TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2023) 47: 412-426

doi:10.55730/1300-011X.3097

Segregation of apple cultivars on the basis of main fruit physical and chemical properties and antioxidant activity

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Received: 14.07.2022 • **Accepted/Published Online:** 24.12.2022 • **Final Version:** 02.06.2023

Abstract: This study aims to analyze the essential physicochemical properties and antioxidant activity of 25 apple commercial cultivars grown in Serbia during 2019 and 2020 in order to segregate the apples with the best size, shape, and also nutritious and health promoting values. The results showed that 'Morens Jonagored' and 'Red Boscop' had similar and the highest fruit weight (FW) whereas 'Golden Delicious', 'Hired', 'Red Chief', and 'Morens Jonagored' had the highest and similar fruit length (L). The lowest both these values were found in 'Akane'. 'Red Boscop' had the highest fruit diameter (D), whereas the lowest had 'Galaxy'. Twenty-two or 88% of the examined cultivars have a D greater than 70 mm. Both fruit shape indexes [sphericity (φ) and aspect ratio (R_a)] were the highest and similar in 'Akane' and 'Budimka' and the lowest in 'Red Chief'. Peel was a better source of dry matter (DM), total phenolic content (TPC), and total flavonoid content (TFC) and had higher antioxidant capacity than flesh. Moroever, pH juice was higher in peel. In contrast, titratable acidity (TA) was higher in flesh in comparison with peel, whereas ash content was statistically similar in both peel and flesh tissues. 'Akane' had the highest total anthocyanin content (TAc), whereas the lowest was found in 'Red Boscop'. Old cultivar 'Budimka' had the highest TPC and TFC in both peel and flesh, whereas 'Ginger Gold' had the lowest TPC and TFC and also the lowest antioxidant power. Peel of 'Red Chief' and flesh of 'Braeburn' had the best antioxidant activity. Among others, correlation analysis showed that the DM content in the peel is a crucial factor that determines the DM, soluble solids content (SSC), TPC, TFC, and DPPH free-radical scavenging activity values in the flesh. The principal component analysis (PCA) showed high discrimination capabilities of variables measured and revealed that a more nutritious peel may be darker or redder while a more nutritious flesh may have a lighter color and lower SSC.

Key words: Bioactive compounds, Free radical scavenging activity, Fruit size and shape, *Malus* × *domestica* Borkh., Soluble solids content, Total anthocyanin content

1. Introduction

Apple tree (*Malus* × *domestica* Borkh.) belongs to the family Rosaceae, Malus genera and pome fruit group. It is widely grown throughout the world thanks to the high degree of adaptability to different environmental conditions. Its fresh fruits are available to consumers 365 days a year. In the last few decades, apple production has been increasing intensively from year to year thanks to the application of modern technological solutions with 3000–5000 trees per hectare, a good choice of highly productive cultivars and a dwarf rootstock such as M.9 with numerous clones. In Serbia, apple is the most important fruit type with an area of 26,360 ha and production of 489,426 t in 2020 (FAOSTAT, 2022). In this country, apple production is one of the active fruit-growing sectors. However, despite the fact that most of the apples produced are either sold in the local Serbian market or exported, many apples remain unsold. Thus, the processing and fermentation of the apples to produce beverages such as cider or other processed products could be a promising perspective to exploit the potential of the unused apples. According to FAOSTAT (2022), apple is ranked as the third fruit produced worldwide with 86.44 million tons during the season of 2020.

In recent years, attention has been focused on the internal apple fruit quality, especially on compounds that promote human health. Mature apple fruits are rich source of primary and secondary metabolite and have high antioxidant potential. They are mainly composed of ≈85% water, 12%–14% carbohydrates, 0.3%–1.0% organic acids, 0.3% proteins, less than 0.1% lipids, as well as starch, tannins, cellulose, enzymes, phytohormones, vitamins and minerals, especially N, P, K, Ca, Mg, S, and Fe (Nour

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et al., 2010; Francini et al., 2022). Apples also contain respectable amounts of secondary metabolites such as flavonols, flavanols, cinnamic acid, dihydrochalcones, and anthocyanins (Persic et al., 2015). Some of them have been proven to have antioxidant activity that inhibits the activity of free radicals in the cells i.e. have a beneficial impact on human health (Persic et al., 2015). On this point of view, consumers are becoming more interested in the content of the health-promoting compounds in fruit because of their antioxidant activity and which do not contain harmful substances and residues of various origins (Milošević et al., 2019a).

Earlier and recent studies reported that peel is a richer source of polyphenolic compounds than flesh (Drogoudi et al., 2008; Leccese et al., 2009; Milošević et al., 2019a). Many studies worldwide have shown that the apple cultivar may have a crucial influence on the phenolic content, total antioxidant activity, and generally fruit chemical composition. Apart from the cultivar, the rootstock, ripeness, physiological condition of a tree as well as soil and weather conditions and cultural practices have an important role in the formation of the internal fruit quality (Markuszewski and Kopytowski, 2008; Milošević et al., 2018, 2019ab). Moreover, the color of the skin and flesh can have a significant impact on the content of organic compounds, because large variations between cultivars with red, yellow, green, or bicolored skin have been found (Drogoudi et al., 2008; Persic et al., 2017).

The physical properties of the fruit, in addition to the chemical ones, are an important link in the chain of consumer demands and different manipulative processes. Among others, one of the fundamental conditions for improvement of quality is proper sorting and handling of the apples for market. Generally, the properties of fruit and vegetable products are the most important parameters to determine the proper standards of design of grading, conveying, processing, and packaging systems (Tabatabaeefar and Rajabipour, 2005). In addition, FW and fruit volume are the most important attributes in determining sizing of processing systems whereas fruit linear dimensions (L and D) are other important parameters determining the fruit size (Mohsenin, 1986). Hence, the fruit physical attributes and their relationships must be known. On this line, several researchers conducted studies about physical properties of different apple cultivars (Demir et al., 2018; Mehdi et al., 2020; Milošević and Milošević, 2021). In support of this, on June 1, 2008, new European Union Commission regulations will come into full effect (EC 85/2004, amended by Commission Regulation EC 1238/2005). According to these regulations, apples were divided into three categories depending on either diameter or weight: Extra, Class I, and Class II. The new regulations lower the minimum size and weight requirements for each category, and lower the minimum size requirements for commercial apple sale.

There are more than 7500 known cultivars of apples (Dobrzański et al., 2006). However, only between 20 and 40 are produced and commercially traded around the world (Strohm, 2013). The review of the literature shows that the information about the physico-chemical properties of the commercial apples and their potential to use for fresh consumption and/or processing industry is available but limited in some cases. On this point of view, this study aimed to determine the main physical properties, chemical composition, and antioxidant activity of 25 commercial apple cultivars grown under Serbian environmental conditions in high density planting system and their segregation with the best performances using multivariate statistical analysis.

2. Materials and methods

2.1. Plant material and orchard layout

The commercial apple orchard was located in Prislonica village (43 °57′N, 20° 26′E) near Čačak city, western Serbia at 320 m a.s.l. It was established in spring of 2006 using 25 cultivars (Table 1) grafted on dwarf M.9 T337 rootstock with exception of 'Idared' and 'Red Chief' which grafted on semidwarf MM.106 rootstock. Planting distance was 3.0 m × 1.2 m and training system was slender spindle. Standard cultural practices (pruning, fertilization, weed control, plant protection), except irrigation, were applied. Fruit thinning was not performed in order to maintain the apparent effect of the cultivar bearing potential. Treatments were distributed using the randomized complete block design with five trees per each cultivar in four replicates (n = 20).

2.2. Fruit physical measurements

For measurements of physical properties, fruits (20 per each cultivar in five replicates, n = 100) were randomly collected on each picking date in 2019 and 2020. Maturity of the apples was determined by dipping one slice from each cultivar into an iodine solution and comparing the color changes to a CTIFL color chart (Table 1). Their physical properties were determined immediately after harvest.

FW (g) was measured with a digital balance MAULsteel 5000 G (Jakob Maul GmbH, Bad König, Germany). Caliper L and D of each apple fruits (Figure 1) were measured using a digital vernier gauge Starrett 727 (Athol, MA, USA). The φ and R_a, as fruit shape indexes, were calculated using equations proposed by Mohsenin (1986) (Equation 1) and Omobuwajo et al. (1999) (Equation 2).

