Croatian Journal of Food Science and Technology
journal homepage: www.ptfos.unios.hr/cjfst/

Review paper *DOI: 10.17508/CJFST.2022.13.2.17*

Polyphenols of Traditional Apple Varieties - The Overview

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ARTICLE INFO

Article history:
Received: March 19, 2021
Accepted: May 28, 2021

Keywords:
polyphenols
traditional apple varieties
antioxidant activity

ABSTRACT

Apples are one of the most popular foods around the world. However, recently there has been a growing interest in the preservation of traditional apple varieties. This interest has grown due to the studies that suggest that traditional apples contain higher amounts of polyphenolics and antioxidant activity compared to commercial ones. Polyphenolics in apples have gained much attention because of their beneficial effects on human health, and thus they became a quality trait of apples. Traditional apple varieties have the same groups of polyphenols as commercial ones and those are flavan-3-ols, phenolic acids, flavonols, dihydrochalcones, and anthocyanins. However, traditional apple varieties proved to be rich in some individual polyphenolics, such as procyanidins B1, B2, A2, epicatechin, quercetin-3-rutinoside, chlorogenic acid, etc. This chapter is underlying the great potential of traditional apple varieties as a source of some individual polyphenolics and natural antioxidants.

Introduction

Apples are the most widespread fruit, numbering over 8000 different varieties. Even though apples have been grown for centuries, the existing apple varieties were developed from *Malus sieversii (Ledeb.) Roem*. *Malus sieversii* is a wild apple that can be found in Central Asia. The nomadic lifestyle of the people living there contributed to the spread of the apple tree to other countries (Luby, 2003). Despite a large number of apple varieties on the European market, their range is significantly reduced to no more than 12 varieties. Growing only a few varieties of apples could lead to a reduction in biodiversity (Lončarić et al., 2014). Due to the diversity of pomological properties, fruit quality, and resistance to adverse abiotic and biotic factors, it is important to preserve traditional apple varieties as a source of genetic variability and as a factor of biodiversity in the area in which they grow. Many traditional apple varieties carry genes that provide resistance to pests and diseases, drought resistance, winter endurance, and unique fruit quality. Preservation of old varieties is possible by restoring existing trees or by planting new seedlings from the same varieties (Skenderović Babobjelić, 2015). Recent scientific research suggests that traditional apple varieties contain more polyphenols and antioxidant activity than commercial ones (Jakobek et al., 2015). It was confirmed that the regular consumption of apples contributes to the intake of polyphenols in a significant way (Arts et al., 2000; Hertog et al., 1992). Polyphenols have several positive effects, such as anticancerogenic properties and beneficial effects in cases of cardiovascular diseases, regulation of plasma cholesterol metabolism, antiviral properties, inhibition of *Helicobacter pylori* growth, and staphylococcal enterotoxin A toxicity (Jakobek and Barron, 2015; Hyson, 2011; Valdenergo et al., 2010). Lončarić et al. (2014) reported that traditional apple varieties are mainly grown in some orchards, mainly in peripheral areas, and that they show good adaptability to the local environment and are a valuable source for genetic variability crops. Traditional apples are not represented on the global market, mainly because they do not meet the market standards (Ceymann et al., 2012). Their fruits may

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not look aesthetically perfect compared to the commercial ones, but they are generally characterized by unusual pomological features and by different fullness or taste, sugar and acid ratio, and some varieties have an especially pronounced fruit aroma. One of the reasons may be that most traditional varieties have a balanced composition of nutrients and bioactive substances (Feliciano et al., 2010).

On the other hand, there is a growing demand for functional foods derived from traditional plant sources, as they are a very valuable source of health promoting bioactive compounds. New knowledge and research into traditional apple varieties would open the market for this fruit, and its products should prevent the loss of this valuable genetic material and contribute to greater biodiversity, in order to promote health and general well-being (Lončarić et al., 2019).

Apple

Chemical composition

Consumers today demand value-added foods (e.g., functional foods) that are sustainably produced and processed, considered safe, are natural and fresh, and have significant nutritional value (Putnik et al., 2018). Francini et al. (2013) concluded that apples have a great potential for the production of functional foods. The chemical composition of apples is extremely complex as it is rich in many nutrients, and thus apples are considered a major source of phytochemicals in the human diet. As a confirmation, Scalbert et al. (2000) discovered several phenolic compounds in apples, specifically (+)- catechin and (-)- epicatechin (flavan-3-ols or flavanols), quercetins (flavonols), cyanidin-3-O-galactosides (anthocyanins), phloridzin (dihydrocalcone glycosides), and hydroxycinnamic acids (chlorogenic acid and *p*-coumaroylquinic acid). Furthermore, Eisele and Drake (2005) analysed 175 traditional apple varieties developed from scion wood collected from twelve countries and several geographical areas in the USA. They found that juices from many of the varieties were high in potassium and malic acid. Phenol content between the various apple sorts was highly variable, with some juices containing little, if any, phenolic compounds. Chlorogenic acid and phloridzin were detected in all varietal samples. Apple fruits contain an average of 85% water, 0.26% protein, 14% carbohydrates, 2.4% plant fibre, citric, malic acid, flavonoids, and aromatic substances. The energy value of 100 grams of apples is about 218 kJ (52 kcal) (USDA, 2018). They also contain vitamins such as carotenoids, B vitamins, vitamin C and vitamin E, and minerals such as potassium, calcium, magnesium, phosphorus, and iron. The chemical composition of apples is shown in Table 1.

