydrochalcone					
	B3	(+)Cat	B2		(-)Epicat	B3	B4	PP	PXG	PG
Roter Delicious typ Star-kinson	11.54 ± 0.09q	14.35 ± 0.11p	347.82 ± 2.78d	181.34 ± 1.45d	255.53 ± 2.04h	104.93 ± 0.84m	498.51 ± 2.89j	9.42d	16.86 ± 0.13i	36.02 ± 0.29g
Roter Herbstkalvill	53.84 ± 0.43d	54.28 ± 0.43c	457.88 ± 3.66b	185.09 ± 1.48c	468.38 ± 3.75b	366.89 ± 2.94b	481.45 ± 2.79k	7.17l	11.51 ± 0.09l	19.29 ± 0.15n
Booskop (Piękna z Boskop)	80.33 ± 0.64a	65.39 ± 0.52b	546.62 ± 4.37a	279.10 ± 2.73a	516.13 ± 4.13a	326.89 ± 2.63a	229.28 ± 1.33t	6.07n	45.23 ± 0.36a	35.54 ± 0.28h
Wintergoldparman	30.89 ± 0.25i	33.10 ± 0.26h	231.66 ± 1.85l	96.43 ± 0.77j	252.03 ± 2.02k	179.80 ± 1.44d	809.29 ± 2.90b	8.83f	21.68 ± 0.17f	83.21 ± 0.67a
Landsbergen Renette	22.20 ± 0.18k	25.41 ± 0.20i	291.60 ± 2.33g	107.64 ± 0.86h	300.05 ± 2.40e	177.74 ± 1.42e	455.71 ± 2.01l	7.81ij	13.11 ± 0.10k	15.73 ± 0.13p
Riesenboiken	8.83 ± 0.07r	12.29 ± 0.10q	136.34 ± 1.09r	37.80 ± 0.30t	168.37 ± 1.35p	79.77 ± 0.64p	767.61 ± 3.31c	9.19e	6.08 ± 0.05r	15.28 ± 0.12q
Gelber Richard	25.17 ± 0.20j	23.79 ± 0.19j	157.32 ± 1.26q	82.69 ± 0.66l	163.36 ± 1.31q	74.35 ± 0.59r	546.70 ± 3.17h	11.00b	38.80 ± 0.31b	71.42 ± 0.57b
Kaiser Alexander	40.44 ± 0.32g	40.15 ± 0.32g	233.58 ± 2.87k	101.54 ± 0.81i	255.70 ± 2.05g	167.68 ± 1.34f	660.64 ± 3.84e	7.96h	19.93 ± 0.16g	68.68 ± 0.55c
Altländer Pfannkuchenapfel	57.72 ± 0.46c	40.81 ± 0.33f	250.56 ± 2.00i	167.61 ± 1.34e	324.03 ± 2.59d	167.47 ± 1.34g	529.08 ± 3.07i	5.35p	17.65 ± 0.14h	21.89 ± 0.18l
Roter Trier Weinapfel	68.78 ± 0.55b	94.02 ± 0.75a	340.05 ± 2.72e	207.92 ± 1.66b	368.55 ± 2.95c	217.00 ± 1.74c	420.75 ± 2.44m	7.34k	33.70 ± 0.27c	57.32 ± 0.46d
Boikenapfel	32.34 ± 0.26h	42.42 ± 0.34e	320.77 ± 0.23f	131.88 ± 1.06g	255.10 ± 2.04i	158.40 ± 1.27h	650.29 ± 3.77f	6.79m	14.81 ± 0.12j	32.37 ± 0.26i
