

## Phenolic Compounds from Apples: Reviewing their Occurrence, Absorption, Bioavailability, Processing, and Antioxidant Activity – a Review

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This review summarises the research on phenolic compounds in apples in relation to their geographical origin, cultivars, technological processes, and human health effects due to their antioxidant activity and bioavailability. Apples are popular among consumers and are known as a rich source of vitamins, minerals, and biologically active compounds. In this review article, we have focused on their phenolic compounds. Some epidemiological studies have confirmed the protective effects of apples against chronic diseases, which can be linked to the high content of phenolics in plant tissues and their bioavailability. However, according to the available literature, the geographical origin and variety of apples influence the content of these bioactive compounds and are highly related to their antioxidant activity fluctuation. The gathered studies have confirmed that the choice of polyphenol-rich raw material as well as proper processing are important to achieve high-quality fruit-based products with a high content of antioxidants after a few stages of production. It was proven that the processing of apples could significantly modify the content of phenolics in the finished products, including juices, concentrates, vinegars, and ciders. For instance, the use of high pressure or vacuum has been proposed as a highly potent solution in inhibiting flavonoid degradation during intensive processing. Moreover, several methods have been screened to monitor the phenolic content and antioxidant activity of apple samples, based on spectrophotometry, HPLC, LC-MS, and LC-MS/MS techniques for chemical compound separation and identification. The main assumptions of these techniques and results obtained are described in this review.

### INTRODUCTION – THE PROTECTIVE EFFECTS OF BIOACTIVE COMPOUNDS FROM FRUITS AND VEGETABLES IN HUMANS

Data published by the World Health Organization (WHO) show that low consumption of fruits and vegetables might be the reason for the increased incidence of chronic diseases, and could be the cause of 14% of deaths from gastrointestinal cancers, 11% of those from coronary heart disease, and 9% of those from strokes [WHO, 2009; Arts & Hollman, 2005]. One of the hypotheses is that vegetables and fruits contain high amounts of biologically active compounds eliciting protective effects against lifestyle-related diseases [Arts & Hollman, 2005]. This effect might be due to the antioxidant properties of certain compounds. Antioxidants inhibit oxidation reactions, thus scavenging oxygen free radicals formed upon these reactions. Free radicals can bind proteins, damage lipids of cell membranes, or disrupt the structure of nucleic acids. They are naturally counteracted by the human organism; however, this natural balance might be upset by illnesses, stress, excessive consumption of highly-processed foods,

and high environmental pollution. The consumption of fruits and vegetables is highly recommended to support the body in this respect. The main antioxidants found in fruits and vegetables are A, E, and C vitamins;  $\beta$ -carotene; lycopene; and a diverse group of compounds named phenolics. Therefore, it is hard to select one specific compound responsible for health benefits. That is why, Ohr [2004] stated that “scientists are currently studying how mixtures of antioxidants ... work together. They are looking for synergistic effects among individual antioxidants.”

The most important group of antioxidants is the phenolic compounds (Figure 1). They are organic chemical compounds with at least one phenol unit, represented by, *i.a.*, tannins (hydrolysable and condensed), flavonoids, and lignans. Phenolic compounds can be found in a variety of plant species. Their high content is correlated with antioxidant activity, which decreases the risk of coronary disease and protects against neurodegeneration [Giacalone *et al.*, 2015]. The positive effect of flavonoids on memory improvement was also studied [Whyte & Williams, 2011]. Of 12 epidemiological surveys about coronary heart disease risk assessment compared by Arts & Hollman [2005], seven demonstrated the protective effects of a diet enriched with polyphenols. An inverse relationship has also been determined between poly-