The sphericity (φ) was calculated from Equation 1.

$$\varphi = \frac{D_g}{L} \quad (1)$$

where D_g is the geometric mean diameter [$D_g = (LD^2)^{1/3}$].

Table 1. Harvest time, fruit weight, fruit linear dimensions and fruit shape indexes (sphericity and aspect ratio) of 25 apple cultivars. Data are the mean ± SE for 2 consecutive years.

Cultivar	Harvest time (Average 2019/20)	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	Sphericity	Aspect ratio
Akane	10 August	104.35 ± 3.95 k	52.03 ± 2.26 j	65.09 ± 0.78 jk	1.16 ± 0.04 a	125.59 ± 6.25 a
Ginger Gold	26 July	196.95 ± 28.23 ef	73.66 ± 2.38 b	74.26 ± 1.40 fg	1.01 ± 0.01 k	101.13 ± 2.24 m
Jonathan Watson	13 September	173.95 ± 4.19 gh	64.32 ± 1.54 fgh	76.82 ± 1.67 e	1.13 ± 0.01 c	119.58 ± 1.76 cd
Galaxy	20 August	121.65 ± 7.49 j	60.53 ± 1.26 i	63.35 ± 2.47 k	1.03 ± 0.01 ij	104.83 ± 2.01 jkl
Gala Mondial	23 August	144.05 ± 6.75 i	62.19 ± 0.27 hi	66.33 ± 0.27 j	1.04 ± 0.00 i	106.87 ± 0.65 ij
Golden Delicious	16 September	208.70 ± 13.83 de	74.78 ± 1.59 ab	76.60 ± 1.04 e	1.02 ± 0.02 jk	102.53 ± 2.46 lm
Hired	17 September	205.95 ± 10.77 de	74.97 ± 4.00 ab	72.81 ± 3.58 gh	0.98 ± 0.01 l	97.27 ± 1.20 n
Fuji	16 October	193.60 ± 12.65 ef	67.63 ± 2.34 e	75.36 ± 1.77 ef	1.08 ± 0.04 fg	111.71 ± 5.48 g
Golden Reinders	15 September	169.20 ± 7.19 gh	65.01 ± 1.18 f	71.74 ± 0.23 hi	1.07 ± 0.01 gh	110.72 ± 1.87 gh
Braeburn	11 October	169.25 ± 4.55 gh	62.21 ± 1.85 ghi	71.46 ± 0.97 hi	1.10 ± 0.02 de	115.11 ± 3.78 ef
Red Chief	15 September	207.50 ± 7.08 de	75.82 ± 2.01 ab	72.82 ± 0.61 gh	0.97 ± 0.02 l	96.28 ± 2.90 n
Starking	17 September	158.25 ± 10.81 hi	64.05 ± 1.41 fgh	70.71 ± 1.26 i	1.07 ± 0.02 gh	110.85 ± 3.33 gh
Morens Jonagored	20 September	253.35 ± 4.13 a	77.18 ± 2.17 a	79.75 ± 2.23 d	1.02 ± 0.01 jk	103.51 ± 1.17 klm
Topred	14 September	181.19 ± 6.12 fg	70.59 ± 2.03 d	72.74 ± 2.12 ghi	1.02 ± 0.01 jk	103.29 ± 1.18 klm
Delbar Jubileum	22 October	215.90 ± 14.58 cd	73.48 ± 2.42 bc	81.00 ± 0.58 bcd	1.07 ± 0.02 gh	110.31 ± 2.76 gh
Unknown	26 September	165.85 ± 22.83 gh	65.86 ± 0.81 ef	76.48 ± 0.97 e	1.10 ± 0.01 de	116.26 ± 1.80 ef
Richared	15 September	197.00 ± 13.94 ef	69.12 ± 1.22 d	75.04 ± 2.57 ef	1.06 ± 0.02 h	108.71 ± 2.91 hi
Hapke	16 September	181.00 ± 11.09 fg	70.11 ± 2.03 d	72.11 ± 2.12 hi	1.02 ± 0.01 jk	105.20 ± 1.78 jk
Stayman Winesap	19 October	195.10 ± 11.10 ef	66.18 ± 0.69 ef	75.51 ± 1.59 ef	1.09 ± 0.02 ef	114.36 ± 3.01 f
Red Boscop	24 October	250.45 ± 13.34 a	69.85 ± 2.87 d	84.44 ± 2.02 a	1.14 ± 0.02 bc	121.11 ± 3.85 bc
Melrose	14 October	202.90 ± 6.94 de	64.60 ± 2.12 fg	79.95 ± 1.10 cd	1.15 ± 0.03 ab	123.00 ± 4.26 b
Granny Smith	14 November	233.25 ± 28.17 b	71.21 ± 1.56 cd	81.98 ± 2.59 bc	1.11 ± 0.03 d	115.56 ± 4.32 ef
Idared	17 October	231.15 ± 38.19 bc	70.45 ± 2.14 d	82.28 ± 1.78 b	1.11 ± 0.04 d	117.35 ± 6.26 de
Budimka	27 October	217.10 ± 20.56 bc	65.39 ± 0.97 ef	81.99 ± 5.27 bc	1.16 ± 0.04 a	125.69 ± 6.11 a
Pink Lady	16 November	175.05 ± 10.14 gh	65.65 ± 2.14 ef	72.20 ± 1.02 hi	1.07 ± 0.02 gh	110.18 ± 2.62 gh

Means within the column followed by different letters differ significantly among cultivars at $p \leq 0.05$, based on LSD test.
Different letters in the same column indicate significant differences according to LSD test ($p \leq 0.05$).

The aspect ratio (R_a) was calculated from Equation 2.

$$R_a = D/L \quad (2)$$

where D is the fruit diameter, and L is the fruit length.

2.3. Fruit chemical analysis

Fruit sampling for chemical analysis, about 50 per each cultivar, was performed at technological (fully) maturity stage i.e. after 5 weeks of storage at 2 °C. The fruits were cored and cut into small portions, homogenized and used for the standard tests for chemical analyses. Chemical analyses were performed separately for the peel and separately for the flesh, except for SSC, which was determined in whole fruits and TAc which was analyzed only in peels (without flesh).

The SSC (°Brix) was determined in fruit juice using hand refractometer Carl Zeiss 32-G (Carl Zeiss, Jena, Germany) at room temperature (20 °C). For the calculation of TA, the fruit juice was titrated with 0.1 NaOH up to pH 8.1. The resulting values were expressed as a % of malic acid as it is the predominant organic acid in the apple. The pH of the juice was determined with an MP 220 pH meter (Mettler Toledo, Columbus, Ohio). The ash content was determined by incinerating the dried sample in muffle furnace at 600 °C for 8 to 10 h as described in AOAC method No.940-26 (AOAC, 1990).

The TPC, TFC, free radical scavenging activity (DPPH assay) and TAc were analyzed using Cary 300 UV/Vis spectrophotometer (Agilent Technologies, Santa Clara, CA).

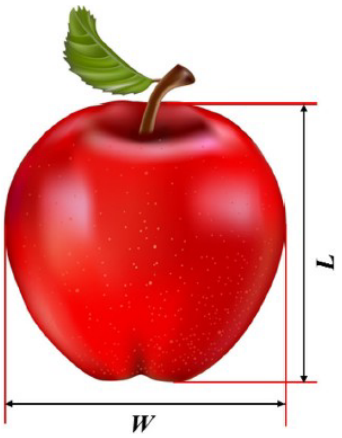

Figure 1. Dimensions of apple fruit; L and W are the length and width (diameter - D) (Demir et al., 2018).

The TPC was determined using the Folin-Ciocalteu colorimetric method (Lima et al., 2005) and the results were expressed as milligrams of gallic acid equivalents per 100 g of fresh weight (mg GAE 100 g^{-1} fw). The TFC was determined by a colorimetric method described by Zhishen et al. (1999), and the results were expressed as milligrams of rutin equivalents per 100 g fw (mg RUE 100 g^{-1} fw). Antioxidant activity was determined using DPPH method reported by Brand-Williams et al. (1995) and modified by Sánchez-Moreno et al. (1998). The results were expressed as Trolox® equivalent (μmol TE 100 g^{-1} fw). The TAc was determined according to the method proposed by Giusti and Wrolstad (2001) based on the pH-differential method previously described by Fuleki and Francis (1968). Content was expressed as milligram of malvidin-3-o-glucoside equivalents per 100 g fw (mg ME 100 g^{-1} fw).