1.1 Commercial apple varieties

In recent decades, new varieties of apples such as ´Braeburn´, ´Elstar´, ´Golden Delicious´, ´Granny Smith´, and ´Jonagold´ led to a reduction in the cultivation of old varieties. The quality traits of

Table 1. Nutritional value of apple fruits per 100g edible parts (USDA, 2018)
IU- international unit; DFE- dietary folate equivalent; RAE- Retinol Activity Equivalents

Nutrients	Unit	Amount	Nutrients	Unit	Amount
Water	g	85.56	Vitamin C (Ascorbic acid)	mg	4.6
Energy	kcal	52.0	Thiamin	mg	0.017
Protein	g	0.26	Riboflavin	mg	0.026
Carbohydrate	g	13.81	Niacin	mg	0.091
Dietary fiber	g	2.4	Vitamin B6	mg	0.041
Total sugar	g	10.39	Folate, DFE	µg	3.0
Calcium	mg	6.0	Vitamin A RAE	µg	3.0
Iron	mg	0.12	Vitamin A IU	IU	54.0
Magnesium	mg	5.0	Vitamin E (α tocopherol)	mg	0.18
Phosphorus	mg	11.0	Vitamin K (phylloquinone)	µg	2.2
Potassium	mg	107.0	Total lipids (Fat)	g	0.17
Sodium	mg	1.0	Saturated fatty acids	g	0.028
Zinc	mg	0.04	Mono unsaturated fatty acids	g	0.007

commercial apples grown in large quantities such as ´Fuji´, ´Golden Delicious´, ´Gala´, and ´Idared´ are well known, and according to consumer acceptance and demands, crunchiness, firmness, and sweetness are highlighted as important quality traits of apples (Harker et al., 2008; Symoneaux et al., 2012). On the other hand, traditional varieties are not well known, have been neglected, and are grown only in limited quantities, mostly in small orchards (Lo Piccolo et al., 2019). Commercial apple varieties have a lower polyphenol content than traditional apple varieties. The content of polyphenolic compounds was reduced by growing new varieties, due to rapid enzymatic browning and bitter taste. It is assumed that this is the reason why the content of polyphenols and AOC in old apple varieties is higher than in new ones (Kschonsek et al., 2018).

Traditional apple varieties

Traditional, old apple varieties are the precious wealth and natural heritage of each country, and they are significant due to economic, agronomic, and health reasons (Skenderović Babobjelić, 2015). Skenderović Babojelić et al. (2015) stated that traditional apple varieties do not look like first-class fruit that we can find in stores. This is the primary reason why such apples are not the first choice for consumers, which is confirmed by Hoehn et al. (2003), who found that consumers are primarily interested in the colour and size of the fruit when they are choosing an apple. Moreover, other properties are less important to them. However, traditional apple varieties are distinguished by their especially expressed aroma and different fullness of taste (Skenderović Babobjelić, 2015).
Some of the old cultivars analysed by Skenderović Babojelić et al. (2015) are: ´Roter Pogatscher´ (´Božićnica´), ´Yellow Bellflower´ (´Lijepocvjetka´), ´Großer Rheinischer Bohnapfel´ (´Bobovec´), etc. In that research, they concluded that acids have an important role in fruit, as they can slow down the harmful effects of bacteria, degradation of ingredients, and spoilage. Jakobek and Barron (2015) analysed the flesh and the peel of traditional apple cultivars from the area of Continental Croatia, with the aim of highlighting and preserving the biological diversity of apple cultivars with greater bioactive potential. They used HPLC (high-performance liquid chromatography) with a diode-array detector (DAD) for the determination of the composition and content of polyphenolic compounds. The results showed that the old cultivars (´Zimnjara´, ´Lještarka´, and ´Adamova Zvijezda´) stood out regarding the amount of polyphenols. The authors concluded that the cultivars differ according to the content of individual polyphenolic groups; some of them are richer in flavan-3-ols, and others in phenolic acids, i.e., cultivars in which flavan-3-ols occupy a larger proportion contain a smaller number of phenolic acids, and vice versa. Therefore, it can be concluded that it is possible to classify old and new apple cultivars based on the predominant proportion of phenolic acids and flavan-3-ols (Ceymann et al., 2012). Old cultivars, when compared to the commercial ones, are more resistant to diseases and pests; they do not require a large number of pesticide applications or intensive care, so they are easily adaptable to organic farming (Hoehn et al., 2003; Purdešova et al., 2017). Unfortunately, old orchards are decaying and causing the loss of older apple cultivars (Vujević et al., 2018). However, consumer demand for traditional ´old´ apples and their products is increasing, as they follow the trend of consuming natural foods, without added pesticides and additives, which may help in the preservation of biodiversity and cultural heritage.

Polyphenols

Polyphenol biosynthesis

Polyphenolic substances form one of the most numerous and widespread groups of compounds in plants, consisting of more than 9000 different compounds (Martens et al., 2005). Their general chemical formula is Ar-OH, wherein Ar is a phenyl, a substituted phenyl or another aryl group. The whole group is named after the basic representative of this sort, the phenol (Figure 1) (Stričević and Sever, 2001). The most important and largest group of polyphenols are flavonoids (Kähkönen et al., 1999, Mattila et al., 2006). Based on numerous studies, it has been established that some of the main nutritional sources of flavonoids are apples, red onions, tea, and red wine. Three subgroups of flavonoids are widespread and dominant in plants. These are flavonols, anthocyanins, and flavan-3-ol (flavanols) (Robards and Antolovich, 1997). Apples contain several major groups of polyphenolic compounds: flavanols (catechin, epicatechin, and procyanidins), phenolic acids (mainly chlorogenic acid), dihydrochalcones (phloretin glycosides), flavonols (quercetin glycosides), and anthocyanins (cyanidin). Furthermore, various studies indicate that a number of variables, such as variety, geographical location, harvest time, and storage conditions, affect the content and the form of polyphenols, and the antioxidant capacity in apples (Kschonsek et al., 2018).