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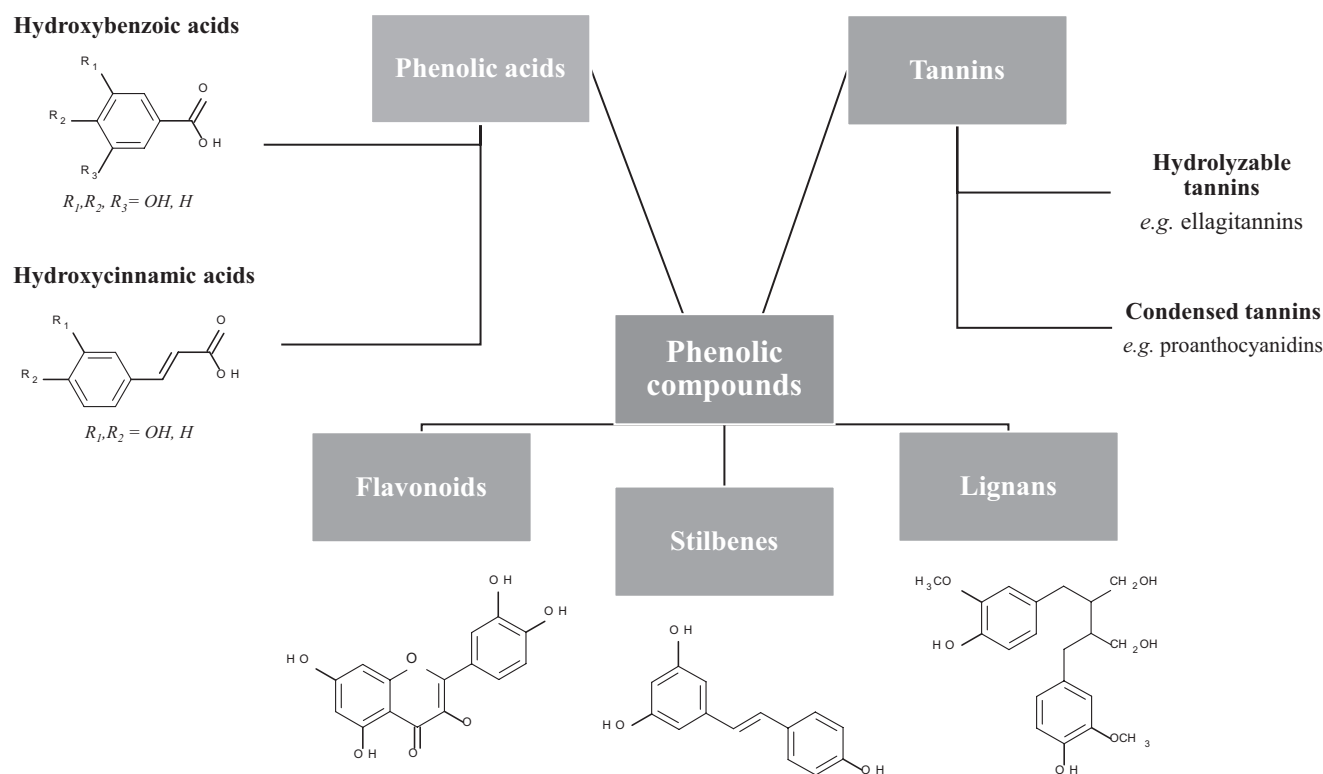


FIGURE 1. Representatives of phenolic compounds with their chemical structures.

phenol intake and stroke and breast cancer risk [Bosetti *et al.*, 2005; Knekt *et al.*, 2002]. In turn, Jiao *et al.* [2019] have demonstrated that phenolics affect obesity by modulating the gut microbiota and prebiotic agents. Moreover, the anti-inflammatory properties of flavonoids were discussed by Maleki *et al.* [2019], who suggested that the possible mechanism of their action is by inhibiting enzyme activity and transcription factors responsible for inflammation. In epidemiological studies conducted on the Finnish population (>10,000 people), it was proven that flavonoid intake might be crucial for decreasing the risk of development of chronic diseases [Knekt *et al.*, 2002]. Also, in clinical studies, a positive correlation was observed between flavonoid administration and the progress of type 2 diabetes [Guo *et al.*, 2019]. Finally, a positive effect of nutritional supplementation with flavonoids was seen in the research on skeletal muscle atrophy to prevent oxidative stress-induced muscle waste [Salucci & Falcieri, 2020].