Kaiser Wilhelm	0.99 ± 0.01t	4.76 ± 0.04s	28.98 ± 1.61w	5.24 ± 0.04u	28.69 ± 0.23w	7.82 ± 0.06w	951.90 ± 2.31a	27.79a	8.13 ± 0.07q	14.99 ± 0.12r
Roter Eiserapfel	6.87 ± 0.05s	12.44 ± 0.10q	200.84 ± 2.33n	71.61 ± 0.57o	140.83 ± 1.13s	50.16 ± 0.40u	419.83 ± 2.44n	8.61g	4.34 ± 0.03t	13.96 ± 0.11s
Rote Sternerenette	12.71 ± 0.10p	16.82 ± 0.13m	290.89 ± 1.47h	79.96 ± 0.64m	197.55 ± 1.58l	99.33 ± 0.79n	260.36 ± 1.51r	6.82m	11.64 ± 0.09l	37.75 ± 0.30f
Geflammtter Kardinal	14.70 ± 0.12n	20.45 ± 0.16k	183.40 ± 1.97o	69.38 ± 0.56p	184.58 ± 1.48o	105.35 ± 0.84l	679.55 ± 1.94d	8.78f	5.04 ± 0.04s	18.22 ± 0.15o
Lausitzer Nelkenapfel	41.17 ± 0.33f	40.82 ± 0.33f	242.22 ± 0.88j	131.85 ± 1.05g	253.58 ± 2.03j	129.72 ± 1.04k	268.00 ± 1.55q	6.75m	23.53 ± 0.19d	51.05 ± 0.41e
Weisser Winterkalvill	30.94 ± 0.25i	15.11 ± 0.12n	109.88 ± 3.05t	76.95 ± 0.62n	186.90 ± 1.50n	59.56 ± 0.48s	230.36 ± 1.34s	5.88o	10.54 ± 0.08o	25.45 ± 0.20j
Dulmener Rosenapfel	47.17 ± 0.38e	46.42 ± 0.10d	380.99 ± 0.69c	150.61 ± 1.20f	275.37 ± 2.20f	158.12 ± 1.04i	51.55 ± 0.30w	7.14l	11.25 ± 0.09m	18.36 ± 0.15o
Horneburger Pfannkuchenapfel	11.45 ± 0.09q	14.64 ± 0.19o	86.68 ± 1.06u	49.38 ± 0.40s	118.47 ± 0.95t	75.83 ± 0.48q	575.74 ± 2.34g	9.99c	11.31 ± 0.18m	9.19 ± 0.07t
Wintergoldparmane	18.60 ± 0.15l	11.94 ± 0.15r	131.99 ± 1.80s	61.19 ± 0.49r	109.68 ± 0.88u	58.89 ± 1.04t	403.31 ± 1.52o	7.78j	22.22 ± 0.07e	23.06 ± 0.18k
Charlamowsky	15.44 ± 0.08m	23.64 ± 0.20j	225.48 ± 1.42m	87.61 ± 0.70k	197.08 ± 1.58m	147.39 ± 0.48j	219.64 ± 1.27u	7.75j	8.78 ± 0.09p	19.47 ± 0.16m
Parkers Pepping	13.17 ± 0.11o	18.38 ± 0.17i	177.57 ± 1.09p	61.93 ± 0.50q	150.92 ± 1.21r	81.33 ± 1.26o	326.18 ± 1.89p	5.38p	10.93 ± 0.09n	5.96 ± 0.05u