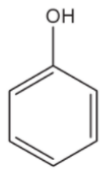

Fig. 1. Chemical structure of phenol (Vermerris and Nicholson, 2006)

Polyphenols are the products of secondary plant metabolism. They are formed from two primary synthetic pathways: the acetate and the shikimate pathways (Lattanzio,2013). Shikimic acid and acetic acid are both derived from glucose metabolism (Ghosh et al., 2012). The active form of acetic acid, acetyl- CoA, and later on the pathway as malonyl- CoA, represents the starting point of fatty acid synthesis in the primary pathway, and also the starting point in the secondary pathway of the synthesis A ring of flavonoids. Aromatic amino acids are products of the primary sciatic pathway, and their degradation leads to the phenylpropanoid pathway, which is a secondary pathway. Furthermore, the phenylpropanoid pathway is omnipresent in higher plants where it forms the nucleus of a series of related pathways leading to a variety of products, including stilbene and flavonoids (Rhodes, 1998). As noted above, by condensing three moles of malonyl- CoA, obtained by glucose metabolism, the A ring was biosynthesized. The C and B ring are also derived from the glucose mechanism in the schikimata and phenylpropanoid pathways, in order to obtain C-9 acids. As CoA is derived from C-9 acids, C-9 acids are also condensed with the C-6 product from malonate to form the C-15 chalcone. Hydration and subsequent ring closure effect flavonoid diversity. **Figure 2** shows their common structure in diphenylpropanes (C_6-C_3-C_6) and consists of two aromatic rings linked through three carbons that usually form an oxygenated heterocycle (Formica and Regelson, 1995). Furthermore, Figure 3 shows a schematic representation of flavonoid biosynthesis.

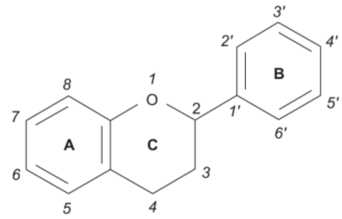

Fig. 2 Basic structure and numbering system of flavonoids (Formica and Regelson, 1995)

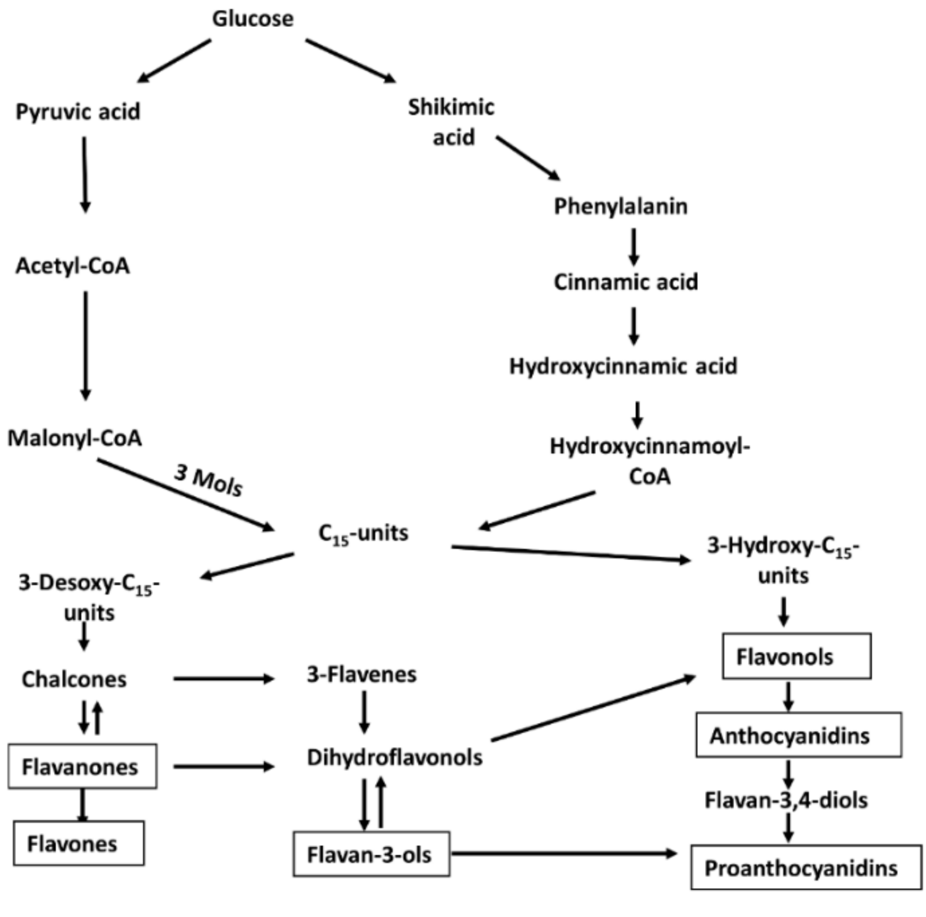

Fig. 3. Schematic outline of the biosynthesis of flavonoids (Wollgast and Anklam, 2000)

Polyphenols in traditional apple varieties

There are three different groups of polyphenolic compounds in the apple peel: dihydrochalcones, flavan-3-ols, and flavanols. On the other hand, apple flesh is rich in chlorogenic acid and the chlorogenic acid isomer (Jakobek et al., 2013). Several main groups of polyphenolic compounds are found in apples: flavanols (catechin, epicatechin, and procyanidins), phenolic acids (mainly chlorogenic acid), dihydrochalcones (phloretin glycosides), flavonols (quercetin glycosides), and anthocyanins (cyanidin). Table 2 shows the result of the determined content of total polyphenols (TPC) and the antioxidant activity (AA) of the examined traditional apple varieties. The results indicate that traditional apple varieties differ from each other in total polyphenol content and antioxidant activity. The highest content of total polyphenols was recorded in the variety ´Božićnica´ (402.12 mg GA/ 100g FW), and the lowest content was in the cultivar ´Lijepocvjetka´ (260.17 mg GA/100 g FW). Furthermore, the highest antioxidant activity was shown by the variety ´Božićnica´ (407.70 mmol trolox/ 100 mL), and the lowest by the variety ´Bobovec´ (Crkvenac M., 2019).