One of the most affordable fruit-based products on the market is juice. Juices have been proved to contain a high number of antioxidants, such as vitamin C, and to be rich in varied polyphenols [Jacob *et al.*, 2008; Martí *et al.*, 2009]. Therefore, it has been experimentally confirmed that the polyphenols from grape juice prevent heart disease and that compounds with high antioxidant activity from berries or apple juice inhibit hypercholesterolemia [Habanova *et al.*, 2019]. In another example, juice from pineapples, oranges, and grapefruits was seen to be a rich source of folic acid derivatives, which help prevent nervous system diseases and malformations, such as spina bifida. In turn, cranberry

juice has been shown to decrease urinary tract infections [Jepson *et al.*, 2004].

Among the polyphenols, considerable attention has been paid to flavonoids, the intake of which varies from 60 to 600 mg per day among different dietary patterns. Ample studies have focused on determining their content in different food products using novel methodologies and advanced equipment. Today, their bioavailability in the human body is a topic related to phenolic compounds' influence on human health [Manach *et al.*, 2005].

#### APPLE PHENOLIC COMPOUNDS – PROFILE AND INTAKE

Apples are a rich source of flavonoids, including flavones, flavanols, anthocyanins, and dihydrochalcones (Figure 2). The major individual flavonoids in apples are quercetin and its glycosides, (–)-epicatechin, (+)-catechin, and phloridzin and its derivatives [Lee *et al.*, 2003; Francini & Sebastiani, 2013]. Cyanidin glycosides can be found only in apples of red varieties. Other apple polyphenols are procyanidins – the oligomers of (–)-epicatechin and (+)-catechin, with their content in apple fresh weight ranging from 38 to 154 mg [Hellstrom *et al.*, 2009]. Phenolic acids, e.g., chlorogenic acid, are also present in apple flesh and skin. However, the quantitative and qualitative profile of phenolic compounds found in apples differs depending on their variety, country of origin, and special pre-treatment, which is summarised in Table 1.

Zhang *et al.* [2019] reported that the positive effect of phenolic content and composition might be due to the pol-

lination of apples with quince pollen. A significant increase in (+)-catechin, (-)-epicatechin, chlorogenic acid, phlorizin, quercetin, and quercetin 3-*O*-galactoside contents was obtained by this modification.

Only a part of phenolic compounds is found in the free form in plant tissues, and most are bound to cell wall components. Important is that apples have the highest portion of free phenolics when compared to other fruits [Sun *et al.*, 2002]. The apples' bound phenolics are less bioavailable than free phenolics since they need to be released from the food matrix during digestion [Williamson *et al.*, 2018]. Therefore, an important aspect of research is to study phenolics occurrence in natural food products and then to determine their intake by consumers. Apples, blackcurrants, strawberries, raspberries, chokeberries, cranberries, blackberries, plums, apricots, pomegranates, oranges, pineapples, and grapefruits are known to be the richest sources of polyphenols [Calderón-Oliver & Ponce-Alquicira, 2018]. Their consumption is estimated to range from 100 to 200 g per day. Apples provide approx. 22% of polyphenols in the diet [Scalbert & Williamson, 2000]. An apple portion contains 400 mg of polyphenols, whereas the average portion of pears or grapes provides 300 mg of polyphenols, and a portion of cranberries, cherries or blueberries provides 200–400 mg [Scalbert & Williamson, 2000]. In general, the intake of polyphenolic compounds varies widely among populations with the average approximating 1 g per day [Scalbert & Williamson, 2000]. For example, the main source of flavonoids in a diet of the Australian population is soy and its products, whereas 55.2% of total flavonoids come from non-alcoholic beverages in a diet of the French population [Del Bo *et al.*, 2019]. In many countries (*e.g.*, Poland), tea and fruits are also a common source

of flavonoids in the diet. According to data selected by Murphy *et al.* [2019], flavonoid intake also varies by gender: women in Australia consume a higher amount of flavonoids than men: 659 vs. 566 mg per day. The interest in purchasing natural food products with a high flavonoid content by consumers might be driven by their positive effects on human health.