Values are mean ± standard deviation. $n = 3$ a–e Mean ± SD followed by different letters within the same line represent significant differences ($p < 0.05$)

B3 B-type procyanidin trimer, (+)Cat (+)Catechin, B2 B-type procyanidin dimer, (–)Epicat (–)Epicatechine, B3 B-type procyanidin trimer, B4 B-type procyanidin tetramer, PP polymer procyanidin, PXG phloretin-2'-O-xyloglucoside, PG phloretin-2'-O-glucoside

Wilhelm'). These values were comparable to some different cultivars of apple (4.2–50.3). The properties of polymeric procyanidins, and especially their susceptibility to oxidation, are dependent on their degree of polymerization [39].

Anthocyanins

The average concentration of total anthocyanins in 22 old apple cultivars was 30.19 mg/100 g dm and depended significantly on the cultivars (Table 4). The content of anthocyanins in fruits of analyzed cultivars of apple ranged from 0.00 for Lausitzer Nelkenapfel to 133.90 mg/100 g dm for Altländer Pfannkuchenapfel. Lower levels, below 5 mg/100 g dm, of anthocyanins were obtained in cvs.

Lausitzer Nelkenapfel, Wintergoldparmane, Horneburger Pfannkuchenapfel, Kaiser Wilhelm, Kaiser Alexander, Gelber Richard, Booskop (Piękna z Booskop), Roter Herbstkalvill and Wintergoldparman. The main anthocyanin compound identified in all old apple cultivars was cyanidin-3-*O*-galactoside (100–85% of total anthocyanins), then cyanidin-3-*O*-glucoside (0–8%), cyanidin-3-*O*-arabinoside (0–7%), and cyanidin-3-*O*-xyloside (0–7%). In the results of Khanizadeh et al. [40] and Awad et al. [41], cyanidin-3-*O*-galactoside was also a major compound in apple fruits. The anthocyanin concentrations in the different apple cultivars depend on growing location, environmental factors, genetic traits, cultivar and the activity of dihydroflavonol reductase (DFR) [42]. The anthocyanins

Table 4 Concentration of phenolic acids and anthocyanins (mg/100 g dm) of all old apple cultivars