2.4. Chemicals and reagents

All chemicals and reagents used were of analytical grade and were used as received without any further purification and were obtained from Sigma-Aldrich (St. Louis, MI, USA). All solutions were prepared with deionized water of resistivity not less than 18.2 MΩ cm^{-1}.

2.5. Statistical analysis

Data for both 2019 and 2020 were pooled and then they were calculated as average values and presented as mean ± SE for each cultivar. All data were statistically evaluated with analysis of variance (one-way ANOVA) procedures of Microsoft Office Excel software (Microsoft Corporation, Redmond, WA, USA). Treatments means were compared using LSD test. *p*-values that are equal to or less than 0.05 were considered statistically significant. Pearson's rank correlation matrix (values were different from 0 with a significance level α = 0.05) was done using the R corrplot package (Wei and Simko, 2017). Principal components analysis (PCA) as well as biplot graphical display was performed using the XLSTAT software package v. 7.0 (Addinsoft, Paris, France).

3. Results and discussion

3.1. Evaluation of fruit physical properties

Data in Table 1 showed that differences in FW and both linear dimensions among cultivars were significant. The highest and similar FW had 'Morens Jonagored' and 'Red Boscop' and the lowest was found in 'Akane'. 'Akane' also had the lowest L, whereas 'Morens Jonagored' alongside with 'Red Chief', 'Hired', and 'Golden Delicious' had statistically similar and the highest L. 'Red Boscop' is the cultivar with the highest D whereas 'Galaxy' had the lowest value. Previous studies on apple also reported a high variability among cultivars regarding these parameters (De Salvador et al., 2006; Leccese et al., 2009; Ozturk et al., 2011; Mehdi et al., 2020; Wicklund et al., 2021; Mureşan et al., 2022). For example, in a study by Dobrzański et al. (2001), FW of nine apples varied between 101 and 256 g, whereas Leccese et al. (2009) reported values of three apple cultivars between 169.2 and 191.7 g. Moreover, Leccese et al. (2009) stated that average FW of 'Golden Delicious B' was 179.4 g which was lower and/or higher than those obtained in our work for both 'Golden Delicious' and 'Golden Delicious Reinders', respectively, probably due to differences in growing technology, rootstock used, crop load and tree age as previously reported by Milošević and Milošević (2021). These authors also found that 'Pink Lady' had FW between 134.35 and 159.55 g and D between 66.01 and 70.22 mm depending on the rootstock, which were lower than those values obtained in the current study whereas our data for 'Granny Smith' for FW, L and D were in a good harmony with results of Ozturk et al. (2022).

The φ and R_a indexes usually are used to describe fruit shape (Mohsenin, 1986). In the present study, values of both these traits significantly varied among cultivars. Local 'Budimka' and two international ('Melrose' and 'Akane') cultivars had the highest φ values and tend towards an obloid shape, whereas the lowest value had 'Red Chief' and tend cylindrical to cylindrical waisted shape according to descriptor list for apple (IBPGR, 1982). Otherwise, φ values close to 1 indicate a globose shape, greater than 1 indicate an obloid shape and less than 1 indicate an elongated shape (cylindrical waisted, conic, ovoid, cylindrical and/or ellipsoid in general). The R_a showed great similarity in tendencies in relation to φ because 'Budimka' and 'Akane' had the highest and statistically similar values, and 'Red Chief' had the lowest. In a study of Ozturk et al. (2011), φ values of three summer apple cultivars varied between 0.883 and 0.958.

The FW is a function of crop load, tree capacity, and preharvest growing conditions due to competition

between fruits for carbohydrates (Milošević et al., 2018). Moreover, FW alongside fruit size and fruit shape is a major quantitative inherited factor and polygenic component that affects yield, visual fruit quality, and consumers' acceptability. On the other hand, all above physical properties are of the paramount importance for proper design of machines and processes to harvest, fruit sizing, sorting and packing, handle and store of apples and to convert it into food and feed requires a knowledge and understanding (Mohsenin, 1986). Additionally, fruit shape is a useful indicator for description of cultivars in applications for plant cultivar rights and many pomological descriptions (Beyer et al., 2002).

In Serbia, apples are sorted into three groups in order to meet certain norms, and in accordance with international protocols, regulations, and standards such as the European Union Commission regulations from 2008 (EC 85/2004, amended by Commission Regulation EC 1238/2005). According to these regulations and standards, large-fruited cultivars when size determined by D and FW, such as 'Braeburn', 'Fuji', 'Golden Delicious', 'Golden Reinders', 'Granny Smith', 'Idared', 'Morens Jonagored', 'Melrose', 'Red Chief', 'Starking', 'Starkrimson', 'Topred', and 'Richared', should be at least 70 mm in diameter or 140 g in order to be classified as "Extra" (i.e. the best quality). However, fruits that are too large suffer from physiological disorders such as bitter pit in 'Idared', especially during storage, and must be harvested and consumed first (Pole et al., 2017). According to Dobrzański et al. (2001, 2006), dividing other apples in classes 65, 60 mm and/or 110 g, etc. (i.e. with a 5 mm spread in each class) enhances marketing capacity and bring a higher price. Small-fruited cultivars like 'Akane', 'Galaxy', and 'Gala Mondial' should be at least 60 mm in diameter or 90 g in order to be classified "Extra". In the last few years, traders in Serbia have been demanding apple fruits with a minimum diameter of 75 mm, which is not well accepted by producers (Milošević et al., 2018).

In our study, 23 cultivars, which were 92% of the total number of the tested, had an FW greater than 140 g (Table 1). The 22 cultivars or 88% had D higher than 70 mm, whereas 13 cultivars or 52% had D higher than 75 mm in average. Only 'Akane' and 'Galaxy' had lower FW than 140 g or diameter close to 65 mm ('Akane') or lower than 65 mm ('Galaxy'). In Serbia, as a rule, fruits with a diameter less than 55 mm and damaged fruits are classified as industrial and are sold at a very low price, which causes great dissatisfaction among apple growers.

3.2. Evaluation of fruit chemical properties

The ANOVA results revealed that the DM, SSC, and TA were found to be significant among cultivars (Table 2). Regarding fruit tissue, the peel was significantly richer in DM than the flesh, while the acidity was higher in the flesh compared to the peel. Namely, the peel had 1.41 times more DM than the flesh, while flesh had 1.78-fold higher TA than peel. Our data were in a good agreement with results of Pissard et al. (2018), who reported that apple peel and flesh contained 224 g kg^{-1} and 155 g kg^{-1} DM, respectively, whereas Oszmiański et al. (2018) reported values between 14.62% and 17.12% in whole fruits of old apples. Similar tendencies have been reported previously by Lata et al. (2005) with values of 229 g kg^{-1} and 159 g kg^{-1} in both peel and whole fruit, respectively. Otherwise, the peel of the fruit can be an important physical feature because it represents a barrier between the environment and the flesh (Gonzalez-Talice et al., 2013).

Results regarding the DM in peel present the highest and similar values in 'Topred' and 'Richared', followed by 'Hapke' (all members of the 'Red Delicious' group), 'Budimka', 'Pink Lady' etc., whereas the lowest and statistically similar content had 'Ginger Gold', 'Melrose', and 'Idared'. In flesh tissue, the highest DM was found in 'Richared', followed by 'Topred' and 'Pink Lady', whereas the lowest value was found in 'Melrose'. Interestingly, 'Jonathan Watson', 'Golden Delicious', 'Morens Jonagored', and 'Budimka' had quite similar DM in flesh. Other authors also reported a strong impact of the apple cultivar per se or genotype on the DM content in apple fruits (Campeanu et al., 2009; Nour et al., 2010; Pissard et al., 2018). The percentage of DM is an important quality attribute, especially in the case of apple cultivars used for production of different products in processing industry.