## APPLE PHENOLIC COMPOUNDS – THE BIOLOGICAL EFFECTS

As mentioned in the Introduction section, dietary phenolics can play a positive role in reducing the risk of development of chronic diseases associated with oxidative stress. The biological effects of apple phenolic compounds are also of interest. Flavonoids extracted from apple peel were implicated in the suppression of breast cancer and the growth of carcinogenic cells in the bronchi [George & Rupasinghe, 2017; Loung *et al.*, 2019]. In turn, Zielińska *et al.* [2019] proved the potential benefits of apple-derived polyphenols in modulating inflammation at the intestinal level. Apple extracts can also regulate carbohydrate metabolism through the inhibition of  $\alpha$ -glucosidase activity, thereby potentially reducing the risk of type 2 diabetes development [Raphaeli *et al.*, 2019]. The newest research from Zou *et al.* [2020] showed that apple phenolics might be effective in the prevention of obesity *via* promoting brown adipogenesis. There is no clear evidence as to whether apple flavonoids protect lipids and proteins in plasma and decrease LDL cholesterol level [Zou *et al.*, 2020]. Zeng *et al.* [2020] explained, with the example of apple proanthocyanidins, that the hypocholesterolemic activity of polyphenols might be related to their ability to bind cholesterol. In their *in vitro* study, tannins were potent

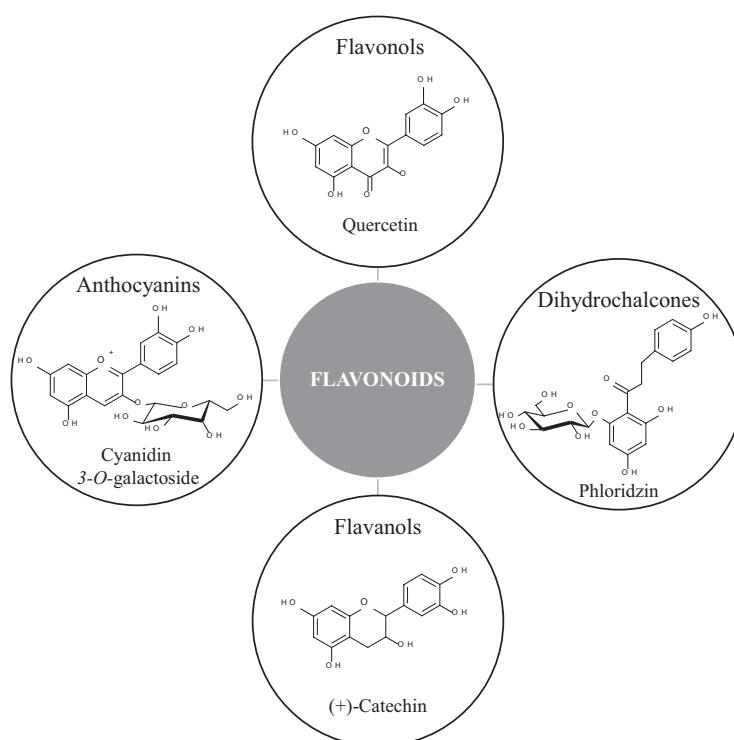


FIGURE 2. Flavonoid classes with examples of each class and their structure.

TABLE 1. The summary of latest studies about major phenolic compounds found in apples according to variety, country of origin, and special pre-treatment.