Flavanols

The name for flavanols is often replaced by the term "flavan-3-ols", where hydroxyl is a group that is attached to the C3 atom. Flavanols are also often called catechins, but they do not have the properties of ketones such as flavanonols. Catechins have two epimers, depending on the spatial configuration of the bonds between the B ring and the 2nd site of the carbon atom, and the hydroxyl group on 3. These two epimers, epicatechin- (-), catechin – (+) and their derivates, epigallocatechin and gallocatechins, are commonly categorized as catechins. Gallocatechin and epigallocatechin contain an additional hydroxyl group on the B ring (Hollman and Arts, 2000). In fruit, flavanols are often found in oligomeric or polymeric forms, in the form of proanthocyanidins or tannins, but it is also possible to find free flavanol monomers. Thus, the monomers (+)- catechin, (-)- epicatechin, (+)- galocatechin, and (-)- epigallocatechin are more often found in fruit in free form, and less in the form of glycosides that differ from other flavonoids (Mattila et al., 2006).

Table 2. Proportion of total polyphenols (TPC) and antioxidant activity (AA) of examined traditional varieties of (Crkvenac, 2019.)

Cultivar	TPC (mg GA/100 g FW)	AA (mmol trolox /100 mL)
´Lijepocvjetka´	260.17 ± 0.00	402.37 ± 2.75
´Bobovec´	289.83 ± 3.67	368.01 ± 6.17
´Zlatna Zimska Parmenka´	387.29 ± 6.36	391.97 ± 1.51
´Božićnica´	402.12 ± 1.83	407.70 ± 8.08

Table 3. The content of dihydrohalcones and flavanols (µg/g dw) in the apple peels (adopted from Lončarić et al., 2020)

Commercial	Catechin	Epicatechin	Phloridzin
`Golden Delicious´	n.d.	260.33 ± 3.71	72.88 ± 0.35
`Idared´	n.d.	144.96 ± 1.94	44.15 ± 0.66
`Jonagold´	n.d.	320.39 ± 4.90	69.15 ± 1.51
`Fuji´	27.28 ± 2.28	256.25 ± 8.83	38.22 ± 2.39
`Granny Smith´	47.45 ± 1.17	201.72 ± 5.61	98.35 ± 2.87
Traditional			
`Ljepocvjetka´	n.d.	371.00 ± 22.67	212.86 ± 6.83
`Crveni Boskop´	74.97 ± 1.67	219.52 ± 10.25	77.48 ± 4.50
`Božićnica´	172.39 ± 1.84	504.37 ± 14.17	577.58 ± 8.80
`Apistar´	512.36 ± 12.20	1194.72 ± 21.41	447.79 ± 10.08
`Bobovac´	184.46 ± 1.12	1317.78 ± 10.80	442.42 ± 935

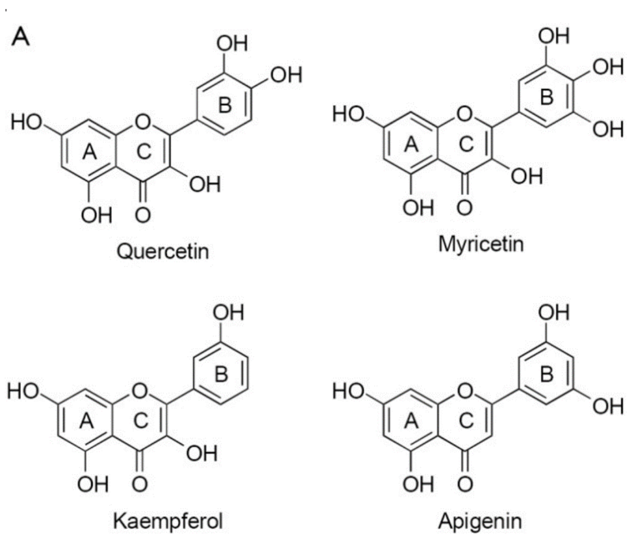

Fig. 4. Chemical structure of basic flavonols (Häkkinen, 2000)

Flavanols (oligomeric procyanidins and catechin) are the major class of apple polyphenols (80%), followed by hydroxycinnamic acids (1-31%), flavonols (2-10%), dihydrochalcones (0.5-5%) and in red apples, anthocyanins (1%) (WojdyŁo et al., 2008). Table 3 shows the content of flavanols and dihydrochalcones in the apple peel from Lončarić et al. (2020). Lončarić et al. (2020) reported that the contribution of epicatechin and catehin in traditional apple varieties was 9.9% of total polyphenols and it was 11.5% in commercial apple varieties. In both varieties, epicatechin was the predominant polyphenol. The varieties `Bobovac´, `Božićnica´, and `Apistar´ had the highest content of epicatechin (1317.78 µg/g dw), phloridzin (577.58 µg/g dw), and catechin (512.36 µg/g dw). Furthermore, Lončarić et al. (2020) showed that traditional apple varieties are dominated by non- flavonoids (chlorogenic acid), while commercial varieties were dominated by flavanols (procyanidins).