The ratio between peel and flesh is consistent with the results of other studies reporting that, depending on the cultivar, apple flesh contains more TA than the peel (Drogoudi et al., 2008). The results in our study showed that peel of 'Red Boscop' had 8.10-fold higher TA than 'Melrose'. Peel of 'Hired', 'Starking', and 'Hapke' (all clones from 'Red Delicious' group), and had similar TA values as 'Melrose'. In the flesh tissue, 'Red Boscop' also had the highest TA, whereas the lowest and statistically similar TA was noted in 'Hired' and 'Starking'. Similar to our findings, Nour et al. (2010) reported that 'Red Boscop' had the highest acidity in comparison with other 14 apples. Other studies also reported high variability among cultivars in acidity. Campeanu et al. (2009) noted values of TA in a range between 0.127% and 0.345%, whereas Mureșan et al. (2022) reported values between 0.18% and 0.55% but in whole fruits. In a study by Mehdi et al. (2020), the highest acidity was present in 'Golden Delicious' (0.26%), while the lowest acidity was 0.16% present in 'Red Delicious'. Some authors have found that nonred apple cultivars had higher acidity compared to red-colored cultivars (Leccese et al., 2009; Campeanu et al., 2009; Kumar et al., 2018). Our results in general coincided with the statements of the mentioned authors. Low acidity determines a good quality for fresh consumption whereas fruits of apples with high

Table 2. Average dry matter content, soluble solids content, and titratable acidity in peel and flesh of 25 apple cultivars. Data are the mean ± SE for 2 consecutive years.

Cultivar	Dry matter content (%)		Soluble solids content (°Brix)	Titratable acidity (%)	
	Peel	Flesh	Flesh	Peel	Flesh
Akane	19.08 ± 0.02 lmn	15.37 ± 0.19 efg	13.47 ± 0.05 n	0.159 ± 0.00 f	0.234 ± 0.00 ef
Ginger Gold	17.41 ± 0.13 o	13.68 ± 0.17 k	13.27 ± 0.09 o	0.192 ± 0.00 e	0.150 ± 0.00 l
Jonathan Watson	22.33 ± 0.36 d-g	16.45 ± 0.02 d	14.20 ± 0.08 hi	0.272 ± 0.00 b	0.314 ± 0.00 d
Galaxy	20.76 ± 0.21 g-k	15.44 ± 0.11 ef	14.27 ± 0.05 gh	0.131 ± 0.00 h	0.217 ± 0.00 fgh
Gala Mondial	19.32 ± 0.22 klm	14.65 ± 0.12 hij	15.40 ± 0.14 c	0.103 ± 0.00 j	0.206 ± 0.00 h
Golden Delicious	22.42 ± 0.08 def	16.46 ± 0.15 d	15.80 ± 0.16 b	0.156 ± 0.00 f	0.242 ± 0.00 e
Hired	22.21 ± 0.18 d-h	14.48 ± 0.17 ij	13.67 ± 0.05 m	0.062 ± 0.00 o	0.103 ± 0.00 n
Fuji	21.97 ± 0.25 d-i	15.44 ± 0.27 ef	14.92 ± 0.05 e	0.102 ± 0.00 jk	0.151 ± 0.00 l
Golden Reinders	20.47 ± 0.09 i-e	14.68 ± 0.14 hij	14.07 ± 0.02 j	0.113 ± 0.00 i	0.175 ± 0.00 jk
Braeburn	20.62 ± 0.17 h-l	14.56 ± 0.10 ij	15.87 ± 0.05 b	0.156 ± 0.00 f	0.350 ± 0.00 c
Red Chief	19.41 ± 0.05 klm	13.56 ± 0.13 k	13.12 ± 0.05 p	0.072 ± 0.00 n	0.127 ± 0.00 m
Starking	22.54 ± 0.18 def	15.72 ± 0.05 e	14.57 ± 0.02 f	0.060 ± 0.00 o	0.106 ± 0.00 n
Morens Jonagored	21.54 ± 0.16 e-j	16.71 ± 0.13 d	12.40 ± 0.08 q	0.093 ± 0.00 l	0.230 ± 0.00 efg
Topred	29.02 ± 0.13 a	20.21 ± 0.12 b	18.20 ± 0.04 a	0.072 ± 0.00 n	0.158 ± 0.00 kl
Delbar Jubileum	20.25 ± 0.18 jkl	14.33 ± 0.06 j	13.80 ± 0.04 l	0.103 ± 0.00 j	0.218 ± 0.00 fgh
Unknown	21.44 ± 0.24 f-j	13.45 ± 0.32 k	14.92 ± 0.05 e	0.134 ± 0.00 h	0.218 ± 0.00 fgh
Richared	28.29 ± 0.12 a	21.40 ± 0.11 a	15.07 ± 0.02 d	0.091 ± 0.00 l	0.182 ± 0.00 ij
Hapke	25.33 ± 2.71 b	15.11 ± 0.17 fgh	14.92 ± 0.05 e	0.062 ± 0.00 o	0.165 ± 0.00 jkl
Stayman Winesap	21.76 ± 0.74 d-j	14.90 ± 0.04 ghi	13.92 ± 0.05 k	0.098 ± 0.00 k	0.201 ± 0.00 hi
Red Boscop	21.93 ± 0.14 d-i	15.37 ± 0.19 efg	15.07 ± 0.05 d	0.470 ± 0.00 a	0.765 ± 0.00 a
Melrose	18.26 ± 0.16 mno	12.34 ± 0.07 l	12.02 ± 0.02 r	0.058 ± 0.00 o	0.211 ± 0.00 gh
Granny Smith	23.31 ± 0.10 cd	15.32 ± 0.33 efg	14.12 ± 0.05 ij	0.254 ± 0.00 c	0.441 ± 0.00 b
Idared	17.64 ± 0.07 no	13.37 ± 0.26 k	12.07 ± 0.05 r	0.081 ± 0.00 m	0.213 ± 0.00 gh
Budimka	24.56 ± 0.09 c	16.32 ± 0.18 d	14.53 ± 0.05 f	0.149 ± 0.00 g	0.336 ± 0.00 c
Pink Lady	23.00 ± 0.27 cde	18.07 ± 0.17 c	14.33 ± 0.05 g	0.221 ± 0.00 d	0.465 ± 0.00 b
Average	21.79 ± 0.28 A	15.49 ± 0.16 B	14.32 ± 0.06	0.139 ± 0.00 B	0.247 ± 0.00 A

Different small letters in the same column indicate significant differences according to LSD test ($p \leq 0.05$).
Different capital letters in the adjacent columns in the last row indicate significant differences between peel and flesh at $p \leq 0.05$, based on LSD test.

acidity are suitable for juice production and the diet of peoples with diabetes.

The fruit SSC is a critical factor in determining fruit quality. Moreover, SSC is a good indicator of sugar content (accounting for 65%–80%) and presumably of sweetness (Brady, 1993). This compound, determined in whole fruits, significantly varied among cultivars with average value of 14.32°Brix for all (Table 2). The highest value had 'Topred', followed by 'Braeburn', 'Golden Delicious', and 'Red Boscop'. The lowest and quite similar values were found in 'Melrose' and 'Idared'. The variability of SSC in peel + flesh observed among cultivars has already been described in the literature. As it has previously been observed, SSC in apples varied from 11.0% to 15.5% (Campeanu et al., 2009), 10.8% to 16.5% (Nour et al., 2010), 10.56% to 13.00% (Mehdi et al., 2020), and 10.13% to 24.03% (Mureşan et al., 2022). Nour et al. (2010) reported that 'Red Boscop' was the cultivar with the highest SSC which partially support our result for this apple. In a study of Leccese et al. (2009), 'Golden Delicious' clone B contained 13.7% soluble solids which was lower than those obtained in our work, whereas De Salvador et al. (2006) reported higher SSC in 'Golden

Delicious' than in 'Red Chief' which was not the case in our trial. These similarities and/or discrepancies with results of other scientists can be connected with cultural practices, crop load, rootstocks, maturity stage, and fruit position on the canopy. Otherwise, SSC in different apple cultivars grown in the world ranged from 8.5 to 17.0 °Brix (Vieira et al., 2009; Mehdi et al., 2020). Our research outcomes showed conformance with those of previous studies.