Apple variety	Phenolic acids					Anthocyanins				
	NCH	CH	CP	PCQ	PCQI	CGAL	CGLU	CARA	CXYL	
Roter Delicious typ Starkinson	7.16 ± 0.06a	2294.03 ± 2.59a	23.28 ± 0.19d	64.95 ± 0.52a	167.81 ± 1.34a	72.70 ± 0.58c	2.20 ± 0.02d	2.10 ± 0.02c	2.79 ± 0.03c	
Roter Herbstkalvill	1.95 ± 0.02i	825.62 ± 1.38d	15.66 ± 0.13j	13.93 ± 0.11c	61.24 ± 0.49j	2.22 ± 0.02o	nd	nd	nd	
Booskop (Piękna z Booskop)	0.98 ± 0.02 k	754.46 ± 1.26f	12.37 ± 0.10 m	8.96 ± 0.07f	45.53 ± 0.36 m	2.73 ± 0.02n	nd	nd	nd	
Wintergoldparman	1.21 ± 0.03jk	754.26 ± 1.65 g	14.43 ± 0.12 k	4.43 ± 0.04p	45.26 ± 0.36n	0.80 ± 0.01q	nd	nd	nd	
Landsbergen Renette	1.16 ± 0.04jk	709.66 ± 1.45 h	31.56 ± 0.25c	7.09 ± 0.06i	77.35 ± 0.62f	14.28 ± 0.11i	0.39 ± 0.06 h	0.33 ± 0.03 h	1.12 ± 0.02e	
Riesenboiken	1.95 ± 0.01i	1031.45 ± 3.09b	16.96 ± 0.14i	13.93 ± 0.11c	65.62 ± 0.51 h	7.42 ± 0.06 l	nd	nd	nd	
Gelber Richard	3.02 ± 0.06e	913.59 ± 2.74c	7.56 ± 0.06q	18.02 ± 0.14b	58.22 ± 0.47 k	0.46 ± 0.01r	nd	nd	nd	
Kaiser Alexander	2.05 ± 0.04 hi	709.17 ± 2.13i	18.05 ± 0.14h	2.31 ± 0.02r	40.68 ± 0.33o	1.46 ± 0.01p	nd	nd	nd	
Altländer Pfannkuchenapfel	1.22 ± 0.01j	267.65 ± 0.80 s	33.00 ± 0.26b	4.78 ± 0.04o	102.82 ± 0.83c	123.33 ± 0.99a	3.61 ± 0.02a	3.15 ± 0.05b	3.81 ± 0.03a	
Roter Trier Weinapfel	1.21 ± 0.01jk	219.90 ± 0.66t	21.91 ± 0.18f	4.11 ± 0.03q	93.09 ± 0.74e	7.72 ± 0.06 k	nd	nd	nd	
Boikenapfel	3.21 ± 0.08d	175.92 ± 0.53u	14.19 ± 0.11 l	4.40 ± 0.04p	28.54 ± 0.23r	72.84 ± 0.58c	2.37 ± 0.03c	2.02 ± 0.03c	2.81 ± 0.02c	
Kaiser Wilhelm	0.37 ± 0.02 l	776.78 ± 2.03e	3.73 ± 0.03u	13.64 ± 0.11d	28.39 ± 0.23r	0.88 ± 0.01q	nd	nd	nd	
Roter Eiserapfel	2.50 ± 0.03 g	675.09 ± 1.69j	20.98 ± 0.17 g	10.58 ± 0.08e	99.58 ± 0.80d	58.95 ± 0.47e	2.17 ± 0.04d	0.54 ± 0.01 g	3.06 ± 0.04b	
Rote Sternerenette	2.53 ± 0.05 g	562.51 ± 1.00 k	10.77 ± 0.09o	5.98 ± 0.05 l	71.64 ± 0.57 g	72.40 ± 0.58d	3.61 ± 0.02a	1.25 ± 0.03e	2.38 ± 0.01d	
Geflammt Kardinal	0.44 ± 0.01 l	332.68 ± 0.93o	18.01 ± 0.14 h	4.55 ± 0.04p	46.81 ± 0.37 l	8.84 ± 0.07j	nd	nd	nd	
Lausitzer Nelkenapfel	2.50 ± 0.03 g	307.67 ± 1.56p	22.52 ± 0.18e	7.74 ± 0.06 g	64.87 ± 0.52i	nd	nd	nd	nd	
Weisser Winterkalvill	2.21 ± 0.04 h	519.55 ± 0.23 l	50.43 ± 0.40a	7.78 ± 0.06 h	167.44 ± 1.34b	75.48 ± 0.60b	2.71 ± 0.03b	3.29 ± 0.02a	nd	
Dulmener Rosenapfel	2.73 ± 0.02f	78.16 ± 1.21w	6.28 ± 0.05r	6.79 ± 0.03j	32.66 ± 0.26q	37.87 ± 0.30f	1.34 ± 0.04f	nd	nd	
Horneburger Pfannkuchenapfel	3.81 ± 0.02c	401.82 ± 0.84 m	5.79 ± 0.05 s	4.53 ± 0.07p	9.75 ± 0.08t	0.74 ± 0.01q	nd	nd	nd	
Wintergoldparmane	4.22 ± 0.06b	280.98 ± 0.88r	8.01 ± 0.03p	4.97 ± 0.02n	5.85 ± 0.05u	3.70 ± 0.03 m	nd	nd	nd	
Charlamowsky	1.04 ± 0.04 k	294.09 ± 1.13q	5.25 ± 0.06t	5.64 ± 0.02 m	17.48 ± 0.14 s	31.12 ± 0.25 g	1.05 ± 0.01 g	0.69 ± 0.03f	nd	
Parkers Pepping	0.31 ± 0.01 l	375.01 ± 1.55n	11.79 ± 0.09n	6.33 ± 0.04 k	35.98 ± 0.26p	16.40 ± 0.13 h	1.63 ± 0.01e	1.36 ± 0.02d	nd	

Values are mean ± standard deviation. *n* = 3

a–e Mean ± SD followed by different letters within the same line represent significant differences (*p* < 0.05)

NCH neochlorogenic acid, CH chlorogenic acid, CP cryptochlorogenic acid, PCQ *p*-coumarylquinic, PCQI *p*-coumarylquinic isomer acid, CGAL cyaniding-3-*O*-galactoside, CGLU cyaniding-3-*O*-glucoside, CARA cyaniding-3-*O*-arabinoside, CXYL cyaniding-3-*O*-xyloside

are responsible for the red to purple color of the skin in some apples. It is important to consume apples with the peel because the skin has much greater total antioxidant properties than the pulp.