Flavonols

Flavonols are characterized by a basic C_6-C_3-C_6 structure (Figure 4). They appear in the cells exclusively as glycosides in which the sugar unit is attached to the C_3 atom and are less common at the position C_7. The most common sugar bound to the flavonol agylocone is glucose. Flavonols can be acylated with various cinnamic or some other phenolic acids (Robards et al., 1999; Hollman and Arts, 2000; Häkkinen, 2000). Flavonol aglycones are quite different in hydroxylation and methylation, so they are also an extremely large subgroup of compounds. WojdyŁo et al. (2008) analysed the phenolic composition of 67 varieties of apple cultivars (new and old varieties). During the research, they concluded that flavonols had the lowest concentration in apples and were fundamentally constituted by quercetin-3-galactoside > 3-rhamnoside > 3-xyloside > 3-arabinoside > 3-glucoside > 3-rutinoside. Dependent upon the genotypes, the proportion of total flavonols varied from 1660.0 mg/kg in the ´Redfree´, to ´Rubinola´ (both are new varieties) having the lowest concentrations of flavonols (80.0 mg/kg) (Table 4). Lončarić et al. (2020) reported that the contribution of flavonols in traditional varieties was only 26.8% and in commercial varieties it was 20.7%, which is lower than reported in other studies, 74% by Jakobek and Baron (2015) and 72.2% by Kschonsek et al. (2018). One of the reasons for the lower amount could be the lower proportion of methanol in the extraction solvent compared to those used in the above studies.

Phenolic acids

Phenolic acids are generally present in nature in various conjugate forms or as esters (Vauzour et al., 2010). They can be divided into hydroxycinnamic and hydroxybenzoic acids (Manach et al., 2004). Hydroxycinnamic acids are present in apples and they account for 1.2% to 31.2%, depending on the apple variety. Furthermore, hydroxycinnamic acid was more dominant in the flesh (40.1%) than in the apple peel (9.3%) (Tsao et al., 2003). The most common hydroxycinnamic acid ester is 5’-Caffeoylquinic acid (CQA) and the second hydroxycinnamic acid was ρ-coumaroylquinic

(PCQ) (Vauzour et al., 2010). The CQA/ PCQ ratio varies depending on the apple variety, ranging from 34.6 to 1146.8 mg/kg dw. This ratio is important when apple fruits are processed into juice and ciders, because CQA is a preferential natural substrate of the catecholase activity of polyphenol oxidase (PPO). Furthermore, PCQ seems to be a competitive inhibitor of enzyme activity (Janovitz-Klapp et al., 1990). These are polyphenolic components with low molecular weight. The basic structure of phenolic acid is shown on Figure 5 (Bravo, 1998). Alonso- Salces et al. (2004) have also observed that the concentration of phenolic acid in cider apples is considerably greater than that found in desert apples. Patrizia et al. (2010) characterized commercial apple varieties as having lower phenolic acid content and antioxidant activity, compared to old varieties.

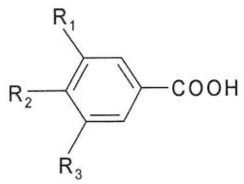

Fig. 5 Basic structure of phenolic acids (Bravo, 1998)

Dihydrochalcones

Dihydrochalcones (DHC) are characteristic polyphenolic compounds in apples and apple products (fruit wine, juice, porridge, bread, etc.). Due to the above facts, the analysis and monitoring of these compounds in apples is recommended for the purpose of identifying counterfeit products (McRae et al.,1990; Amiot et al., 1992; Burda et al.,1998). They are commonly found in apple peel and seeds, where they can represent up to 60% of total polyphenols, compared to apple flesh, where they represent up to 3% of total polyphenols (Guyot et al., 1998). Jakobek et al. (2017) published a study concerning the dihydrochalcones in Croatian traditional apple varieties such as ´Lještarka´, ´Ljetna rebrača´, ´Slavonska srčika´, ´Zimnjara´, and ´Adamova zvijezda´. Dihydrochalcones were identified as phloretin-2´-xyloglucoside and phloretin-2´-glucoside in the flesh, and phloretin-2´-glucoside in the peel. In the studies, dihydrochlacones showed a characteristic UV/Vis absorption at 276 nm that agrees with the literature data (Tsao et al., 2003). In the apple peel, the dihydrochalcone percentage was between 0.8 and 10.8%. Varieties with the highest dihydrochalcone percentage were ´Slavonska srčika´ and ´Adamova zvijezda´. Varieties with lower dihydrochalcone percentage were ´Zimnjara´, ´Lještarka´ and ´Ljetna rebrača´. In the flesh, the dihydrochalcone percentage varied from 3.7 to 12.4%. Varieties that had high dihydrochalcone in the flesh were ´Zimnjara´, ´Slavonska srčika´, ´Lještarka´, and ´Adamova zvijezda´. In conclusion, two varieties with somewhat higher dihydrochalcone percentages in both peel and flesh can be highlighted: ´Slavonska srčika´ and ´Adamova zvijezda´. Moreover, the content of dihydrochalcones can be 4 to 9 times higher in the peel than in the flesh of the apple (Tsao et al., 2003; Khanizadeh et al., 2008). Most people peel an apple, which leads to the loss of dihydrochalcones. Since dihydrochalcones have shown many potential bioactivities and they are studied as a possible drug, their content in natural food sources could be important (Jakobek et al., 2017). WojdyŁo et al. (2008) examined dihydrochalcones in 67 varieties of apple cultivars (new and old varieties). The Macfree variety showed the highest content of dihydrochalcones (434.3 mg/kg dw) and the lowest level was observed for the Topaz variety (49.2 mg/kg dw). Table 4 shows some apple varieties from the WojdyŁo et al. (2008) research, where the varieties Golden Delicious and Jester (commercial apple variety) had the highest content of dihydrochalcones.