Apple peel had significantly higher pH juice value than flesh (Table 3). Moreover, differences were observed within cultivars. Juice pH values were the highest in both peel and flesh in fruits of 'Hired' and the lowest in 'Red Boscop'. The *cv*. 'Pink Lady' had a similar value of pH juice in flesh as 'Red Boscop'. Mehdi et al. (2020) reported pH juice in six apples between 3.50 and 3.89. According to the same authors, 'Red Delicious' had a higher pH juice value compared to 'Golden Delicious', which was confirmed by our results. Previous studied showed that pH values of different apple varieties ranging from 3.55 to 4.27 (Vieira et al., 2010) or between 2.9 and 3.4 (Wicklund et al., 2021). In general, our results are in good harmony with the data provided by the above mentioned authors.

Table 3. Average pH juice and ash content in peel and flesh of 25 apple cultivars. Data are the mean ± SE for 2 consecutive years.

Cultivar	pH juice		Ash content (%)	
	Peel	Flesh	Peel	Flesh
Akane	4.06 ± 0.02 k	3.56 ± 0.01 fg	5.73 ± 0.12 a	4.67 ± 0.09 b
Ginger Gold	3.91 ± 0.01 n	3.87 ± 0.00 cde	5.81 ± 0.04 a	5.48 ± 0.13 a
Jonathan Watson	3.43 ± 0.01 q	3.28 ± 0.00 ij	1.21 ± 0.03 ijk	1.22 ± 0.03 lm
Galaxy	3.99 ± 0.01 l	3.74 ± 0.01 ef	1.16 ± 0.02 jk	1.24 ± 0.02 klm
Gala Mondial	4.18 ± 0.01 i	3.72 ± 0.02 ef	0.81 ± 0.01 m	0.76 ± 0.02 op
Golden Delicious	3.55 ± 0.02 p	3.42 ± 0.01 ghi	0.72 ± 0.01 n	0.35 ± 0.01 r
Hired	4.88 ± 0.01 a	4.77 ± 0.02 a	0.61 ± 0.01 o	1.16 ± 0.04 m
Fuji	4.15 ± 0.02 j	3.68 ± 0.03 efg	2.05 ± 0.01 de	3.92 ± 0.10 d
Golden Reinders	4.84 ± 0.01 b	3.74 ± 0.01 ef	1.13 ± 0.03 k	0.85 ± 0.02 no
Braeburn	4.45 ± 0.02 e	3.85 ± 0.02 de	0.74 ± 0.01 mn	1.98 ± 0.05 f
Red Chief	4.43 ± 0.01 e	4.12 ± 0.01 c	0.90 ± 0.01 l	0.95 ± 0.02 n
Starking	4.85 ± 0.02 b	4.50 ± 0.01 b	0.50 ± 0.01 p	0.56 ± 0.04 q
Morens Jonagored	3.96 ± 0.01 m	3.55 ± 0.02 fg	1.51 ± 0.02 gh	1.35 ± 0.07 jkl
Topred	4.38 ± 0.01 f	3.88 ± 0.01 cde	1.20 ± 0.05 jk	1.75 ± 0.05 g
Delbar Jubileum	3.64 ± 0.01 o	3.05 ± 0.01 jk	1.29 ± 0.04 i	0.89 ± 0.03 no
Unknown	4.01 ± 0.02 l	3.50 ± 0.45 fgh	1.97 ± 0.02 e	3.20 ± 0.20 e
Richared	4.21 ± 0.02 h	3.83 ± 0.02 e	1.23 ± 0.04 ij	1.39 ± 0.01 ijk
Hapke	4.57 ± 0.01 d	4.11 ± 0.01 cd	0.59 ± 0.02 o	0.68 ± 0.03 pq
Stayman Winesap	4.06 ± 0.01 k	3.73 ± 0.01 ef	1.43 ± 0.02 h	1.47 ± 0.02 hij
Red Boscop	3.24 ± 0.01 s	2.92 ± 0.01 k	3.90 ± 0.11 c	4.19 ± 0.13 c
Melrose	4.30 ± 0.02 g	3.23 ± 0.01 ij	1.56 ± 0.04 g	1.47 ± 0.09 hij
Granny Smith	3.95 ± 0.01 m	3.13 ± 0.01 jk	4.41 ± 0.05 b	4.16 ± 0.21 c
Idared	4.78 ± 0.01 c	3.73 ± 0.01 ef	0.82 ± 0.01 lm	0.57 ± 0.06 q
Budimka	4.22 ± 0.01 h	3.64 ± 0.01 efg	1.88 ± 0.03 f	1.55 ± 0.01 hi
Pink Lady	3.28 ± 0.02 r	2.94 ± 0.02 k	2.09 ± 0.06 d	1.61 ± 0.02 gh
Average	4.13 ± 0.01 A	3.66 ± 0.03 B	1.81 ± 0.03 A	1.90 ± 0.06 A

Different small letters in the same column indicate significant differences according to LSD test ($p \leq 0.05$).
Different capital letters in the adjacent columns in the last row indicate significant differences between peel and flesh at $p \leq 0.05$, based on LSD test.

Apples are considered a good source of ash i.e. dietary minerals (Nour et al., 2010; Francini et al., 2022). The peel contained lower ash content than flesh, but differences were not significant (Table 3). However, differences among and within cultivars were very pronounced and significant in both peel and flesh tissues. In peel tissue, 'Ginger Gold' and 'Akane' had statistically similar and the highest ash content, whereas the lowest was found in 'Starking'. The above mentioned two cultivars had approximately 11.5 times higher ash content compared to 'Starking'. In flesh, 'Ginger Gold' as a summer and nonred cultivar also had the highest ash content, whereas the lowest had 'Golden Delicious'. Mehdi et al. (2020) reported that ash content ranged from 0.35% to 2.92%. In work of these authors, 'Golden Delicious' had lower ash amount than 'Red Delicious' which was confirmed by data in our trial for some members of the 'Red Delicious' family such as 'Red Chief', 'Topred', and 'Richared'. In a study of Mureşan et al. (2022), differences in ash content of 22 apple cultivars were visible but were not significant although varied from 1.47% to 3.38%. In this work, ash amounts of whole fruits of 'Golden Delicious' and 'Akane' were 2.91% and 3.09%, respectively, which is a much higher value for 'Golden Delicious' but much lower for 'Akane' in comparison with our results. Similar tendencies were obtained by Campeanu et al. (2009).

3.3. Evaluation of phenolic compounds and total antioxidant capacity

The redness of apple peel is due to the accumulation of anthocyanins, which are water-soluble plant pigments responsible for the blue, purple, and red colors in many plant tissues of fruits, flowers, and vegetables (Scalzo et al., 2005). Table 4 lists the results for TAc in the studied fruits. This compound in peel was the greatest in summer matured and intensively red-colored 'Akane', followed by 'Jonathan Watson', 'Galaxy', 'Red Chief', and 'Idared', also with red-colored peel. The lowest content was observed in slightly bicolored fruits of 'Red Boscop'. In apples with yellow and green skin such as 'Ginger Gold', 'Golden Delicious', 'Golden Reinders', 'Granny Smith', and 'Budimka', anthocyanins were not detected. They were also not detected in flesh in our study due to this tissue of evaluated cultivars have more or less bright coloration which is in agreement with previous work on apple (Drogoudi et al., 2008), although recent studies showed that white-flesh colored apples contain anthocyanidins (Giomaro et al., 2014). For these reasons, red-flesh colored apples in study of Honda and Moriya (2018) have attracted increasing attention and redder fruits are more marketable. Recent studies have shown that the apple cultivar type may substantially influence the fruit TAc, followed by harvest stage, skin color, cultural practice (pruning, thinning, fertilization), plant hormones and environmental factors, especially temperature and sunlight irradiation (Scalzo et al., 2005; Azuma et al., 2012; Honda and Moriya, 2018). Merzlyak et al. (2002) reported that anthocyanins in whole fruits of 'Granny Smith' were small; however, in our study they were not detected. Giomaro et al. (2014) reported that the peel of purple red colored apple *cv*. 'Pelingo' contained 124.60 μg g^{-1} anthocyanins on fresh weight basis, whereas Oszmiański et al. (2018) found that average TAc in whole fruits of 22 old apple cultivars ranged from 0 to 133.90 mg 100 g^{-1} on dry weight basis. In a study by Katiyo et al. (2018), red-fleshed *M. pumila* Niedzwetzkyana Dieck contained 195.45 mg kg^{-1} in peel and 84.28 mg kg^{-1} in flesh. The results in our study and data of previous authors confirm the fact that the peel tissue is the fruit portion with the highest bioactivity. These authors also reported that cultivars are crucial factor determined content of this pigment.