Flavonols

The next group belonging to polyphenol compounds was flavonols, found in 22 old apple cultivars (Table 5). In apple fruits, eight compounds were identified (six quercetin derivatives and two isorhamnetin derivatives) belonging to flavonols. The major compound was quercetin-3-*O*-galactoside (4–43% of total flavonols). In the results of

Ceymann et al. [11] and Khanizadeh et al. [40], quercetin-3-*O*-galactoside was also a major compound in apple fruits. The content of flavonols in 22 old apples of the tested cultivars ranged from 16.01 (cv. Kaiser Alexander) to 220.45 mg/100 g dm (cv. Wintergoldparmane); the average concentration was 106.78 mg/100 g dm. The volume of these compounds in all the analyzed old apple cultivars in our study was similar to those obtained in different cultivars of apple [10, 40].

Flavonols, especially quercetin derivatives, significantly increase the total antioxidant properties of apples. These are mainly anti-inflammatory, antitumor, anticoagulant, anti-allergic and antiviral properties [21].

Table 5 Concentration of flavonols (mg/100 g dm) of all old apple cultivars

Apple variety	Flavonols							
	QRUT	QGAL	QGLU	QARA	QXYL	QRHM	IGAL	IGLU
Roter Delicious typ Starkinson	9.73 ± 0.08a	81.18 ± 0.65a	28.30 ± 0.23c	18.34 ± 0.15f	48.39 ± 0.39d	20.66 ± 0.17h	nd	nd
Roter Herbstkalvill	2.70 ± 0.02e	46.60 ± 0.37g	10.71 ± 0.09h	20.80 ± 0.17e	41.40 ± 0.33f	26.66 ± 0.21e	0.45 ± 0.03de	0.54 ± 0.03k
Booskop (Piękna z Booskop)	2.56 ± 0.02ef	38.86 ± 0.31h	12.28 ± 0.10g	21.69 ± 0.17d	55.18 ± 0.44b	36.08 ± 0.29b	0.24 ± 0.02fgh	2.00 ± 0.06e
Wintergoldparman	0.28 ± 0.00klm	7.60 ± 0.06r	1.71 ± 0.01r	6.66 ± 0.05o	9.25 ± 0.07s	6.39 ± 0.05p	nd	nd
Landsbergen Renette	1.29 ± 0.01i	74.24 ± 0.59b	16.61 ± 0.13d	30.17 ± 0.24a	51.03 ± 0.41c	34.03 ± 0.27c	0.55 ± 0.05d	3.13 ± 0.03c
Riesenboiken	0.45 ± 0.00k	6.81 ± 0.05s	7.83 ± 0.06l	7.57 ± 0.06n	13.02 ± 0.10no	12.21 ± 0.10l	nd	nd
Gelber Richard	9.43 ± 0.08b	56.88 ± 0.48f	32.73 ± 0.26a	17.52 ± 0.14g	40.10 ± 0.32g	33.24 ± 0.27d	2.16 ± 0.09a	1.83 ± 0.04f
Kaiser Alexander	0.18 ± 0.00m	1.42 ± 0.01u	1.01 ± 0.01r	1.65 ± 0.01s	2.69 ± 0.02t	3.34 ± 0.03r	2.15 ± 0.08a	3.57 ± 0.05b
Altländer Pfannkuchenapfel	2.50 ± 0.07f	62.06 ± 0.50e	31.37 ± 0.25b	23.90 ± 0.19b	47.12 ± 0.38e	19.17 ± 0.15i	nd	nd
Roter Trier Weinapfel	0.29 ± 0.00klm	19.21 ± 0.15m	9.43 ± 0.18j	12.77 ± 0.10j	34.72 ± 0.23h	10.84 ± 0.09n	1.71 ± 0.10b	2.68 ± 0.03d
Boikenapfel	nd	31.28 ± 0.25j	2.68 ± 0.16q	14.13 ± 0.11h	25.79 ± 0.21j	13.41 ± 0.11k	nd	nd
Kaiser Wilhelm	1.36 ± 0.04i	22.58 ± 0.18k	9.84 ± 0.26i	5.43 ± 0.04r	9.47 ± 0.08r	12.12 ± 0.10l	nd	nd
Roter Eiserafel	0.85 ± 0.02j	19.52 ± 0.16l	4.34 ± 0.08p	7.48 ± 0.06n	13.18 ± 0.18n	19.04 ± 0.15i	0.33 ± 0.03ef	1.68 ± 0.02g
Rote Sternerenette	2.13 ± 0.05g	32.93 ± 0.26i	7.76 ± 0.02l	10.55 ± 0.08l	20.58 ± 0.14l	21.29 ± 0.17f	0.24 ± 0.01fgh	2.60 ± 0.06d
Geflammer Kardinal	1.45 ± 0.03hi	10.62 ± 0.08p	7.20 ± 0.09m	5.68 ± 0.05q	10.39 ± 0.16q	7.63 ± 0.06o	0.13 ± 0.01ghi	0.73 ± 0.03j
Lausitzer Nelkenapfel	0.41 ± 0.01kl	2.21 ± 0.02t	8.06 ± 0.07k	8.30 ± 0.07m	22.26 ± 0.24k	11.16 ± 0.09m	0.28 ± 0.02fg	1.25 ± 0.07h
Weisser Winterkalvill	2.96 ± 0.06d	11.21 ± 0.09o	6.49 ± 0.06n	6.27 ± 0.05p	17.03 ± 0.56m	5.19 ± 0.04q	nd	nd
Dulmener Rosenapfel	7.86 ± 0.09c	73.42 ± 0.59c	13.85 ± 0.11f	11.43 ± 0.09k	20.48 ± 0.21l	38.64 ± 0.31a	1.54 ± 0.02c	3.51 ± 0.05b
Horneburger Pfannkuchenapfel	0.26 ± 0.02lm	9.70 ± 0.08q	8.21 ± 0.08k	13.58 ± 0.01li	29.55 ± 0.13i	13.70 ± 0.11j	0.14 ± 0.04ghi	0.66 ± 0.01jk
Wintergoldparmane	2.45 ± 0.04f	69.99 ± 0.56d	14.61 ± 0.12e	23.22 ± 0.19c	69.40 ± 0.17a	33.09 ± 0.26d	0.33 ± 0.03ef	7.36 ± 0.09a
Charlamowsky	0.90 ± 0.01j	16.96 ± 0.14n	5.95 ± 0.05o	7.59 ± 0.06n	12.90 ± 0.10o	20.94 ± 0.17g	0.17 ± 0.02gh	0.28 ± 0.04l
Parkers Pepping	1.58 ± 0.05h	19.32 ± 0.15m	8.22 ± 0.10k	5.52 ± 0.04qr	11.05 ± 0.09p	6.50 ± 0.05p	0.11 ± 0.02hi	0.94 ± 0.03i