Anthocyanins

Anthocyanin is one of the flavonoid compounds and it has a strong antioxidant and anticancer effect, as well as the ability to remove free radicals (Xiang et al., 2016). Apples with red flesh are precious because their flowers, leaves, and fruits are rich in anthocyanin (Zhang et al., 2013). Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of 2- phenylbenzopyrylium cation. They have a C_6-C_3-C_6 skeleton typical for flavonoids (Wallace et al., 2015). The main part of anthocyanin is its aglycone, flavilium cation, which contains conjugated double bonds responsible for light absorption at 500 nm. They are plant pigments that allow flowers, vegetables, and fruits to turn blue, purple, or red. There is six basic anthocyanidins: cyanidin, delfinidine, pelargonidine, peonidine, petunidin, and malvidin (Francis, 1989). Figure 7 shows the chemical structure of 6 basic anthocyanin glucosides. They differ from each other in the number of hydroxyl and methoxyl groups on the B ring of the flavilium cation. Anthocyanins are much more stable and soluble in water than anthocyanidins, due to glycosylation (Rein, 2005). Anthocyanins in apples represent less than 1% of total polyphenols and they are essentially located in the apple peel. Apple anthocyanins are a mix of two different cyanidin glycosides, of which cyanidin-3-galactoside is more common than cyanidin-3-glucoside. The content of cyanidin-3- glucoside and 3-galactoside ranged from 10.0 to 5500.9 mg/kg dw, with the lowest observed concentration in the ´Mutsu´, and the highest concentration the ´Geneva Early´. The results are consistent with what was observed in other studies (Tsao et al., 2003). These apple varieties had red or dark red peel. Furthermore, Lata (2009) demonstrated that the richest sources of anthocyanins were the varieties ´Gloster´, ´Idared´, and ´Starking Delicious´. On the other hand, ´Priscilla´, which had a red coloured peel, showed trace amounts of anthocyanins, much below the average concentration. ´Mutsu´, ´Golden Delicious´, ´Shampion´ and ´Freedom´ have typically green skin and the lowest anthocyanin content from 10 to 30 mg/kg dw. In the ´Shampion´ variety, which is a new apple variety, the content of anthocyanin was 0.0 mg/kg dw, it is assumed that it did not get a red colour of the peel due to the short vegetation in our climatic conditions (Table 4). In conclusion, apples with anthocyanins in the peel often have a mixture of yellow and red colour, but also red, yellow, and green colour. Varieties without anthocyanins are green/yellowish (Jakobek et al., 2020).

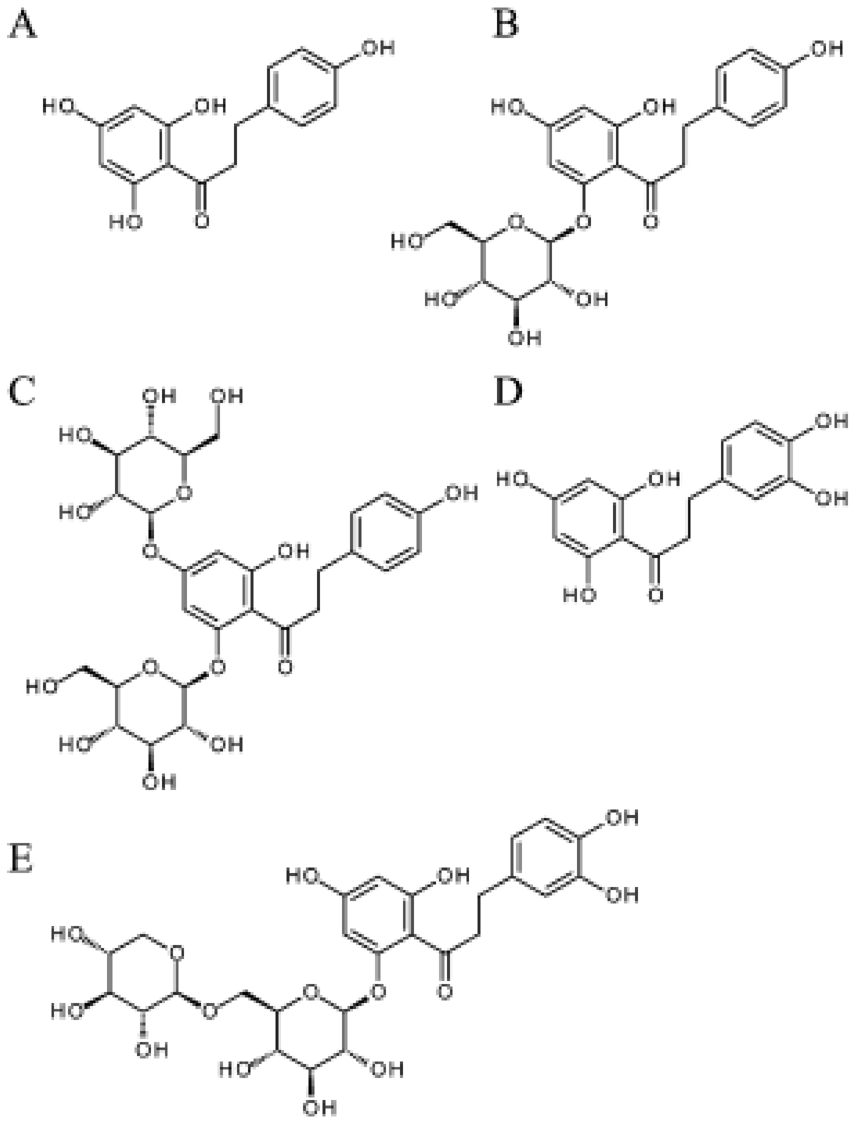

Fig. 6. Structures of apple-derived dihydrochalcones. (A) phloretin; (B) phloridzin; (C) phloretin-2´,4´-di(ß-o-glucoside); (D) 3-OH- phloretin; and 3-OH- phloretin-2´-O-xyloglucoside (Esselen er al., 2013)