Apart from the health-promoting role for the human body, phenolic compounds are important antifungal substances (Schovánková and Opatová, 2011). In the present study, the TPC, TFC, and antioxidant activity were 2.3-, 3.1-, and 1.9-fold higher in the peel than in the flesh (Table 4), which is in agreement with previous studies on apple (Drogoudi et al., 2008; Leccese et al., 2009; Milošević et al., 2018, 2019ab; Butkeviciute et al., 2022; Francini et al., 2022). Chinnici et al. (2004) noted that the total antioxidant capacity of peels were about 2.5 times higher than those found in flesh, probably due to the higher content of polyphenols in the skin.

As regards TPC and TFC, the lowest values in both peel and flesh were found in 'Ginger Gold'. This cultivar also had the lowest DPPH values in both peel and flesh. Interestingly, the old local (autochthonous) Serbian cultivar 'Budimka' had the highest content of total phenols and flavonoids, suggesting its optimal suitability for phenolic extraction among the analyzed apple cultivars (Persic et al., 2017). 'Budimka' has a yellow-greenish ground skin color, which is slightly covered with over-ground red color on the sun-exposed side of the fruit. Oszmiański et al. (2018) also reported that old apples had higher amount of phenolic compounds than newly breed and commercial cultivars. According to these authors, TPC in 22 old apples varies from 1348.40 to 4310.52 mg 100 g^{-1} on dw basis. Similar tendencies of old cultivars *versus* new cultivars were reported previously by Wojdyło et al. (2008), Vieira et al. (2009), and Panzella et al. (2013). In a study of Drogoudi et al. (2008), the TPC in the peel ranged from 8.4 to 19.09 mg GAE g^{-1} dw, whereas in the flesh these values ranged from 3.5 to 9.8 mg GAE g^{-1} dw. In their work, 'Golden Delicious' and 'Granny Smith' also had small amounts of total phenolic compounds, whereas the highest was found in old local Greece cultivar 'Fyriki' with yellowish-green skin color and some reddish patches and

Table 4. Average total anthocyanin, total phenolic, and total flavonoid contents and antioxidant capacity in peel and flesh of 25 apple cultivars. Data are the mean ± SE for 2 consecutive years.

Cultivar	Total anthocyanin content (mg ME 100 g^{-1})	Total phenolic content (mg GAE 100 g^{-1})		Total flavonoid content (mg RUE 100 g^{-1})		DPPH (μmol TE 100 g^{-1})	
		Peel	Flesh	Peel	Flesh	Peel	Flesh
Akane	29.23 ± 0.18 a	357.50 ± 0.54 b	109.26 ± 2.41 h	205.41 ± 0.93 b	53.42 ± 0.12 g	420.52 ± 0.27 g	208.47 ± 1.47 h
Ginger Gold	nd	72.50 ± 0.05 r	9.17 ± 0.13 p	59.31 ± 0.44 u	6.40 ± 0.10 p	159.60 ± 4.41 u	53.76 ± 2.57 v
Jonathan Watson	24.26 ± 0.37 b	227.65 ± 1.05 j	57.04 ± 1.41 o	83.38 ± 0.18 s	28.46 ± 0.33 m	293.02 ± 2.06 q	118.98 ± 4.04 t
Galaxy	13.98 ± 0.16 c	273.17 ± 2.20 g	91.95 ± 1.99 jk	145.13 ± 1.01 h	39.11 ± 0.87 j	402.81 ± 2.11 i	156.79 ± 0.59 n
Gala Mondial	4.90 ± 0.33 j	249.35 ± 2.39 h	64.63 ± 1.06 n	141.29 ± 0.90 i	17.95 ± 0.46 o	345.47 ± 0.48 m	103.30 ± 0.40 u
Golden Delicious	nd	204.91 ± 2.82 k	65.87 ± 1.36 n	129.48 ± 0.89 k	27.10 ± 0.42 n	312.30 ± 0.42 n	134.09 ± 0.67 q
Hired	8.66 ± 0.02 f	283.27 ± 1.71 f	110.04 ± 1.58 h	167.13 ± 1.01 d	44.42 ± 0.37 i	432.23 ± 0.09 d	240.62 ± 3.91 f
Fuji	3.35 ± 0.18 l	243.46 ± 2.32 i	94.02 ± 1.86 j	148.39 ± 0.52 g	48.88 ± 1.01 h	423.62 ± 0.27 f	198.10 ± 0.15 j
Golden Reinders	nd	150.37 ± 1.31 q	83.98 ± 0.78 l	78.91 ± 0.68 t	30.39 ± 0.62 l	277.91 ± 0.10 r	152.83 ± 1.08 o
Braeburn	6.17 ± 0.08 i	274.74 ± 1.41 g	135.63 ± 1.56 d	164.06 ± 1.49 e	64.73 ± 0.44 c	435.68 ± 0.24 s	305.33 ± 0.19 a
Red Chief	11.79 ± 0.07 d	326.06 ± 2.77 c	100.14 ± 2.96 i	194.04 ± 1.36 c	38.13 ± 0.43 j	446.22 ± 0.16 a	208.47 ± 0.08 h
Starking	6.25 ± 0.10 i	240.45 ± 1.11 i	90.53 ± 1.28 k	133.01 ± 0.61 j	34.88 ± 0.59 k	418.58 ± 0.18 h	203.58 ± 0.64 i
Morens Jonagored	4.13 ± 0.13 k	320.79 ± 1.77 d	115.93 ± 0.80 g	164.47 ± 0.68 e	35.41 ± 0.47 k	423.31 ± 0.12 f	190.52 ± 1.33 l
Topred	8.04 ± 0.14 gh	251.77 ± 1.26 h	127.51 ± 1.01 e	145.46 ± 1.01 h	61.65 ± 0.84 d	441.02 ± 0.20 bc	268.20 ± 0.79 d
Delbar Jubileum	2.80 ± 0.13 m	166.49 ± 0.83 p	94.48 ± 0.34 j	79.22 ± 0.64 t	30.95 ± 0.50 l	246.20 ± 0.76 s	145.68 ± 2.49 p
Unknown	2.78 ± 0.13 m	175.67 ± 1.32 o	120.96 ± 1.10 f	85.99 ± 0.83 r	38.18 ± 0.85 j	277.46 ± 1.42 r	189.40 ± 0.82 l
Richared	7.81 ± 0.43 h	230.83 ± 1.58 j	136.29 ± 1.25 d	125.71 ± 1.01 l	68.02 ± 0.74 b	378.50 ± 1.10 j	284.38 ± 2.16 c
Hapke	8.18 ± 0.05 g	171.97 ± 2.18 o	146.42 ± 1.06 b	93.32 ± 0.91 q	57.54 ± 1.27 f	371.78 ± 1.15 k	299.77 ± 1.29 b
Stayman Winesap	4.81 ± 0.03 j	195.08 ± 0.64 l	71.08 ± 1.06 m	130.38 ± 0.68 k	26.70 ± 0.36 n	298.98 ± 1.15 p	121.54 ± 1.10 s
Red Boscop	1.76 ± 0.05 o	181.52 ± 0.94 n	110.87 ± 0.68 h	99.05 ± 1.87 p	59.39 ± 1.54 e	238.83 ± 1.02 t	180.11 ± 0.79 m
Melrose	2.30 ± 0.02 n	203.70 ± 1.53 k	110.83 ± 0.46 h	105.05 ± 0.80 o	48.41 ± 0.52 h	367.27 ± 0.35 l	193.19 ± 1.04 k
Granny Smith	nd	187.04 ± 1.03 m	140.24 ± 1.17 c	117.71 ± 1.85 m	68.30 ± 0.77 b	304.73 ± 0.68 o	223.69 ± 0.68 g
Idared	11.22 ± 0.47 e	296.04 ± 1.49 e	118.35 ± 0.50 fg	161.79 ± 1.86 f	45.08 ± 0.09 i	428.43 ± 0.15 e	204.92 ± 1.08 i
Budimka	nd	435.53 ± 2.21 a	178.91 ± 1.47 a	263.02 ± 1.72 a	73.23 ± 0.70 a	442.07 ± 0.53 b	264.48 ± 0.84 e
Pink Lady	4.20 ± 0.07 k	186.67 ± 0.87 m	92.66 ± 0.46 jk	111.90 ± 0.90 n	30.61 ± 0.36 l	276.48 ± 1.10 r	132.66 ± 0.85 r
Average	8.62 ± 0.16	236.27 ± 1.49 A	103.07 ± 1.19 B	133.30 ± 0.99 A	43.09 ± 0.59 B	354.22 ± 0.82 A	191.31 ± 1.24 B

nd: not detected

Different small letters in the same column indicate significant differences according to LSD test ($p \leq 0.05$).