Values are mean ± standard deviation. $n = 3$

a–e Mean ± SD followed by different letters within the same line represent significant differences ($p < 0.05$)

nd not identified, QRUT quercetin-3-*O*-rutoside, QGAL quercetin-3-*O*-galactoside, QGLU quercetin-3-*O*-glucoside, QARA quercetin-3-*O*-arabinoside, QRHM quercetin-3-*O*-rhamnoside, IGAL isorhamnetin-3-*O*-galactoside, IGLU isorhamnetin-3-*O*-glucoside

Dihydrochalcone

The last group belonging to polyphenol compounds was dihydrochalcones, found in the 22 old apple cultivars (Table 3). In apple fruits, two dihydrochalcone compounds were identified. The content of dihydrochalcone in the 22 tested old apple cultivars ranged from 16.89 (cv. Parkers Pepping) to 110.22 mg/100 g dm (cv. Gelber Richard); the average concentration was 48.24 mg/100 g dm. Similar

results to a lesser extent were obtained by Wojdyło et al. [10] and Khanizadeh et al. [40] Dihydrochalcone, especially phloretin, strengthens the action of active ingredients adhering to the surface of lipids and changes the bipolar potential of the lipid bilayer. In addition, it inhibits active glucose transport into SGLT1 and SGLT2 cells, inhibits various urea transporters, and has strong antioxidant properties [43].