Table 4. Dihydrochalcones, Flavonols, and Anthocyanins Content of Apple Varieties (Data Expressed as mg/kg ± Standard Error (n=3) [a] (adopted from WojdyŁo et al., 2008)

	Dihydrochalcones		Flavonols						Anthocyanins		
Commercial	PLXG	PLG	QRUT	QGAL	QGLU	QARA	QXYL	QRHM	CGAL	CGLU	TPC
´Golden Delicious´	7.3.0 ± 0.7	146.8 ± 1.5	7.0 ± 0.2	92.4 ± 1.3	13.2 ± 0.1	18.2 ± 0.1	27.3 ± 0.7	170.1 ± 1.7	1.1 ± 0.1	12.8 ± 0.2	707.1
´Idared´	1.7.2 ± 1.1	114.3 ± 1.4	5.3 ± 0.4	165.9 ± 1.4	18.0 ± 0.5	70.5 ± 0.5	151.9 ± 1.2	90.8 ± 1.2	90.4 ± 1.3	23.5 ± 0.3	1334.3
´Jonagold´	7.7.7 ± 1.7	1354 ± 1.3	4.6 ± 0.0	72.1 ± 1.1	08.9 ± 0.0	27.5 ± 0.5	52.3 ± 1.1	62.3 ± 1.0	36.9 ± 0.2	17.0 ± 0.4	800.4
´Geneva Early´	14.9.4 ± 1.4	13.52 ± 1.3	1.4 ± 0.0	147.4 ± 1.0	97.4 ± 0.2	89.6 ± 1.3	233.6 ± 3.1	109.7 ± 1.0	500.0 ± 2.4	3500.0 ± 3.6	1215.5
´Mutsu´	57.6 ± 1.3	99.3 ± 1.9	18.0 ± 0.4	289.7 ± 2.3	48.0 ± 0.4	73.2 ± 1.5	119.7 ± 1.0	154.7 ± 1.2	1.1 ± 0.0	10.9 ± 1.2	905.0
´Katja´	9.0.9 ± 0.5	60.1 ± 1.1	0.0 ± 0.0	54.1 ± 2.5	9.1 ± 0.1	34.5 ± 0.1	86.9 ± 1.2	21.1 ± 0.5	400.1 ± 2.3	400.0 ± 2.9	709.2
´Shampion´	82.8 ± 1.3	107.4 ± 1.5	0.0 ± 0.0	268.6 ± 1.1	60.2 ± 0.5	77.8 ± 0.4	174.2 ± 1.1	106.8 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	1412.0
´Redfree´	123.9 ± 1.4	107.8 ± 1.3	1.4 ± 0.0	402.7 ± 1.8	37.3 ± 1.5	189.9 ± 1.2	342.1 ± 2.4	692.2 ± 3.4	0.0 ± 0.0	1000.0 ± 3.1	878.3
´Jester´	7.9.4 ± 1.5	166.9 ± 1.5	11.5 ± 0.2	183.3 ± 1.7	23.9 ± 0.2	73.6 ± 0.1	149.5 ± 2.1	99.6 ± 1.0	114.9 ± 0.3	04.2 ± 0.1	1567.4
´Discovery´	14.6.4 ± 1.4	14.92 ± 1.7	0.0 ± 0.0	288.9 ± 2.1	38.6 ± 0.2	89.9 ± 1.1	183.1 ± 2.3	75.2 ± 0.5	1100.0 ± 4.3	800.0 ± 2.5	1370.5
´Freedom´	5.8.9 ± 0.9	10.36 ± 1.0	04.7 ± 0.0	63.9 ± 1.5	06.7 ± 0.1	17.7 ± 0.3	33.3 ± 1.2	160.3 ± 1.2	22.8 ± 0.1	23.0 ± 0.1	976.2
´Rubinola´	39.0 ± 0.3	66.1 ± 1.4	0.7 ± 0.0	8.4 ± 0.0	2.4 ± 0.0	09.6 ± 0.7	14.8 ± 0.8	51.5 ± 1.2	100.0 ± 1.1	100.0 ± 0.0	888.2

PLXG, phloretin 2´- xyloglucose; PLG, phloridzin; QRUT, quercetin 3-rutinoside; QGAL, quercetin 3-galactoside; QGLU, quercetin 3-glucoside; QARA, quercetin 3- arabinoside;QXYL, quercetin 3-xyloside; QRHM, quercetin 3-rhamnoside; CGAL, cyanidin 3-galactoside; CGLU, cyanidin-3-glucoside; TPC – total polyphenol content

Table 5. Positive Correlation between Phenolic Compounds and Antioxidant Activity (WojdyŁo et al., 2008)

Variables	***ABTS**	**DPPH**	**FRAP**
Total of polyphenols	0.871	0.839	0.804
Dihydrochalcones	0.260	0.198	0.256
Flavonols	0.279	0.143	0.175
Anthocyanins	0.146	0.035	0.095
Procyanidins	0.690	0.591	0.633
Hydroxycinnamic acid	0.542	0.636	0.549