Different capital letters in the adjacent columns in the last row indicate significant differences between peel and flesh at $p \leq 0.05$, based on LSD test.

red-colored 'Starkrimson'. In flesh of several apple cultivars investigated by Leccese et al. (2009), TPC ranged from 0.3 to 0.84 mg GAE g^{-1} whereas in the peel, it ranged from 1.72 to 3.75 mg GAE g^{-1} fw. Butkeviciute et al. (2022) noted values from 3380 to 6434 μg g^{-1} in peel and from 1278 to 3715 μg g^{-1} in flesh depending of the rootstock, whereas Mehdi et al. (2020) reported values between 151.27 and 203.03 mg GAE 100 g^{-1} with very small to small amount found in 'Golden Delicious' and 'Golden Delicious Hard' which confirm the results of our study.

Regarding TFC, besides 'Ginger Gold', low TFC in both peel and flesh was found in yellowish 'Golden Reinders', bi-colored 'Delbar Jubileum', 'Unknown', and 'Red Boscop' and quite unexpected in red-skinned 'Jonathan Watson', 'Starking', 'Hapke', and 'Stayman Winesap' (Table 4). Other authors also reported high variability of this compound among cultivars and between fruit tissues (Boyer and Liu, 2003/04; Agnolet et al., 2015). These authors also reported that, for example, yellowish and green colored apples such as 'Golden Delicious' and 'Granny Smith' had the lowest TFC in comparison with red-colored 'Red Delicious' which is the case in our experiment. Similar tendencies were noted earlier by Escarpa and Gonzalez (1998), Eberhardt et al. (2000) and recently by Milošević et al. (2019a) and Butkeviciute et al. (2022). In our earlier study on apple, TFC of 'Red Chief' depending on the rootstock varied from 35.14 to 57.43 mg RUE 100 g^{-1} dw in flesh and from 48.34 to 77.42 mg RUE 100 g^{-1} dw in peel (Milošević et al., 2018). Earlier, Wolfe et al. (2003) reported that 'Rome Beauty' (306.1 mg CAE 100 g^{-1}) and 'Idared' (303.2 mg CAE 100 g^{-1}) contained higher TFC in peel in comparison with 'Cortland' and 'Golden Delicious'. Similar results were found in work of Katiyo et al. (2018) who reported TFC in the peel of 1544.50 mg RUE kg^{-1} and 1156.62 mg RUE kg^{-1} in flesh. Data in Table 4 revealed that peel of 'Red Chief' had the highest antioxidant activity, followed by 'Budimka', 'Topred', 'Hired', and 'Idared'. Interestingly, other members of 'Red Delicious' group i.e. dark red-skinned clones had high antioxidant power. In the flesh, the highest DPPH value was found in 'Braeburn', followed by 'Hapke', 'Richared', and 'Topred'. These data are in high harmony with our earlier study on apple (Milošević et al., 2018, 2019a). A similar trend in the DPPH of both peel and flesh was found among the studied cultivars by Drogoudi et al. (2008), who reported that peel of 'Starkrimson' had the highest antioxidant capacity whereas the lowest were found in 'Golden Delicious' and 'Granny Smith', respectively. Wolfe et al. (2003) and Agnolet et al. (2015) also reported that red-skinned and old apples had better antioxidant potential than yellow-skinned. Leccese et al. (2009) reported values in the peel of several apples from 17.5 to 41 μmol TE g^{-1} and in the flesh from 3.5 to 6.0 μmol TE g^{-1} fw, whereas Al Daccache et al. (2020) noted DPPH free radical scavenging activity values between 93.15% and 95.97%. In a study of Vieira et al. (2009), antioxidant capacity values in peel of several apple cultivars ranged from 335 to 739 μmol TEAC 100 g^{-1} fw, whereas Katiyo et al. (2018) reported DPPH values in both peel and flesh between 2266.28 and 883.60 mg kg^{-1} (vitamin C equivalent). All mentioned authors suggested that genotype is the main factor that determines the composition of bioactive compounds in apples which confirmed our data. However, comparison of the TPC, TFC, and DPPH values obtained in our work with those of other studies suggests similar results and tendency although differences in the units reported, methodology used, and spectophotometric standards employed make a direct comparison difficult.

3.4. Correlations among variables and segregation of apples using PCA

In general, correlation analysis helps to determine effective traits in order for indirect selection superior genotypes. Data from Figure 2 revealed that FW significantly and positively correlated with both L and D and negatively with TAc. Fruit L positively correlated with D, but negatively with φ, R_a, and TAc. Fruit D positively correlated with TA in flesh, and negatively with flesh pH juice and TAc suggesting that all three variables can be used to predict each other (Agnolet et al., 2015; Persic et al., 2017; Mureșan et al., 2022). On the other hand, fruits with higher values of D, φ, and R_a increased TA in flesh and decreased pH juice in flesh due to positive or negative correlations between them. The DM in the peel positively correlated with SSC, DM, TPC, TFC, and DPPH values, all in the flesh, indicating that DM in the peel is crucial factor determined these compounds in the flesh. The DM in the flesh only correlated with SSC, which was expected.

The TA in the peel positively correlated with TA in flesh and ash in both peel and flesh, and negatively with pH juice and DPPH, both in the peel. This could suggest that fruits with more acidic peel induced higher acidity and ash contents, and decreased pH juice and antioxidant activity of the peel. The TA in the flesh negatively correlated with pH juice in both peel and flesh. As expected, juice pH in peel positively correlated with pH juice in the flesh and antioxidant capacity in both peel and flesh, suggesting that cultivars with higher pH juice in peel induced better antioxidant power. In addition, pH juice in flesh positively correlated with DPPH values in peel. Ash content in the peel significantly correlated with ash in the flesh and negatively with DPPH values in the peel.

The TPC in the peel positively correlated with TPC in the flesh, TFC, DPPH in both peel and flesh, and TAc. Moreover, TPC in the flesh significantly correlated with above numerated variables with exception of TAc. This could suggest that phenolic compounds in predominantly red-fleshed skin apples contribute significantly to their antioxidant activity, as previously reported by Drogoudi