Fig. 2 Content of triterpenoid compounds ($\mu\text{g/g dm}$) of all old apple cultivars

Identification and quantification of triterpenoids

Figure 2 shows the data after determination of triterpenoids in the fruits of three cultivars of cranberry fruits of different maturity stages. The detected compounds were identified as betulinic, ursolic and oleanolic acids based on their molecular ion $[M-H]^-$ at m/z 455.3, MS profiles with the fragmentation pathways, UV–Vis spectra, and the retention times (R_t) of authentic standards.

Triterpene compounds on the other hand are present in the resin, peel and cuticular waxes, fruit and vegetable extracts, and exhibit anticancer, antioxidative, anti-inflammatory, antibacterial, antifungal and antiprotazoal properties [44]. The average amount of triterpenoids in all tested old apple cultivars ranged from 466.3 in cv. Wintergoldparmane to 3753.6 $\mu\text{g/g dm}$ in cv. Gelber

Richard. The content of triterpenoids in ten cultivars of pear was approximately 3–37% lower than that of all old apple cultivars [45]. The main group of triterpenoids in all old apple cultivars was ursolic acid (from 32 to 96% of all triterpenoids), followed by oleanolic acid (from 2 to 20%) and betulinic acid (from 1 to 14%), with significant differences among the cultivars. The exceptions were the two cultivars Wintergoldparman, where the main acid of triterpenoids was oleanolic acid (57%), and Dulmener Rosenapfel, where betulinic acid constitutes 36%. According to He and Liu [46] and Szakiel et al. [44], ursolic acid is the predominant triterpenoid compound present in apple (98%). In addition, ursolic acid content in cranberry was 20% of all wax extract and in sweet cherry was 60% [47]. Betulinic acid has not been determined in apple fruits yet.

Fig. 3 Antioxidant activity (mmol Trolox/100 g dm) of different old apple cultivars. Values are mean $\pm$ standard deviation. $n = 3$

Antioxidant activity

The results of the antioxidant activity of all the old apple cultivars measured by the ABTS and FRAP assay are presented in Fig. 3. Significant differences in the antioxidant properties were observed between analyzed cultivars using these assays. The average result of the antioxidant activity, according to the ABTS and FRAP assay, in the apple cultivars was 72.14 and 46.77 $\mu\text{mol Trolox}/100\text{ g dm}$, respectively. The level of antioxidant activity measured by ABTS assay in all the old apple cultivars ranged from 24.58 to 124.71 $\mu\text{mol Trolox}/100\text{ g dm}$, but the content of antioxidant activity measured by the FRAP assay in apple cultivars ranged from 15.79 to 80.15 $\mu\text{mol Trolox}/100\text{ g dm}$. These amounts were found in cvs. Landsbergen Renette and Wintergoldparmane, respectively. The level of antioxidant capacity in different apple cultivars grown in Poland, analyzed by the ABTS and FRAP assay, was 1.8 times lower and 1.5 times higher, respectively, than old cultivars grown in Poland [8]. According to Ceymann et al. [11], the concentration of antioxidant capacity in analyzed apple cultivars grown in Germany according to the FRAP assay was 2 times lower than in the old apple cultivars grown in Poland. Furthermore, in research by Panzella et al. [34] and Feliciano et al. [36], all the old apple cultivars from Southern Italy and Portugal showed higher antioxidant capacity than the commercial fruits.

Principal component analysis (PCA)

The average results of our studies obtained for 22 old apple cultivars regarding their individual polyphenol profiles and the antioxidant capacity using PCA analysis are presented in Fig. 4. Two main PCAs for the analysis of seven genotypes grown in Poland accounted for 64.20% of the total variability, PC1 for 42.80% and PC2 for 21.38% (Fig. 3). The results obtained from PCA using the linkage method among groups indicated the presence of four clusters:

1. 5, 8, 9, 12, 18 with the highest concentrations of procyanidins polymeric (PP), total acidity (TA) and total sugar (TS) and with a negative correlation with antioxidant capacity;
2. 1, 7, 11, 14, 15, 21, 22 with the highest concentrations of anthocyanins (ANT), total triterpenoids (TT) and ursolic acid (UA) and with the high contents and a positive correlation with antioxidant capacity;
3. 3, 4, 6, 10, 20 with the highest concentrations of total phenolic compounds (TPC), dihydrochalcone (DHC), phenolic acids (PA) and betulinic and oleanolic acid (BA, OA);

Fig. 4 PCA mean showing the relationship among polyphenolic compounds and antioxidant capacity in 22 old apple cultivars grown in Poland. 1, Roter Delicious type Starkinson; 2, Roter Herbstkalvill; 3, Booskop (Piękna z Booskop); 4, Wintergoldparman; 5, Landsbergen Renette; 6, Riesenboiken; 7, Gelber Richard; 8, Kaiser Alexander; 9, Altländer Pfannkuchenapfel; 10, Roter Trier Weinapfel; 11, Boikenapfel; 12, Kaiser Wilhelm; 13, Roter Eiserapfel; 14, Rote Sternrenette; 15, Geflammtter Kardinal; 16, Lausitzer Nelkenapfel; 17, Weisser Winterkalvill; 18, Dulmener Rosenapfel; 19, Horneburger Pfannkuchenapfel; 20, Wintergoldparmane; 21, Charlamowsky; 22, Parkers Pepping; FL, flavonols; ANT, anthocyanins; PA, phenolic acid; F3O, flavan-3-ols; PP, procyanidins polymeric; TS, total sugar; TA, total acidity; TT, total triterpenoids; OA, oleanolic acid; UA, ursolic acid; BA, betulinic acid; TPC, total phenolic acid; Pectins

4. 2, 13, 16, 19 with high antioxidant activity (ABTS and FRAP), and high contents of flavonols (FL), flavan-3-ols oligomers (F3O oligomers) and pectins.

Cluster analysis

Cluster analysis is an unsupervised data analysis method, meaning that prior knowledge of the sample is not required. HCA enables interpretation of the results in a fairly intuitive, graphic way.

Cluster analysis of the 22 old apple cultivar samples, according to their phenolic compounds, was used as an additional exploratory tool to assess heterogeneity among different quality parameters of old apple cultivars grown in Poland. Generally, HCA showed ten clear similarity clusters (Fig. 5). The highest similarity of old apple cultivars was obtained for Roter Trier Weinapfel. The lowest similarity (below 15%) was obtained in old apple cultivars between Kaiser Alexander, Altländer Pfannkuchenapfel, Charlamowsky, Boikenapfel, Rote Sternrenette, Geflammtter Kardinal and Parkers Pepping. The rest of the analyzed old apple cultivars showed similarities between 17 and 47%.

Fig. 5 Hierarchical cluster analysis of 22 old apple cultivars grown in Poland based on group average cluster of phenolic profile

Conclusions

The results showed an important effect of the 22 analyzed old cultivars on the content of polyphenolic compounds in the apple fruits. The concentration of polyphenols in 22 apple cultivars ranged from 1348.40 to 4310.52 mg/100 g dm in fruits of the cvs. Altländer Pfannkuchenapfel and Roter Trier Weinapfel; triterpenoids ranged from 466.30 to 3753.60 µg/g dm in fruits of the cvs. Gelber Richard and Wintergoldparmane. The highest values of the ABTS and FRAP assay were observed in cvs. Wintergoldparmane (124.71 and 80.15 µmol Trolox/g dm) and Horneburger Pfannkuchenapfel (117.93 and 78.63 µmol Trolox/g dm). Additionally, apple cultivars were found to be a good source of sugar (7.41–11.99 g/100 g) and pectins (0.64–2.24 g/100 g). Some old cultivars of apple fruits, especially cvs. Roter Trier Weinapfel, Wintergoldparmane and Horneburger Pfannkuchenapfel, are characterized by the highest amounts of bioactive compounds and antioxidant properties and may be selected for their potential application in commercial cultivation to produce fruits with valuable health-promoting nutritional effects on human health. Therefore, the old apple cultivars could be a promising source of pro-healthy compounds with potential health benefits.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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