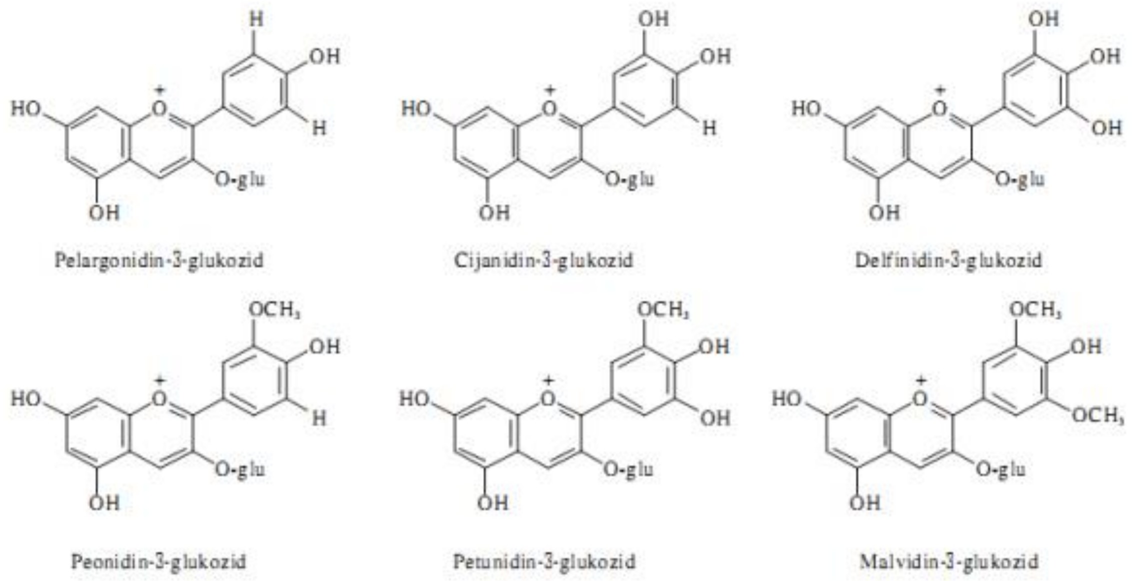

Fig. 7. Chemical structure of 6 basic anthocyanin glucoside (Clifford, 2000)

Antioxidant activity

Antioxidants are substances that prevent the oxidation of other substances. Some of the antioxidants are also enzymes, such as peroxidases, catalases, superoxide dismutases, and proteins, while some compounds have very small molecules (Shi et al., 2001). Antioxidant activity is the ability of certain substances to pass through different mechanisms that are stabilized by substances that lead to oxidative changes. Antioxidant activity depends not only on the structural properties of antioxidants, but also on many other factors, such as temperature, light, substrate type, the physical state of the system, as well as numerous micro components that act as prooxidants or synergists (Yanishlieva-Maslarova and Heinonen, 2001). Antioxidants can slow or inhibit oxidation in two ways: removing free radicals, where the ingredient is defined as a primary antioxidant, or by a mechanism where direct free radical scavenging is not involved, and where the ingredients are defined as secondary antioxidants. Phenolic substances are the primary antioxidants and secondary antioxidants act through a variety of mechanisms. It is common that secondary antioxidants show antioxidant activity only in the presence of some other minor components, e.g., citric acid becomes active only in the presence of metal ions, ascorbic acid is active in the presence of tocopherols or some other primary antioxidants (Gordon, 2001). In recent times, there is a growing interest in natural antioxidants that would lead to a reduction in the use of artificial additives. An unappetizing property of natural antioxidants is their sensitivity to oxygen, especially when there are exposed to higher temperatures, light, and drying (Pichler, 2011). Krasnova and Seglina (2019) published a review about the results of the chemical composition and antioxidant activity of analysed fresh apple samples that showed differences between autumn-winter (AW), winter (W), and late winter cultivars (LW). For W and AW apple cultivars, they found that the total phenol content and antioxidant activity were significantly different, while no differences were found for total flavonoid and tannin content. When results were compared between W and LW, differences were found for total phenol and flavonoid content, but there were no significant differences for tannin content and antioxidant activity. Differences were found in the content of polyphenols in fresh fruits: W cultivars had 1.5 times more polyphenols than AW cultivars, while on the other hand, LW cultivars had 2 times more polyphenols than AW cultivars. In conclusion, the order of polyphenol content goes from higher to lower: AW, W, and LW cultivars. The results indicate that the LW varieties had higher antioxidant activity compared to others. In the literature, the relationship between food concentration of phenolic compounds and antioxidant activity is highly disputed. Van der Sluis et al. (2001) did not find any correlation between the concentration of phenolic constituents and antioxidant activity in apple extracts. On the other hand, others found a strong correlation between total phenols and antioxidant activity (Lee et al., 2000; Sun et al., 2002). A good correlation was observed between the antioxidant potentials determined by ABTS, FRAP, DPPH, and procyanidins and hydroxycinnamic acid

(Table 5). The results showed that the most important compounds for the antioxidant activity of apples were flavan-3-ols, including monomers, dimers, and oligomers. Other polyphenols showed lower correlation coefficients. Tsao et al. (2010) demonstrated that anthocyanins had the highest antioxidant activity among all tested standards. However, it accounted for only 1% of the total polyphenolics and its concentration did not correlate with the antioxidant activity (Table 5). In conclusion, the concentration of procyanidins/flavan-3-ols is the most important contributor to the in vitro antioxidant activity (WojdyŁo, 2008).

Conclusions

Although a large number of traditional apple varieties have been lost forever, there is still hope for the salvation of those still planted in private orchards. In addition to cultural heritage, this paper shows the advantages of old traditional apple varieties over commercial ones. Apart from the fact that the old varieties are richer in polyphenols than the commercial ones, they are also richer in some individual polyphenols, such as epicatechin, chlorogenic acid, procyanidins B1, B2, A2, etc. They all contribute to the improvement of human health. This brings us to the words of our ancestors ″an apple a day keeps the doctor away″. Furthermore, apples have great potential as a source of individual polyphenols and natural antioxidants that should reduce the use of artificial supplements. In conclusion, this is also a good start for further research such as: Traditional apple cultivars as a source of bioactive compounds.

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Funding: This work has been supported by Croatian Science Foundation under the project "Possibility of taking advantage of traditional apple cultivars for the production of apples and apple juice with reduced patulin content" UIP-2020-02-8461.

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