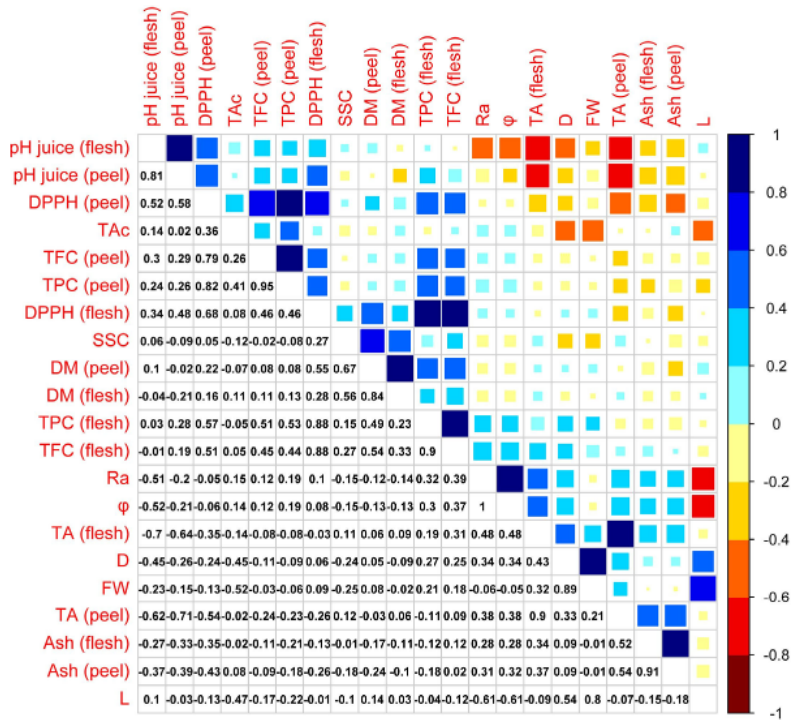

Figure 2. Correlation matrix analysis among marketable apple fruit quality attributes. The size and color intensity of quadrants are proportional to Pearson's correlation coefficient at α = 0.05. Yellow to dark red quadrants indicate negative correlations, whereas blue are positive correlations. In the correlogram scale from −1 to +1, Pearson's correlation coefficient for variables is on the vertical and horizontal axis.

et al. (2008) and Katiyo et al. (2018). The contribution of phenolics to the antioxidant capacity confirmed their important role in the "bioactivity" of apples (Leccese et al., 2009; Milošević et al., 2019a). Hence, the apple cultivars with higher phenolics tended to have higher antioxidant activity and vice versa (Agnolet et al., 2015). Moreover, the TFC both in the peel and flesh significantly and positively correlated with antioxidant activity. Our results are in agreement with data of Wojdyło et al. (2008), Drogoudi et al. (2008), and Katiyo et al. (2018), who found that the strong correlation existed between total polyphenols and antioxidant activity. From a nutritional point of view, the above findings suggest that regular consumption of apples with peel is recommended to enhance the dietary intake of antioxidant compounds. However, the correlations between fruit size and other fruit quality parameters in our study were not clear or were controversial in some cases and are the subject of ongoing discussions (Link, 2000).

PCA is an important statistical tool for analysis of multivariate data which transforms a large number of correlated variables into a smaller number of uncorrelated variables, called principal components with minimal information loss. Other authors have also used PCA to segregation apple cultivars with the best fruit characteristics (Drogoudi et al., 2008; Agnolet et al., 2015; Oszmiański et al., 2018; Wicklund et al., 2021; Mureşan et al., 2022).

In the present study, the first five principal components accounted for 82.91% of the total variation for fruit physicochemical properties and exhibited a very high correlation among them. PC1 explains 27.22% of the total variability (Figure 3) and it is represented by 15 cultivars that correspond to 9 variables. It has large positive loading to juice pH in both peel and flesh, TPC (peel), TFC (peel), DPPH in both peel and flesh and negative loadings to TA (peel), and ash content in both peel and flesh. This component singled out 'Hired', 'Braeburn', and 'Red Chief' as cultivars with higher DPPH activity in both peel and flesh and higher juice pH values. Contrarily, it suggested that *cv*. 'Ginger Gold' had the smallest TPC (peel), TFC (peel), and DPPH in both peel and flesh tissues. PC2 accounted for 21.56% of the variability and was constituted by 5 cultivars that correspond to 5 variables. It is positively correlated with φ, R_a, TA (flesh), TPC (flesh), and TFC (flesh) suggesting that 'Budimka' had the largest values of these parameters, while 'Gala Mondial', 'Golden Reinders', and 'Stayman Winesap' were among cultivars with the smallest of these values. PC3 explains 15.68% and it is represented with 5 cultivars corresponding to 5 variables. This component is in positive correlation with FW, L, D, DM (peel), DM (flesh) and SSC and in negative correlation

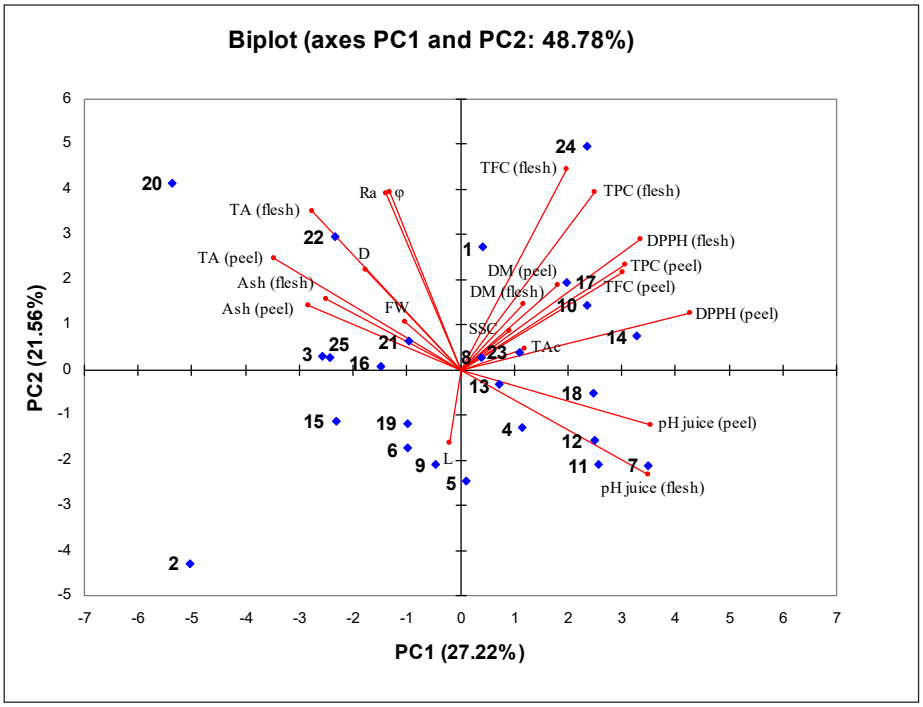

Figure 3. Principal component analysis (PCA) biplot for the 25 studied apple cultivars based on fruit physical properties, chemical composition, and antioxidant activity. The numbers within the biplot indicate the ordinal numbers of the apple cultivars presented in Tables 1–4.

with TAC. PC3 revealed that 'Akane' had the largest TAC but the smallest FW and L, while the 'Morens Jonagored' had the largest FW and L, but relatively small TAC.

4. Conclusion

From the present study, we found large and significant variation in essential physical properties, chemical composition and antioxidant activity among apple cultivars grown under intensive growing technology. A great diversity of fruit shapes and sizes was noticed. Several cultivars, such as 'Morens Jonagored', 'Red Boscop', 'Granny Smith', 'Idared', and 'Delbar Jubileum' can be classified as large fruited genotypes which must be consumed or processed the first due to having a weak storage capacity. Regarding chemical composition, it was suggested that fruits of all apple cultivars would be more beneficial when consumed with their peel, as discarding this part means great beneficial substances loss. Only acidity content was higher in flesh than in peel, whereas ash content was similar in both tissues. Cultivars, i.e. clones from the 'Red Delicious' group, especially 'Topred' and 'Richared', can be associated with extremely high DM and SSC content, whereas summer harvesting 'Ginger Gold' and 'Akane' can be associated with high ash content. 'Budimka' classified as a local (folk, autochthonous) Serbian cultivar showed the best TPC and TFC in both peel and flesh which could compensate for its less acceptable appearance, owing to the presence of attractive fruit shape, ground color and large lenticels on the peel. Moreover, the valuable fruit size and good nutraceutical traits of this apple should be considered in specific breeding programs. 'Red Boscop', alongside 'Jonathan', 'Granny Smith', and 'Pink Lady', was high in acidity and also in bioactive compounds and can be characterized as "sharp" apple cultivars. These cultivars can be used to balance an apple juice mixture. Evaluated apples in this study could be selected with the best antioxidant properties, providing functional food and/or raw material for processing industry to satisfy increasing consumer demands for foods with health-protecting compounds. The correlation and PCA models indicated a clear separation of the cultivars' inner-genus and between unpeeled and peeled fruits inner-cultivar.

Conflict of interest

T. Milošević, N. Milošević, and N. Miletić declare that they have no conflicts of interests.

Ethical approval

Ethics committee approval is not required.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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