Material	Country of origin	Apple variety	Content of major phenolic compounds	Main remarks	Reference
Apple peel	Croatia	'Apistar' 'Bobovac' 'Božićnica'	(-)-epicatechin: <b>119.5</b> mg/100g DW (-)-epicatechin: <b>131.8</b> mg/100g DW (-)-epicatechin: <b>50.4</b> mg/100g DW	The content of phenolics in peel differs among varieties	Lončarić <i>et al.</i> [2020]
Raw apple			procyanidin B <sub>2</sub> : <b>91.55</b> mg/100g DW (-)-epicatechin: <b>76.30</b> mg/100g DW quercetin 3- <i>O</i> -rhamnoside: <b>8.35</b> mg/100g DW		
High-pressure treated apple (400 MPa at 35°C for 5 min)	Spain	'Golden Delicious'	procyanidin B <sub>2</sub> : <b>95.02</b> mg/100g DW (-)-epicatechin: <b>73.08</b> mg/100g DW quercetin 3- <i>O</i> -rhamnoside: <b>11.08</b> mg/100g DW	High-pressure releases flavonoids from the matrix	Fernández-Jalao <i>et al.</i> [2019]
Raw apple			procyanidin B <sub>2</sub> : <b>74.51</b> mg/100g DW (-)-epicatechin: <b>67.89</b> mg/100g DW quercetin 3- <i>O</i> -rhamnoside: <b>9.73</b> mg/100g DW		
High-pressure treated apple (600 MPa at 35°C for 5 min)	Italy	'Golden Delicious'	procyanidin B <sub>2</sub> : <b>103.57</b> mg/100g DW (-)-epicatechin: <b>98.58</b> mg/100g DW quercetin 3- <i>O</i> -rhamnoside: <b>20.15</b> mg/100g DW		
Raw apple	Italy	'Golden Delicious' organic	(-)-epicatechin: Before frozen storage: <b>19.3</b> mg/100g DW Dipped: <b>21.2</b> mg/100g DW Vacuumed with lemon juice: <b>18.1</b> mg/100g	Vacuum pre-treatment method in acid environment inhibits degradation of flavonoids during storage	Santarelli <i>et al.</i> [2020]
Raw apple	Italy	'Golden Delicious' conventional	After frozen storage: <b>19.4</b> mg/100g DW Dipped: <b>16.7</b> mg/100g DW Vacuumed with lemon juice: <b>18.0</b> mg/100g (-)-epicatechin: Before frozen storage: <b>19.6</b> mg/100g DW Dipped: <b>20.0</b> mg/100g DW Vacuumed with lemon juice: <b>25.4</b> mg/100g After frozen storage: <b>15.0</b> mg/100g DW Dipped: <b>16.9</b> mg/100g DW Vacuumed with lemon juice: <b>19.0</b> mg/100g		
Raw apple	Japan	'Golden Delicious' 'Red Delicious' 'Royal Delicious'	(-)-epicatechin: peel: <b>20.37–41.24</b> mg/100g DW pulp: <b>10.36–22.21</b> mg/100g DW (-)-epicatechin: peel: <b>37.74–52.97</b> mg/100g DW pulp: <b>13.21–22.45</b> mg/100g DW Total tannin content (TTC): peel: <b>16.03–33.29</b> TAE/g FW pulp: <b>11.64–25.83</b> TAE/g FW	The profile of phenolics differs among apple parts and growing locations at different altitudes	Dhyani <i>et al.</i> [2018]
Crabapple	China	Malus 'Red splendor' M. micromalus 'Haihongguo' M. micromalus	procyanidin B <sub>1</sub> : <b>103.57</b> mg/kg DW (-)-epicatechin: <b>36.34</b> mg/kg DW phloridzin: <b>24.57</b> mg/kg DW hyperin: <b>72.09</b> mg/kg DW isoquercitrin: <b>25.01</b> mg/kg DW procyanidin B <sub>1</sub> : <b>847.88</b> mg/kg DW (+)-catechin: <b>430.96</b> mg/kg DW procyanidin B <sub>2</sub> : <b>203.47</b> mg/kg DW (-)-epicatechin: <b>1220.76</b> mg/kg DW phloridzin: <b>94.42</b> mg/kg DW hyperin: <b>72.09</b> mg/kg DW isoquercitrin: <b>49.06</b> mg/kg DW procyanidin B <sub>1</sub> : <b>1534.78</b> mg/kg DW (+)-catechin: <b>1326.33</b> mg/kg DW procyanidin B <sub>2</sub> : <b>2151.86</b> mg/kg DW (-)-epicatechin: <b>2727.69</b> mg/kg DW cyanidin 3- <i>O</i> -galactoside: <b>190.17</b> mg/kg DW	Total polyphenol content is the highest in apple cultivar 'Red splendor'	Han <i>et al.</i> [2019]

DW – dry weight; FW – fresh weight; TAE – tannic acid equivalent.

to precipitate cholesterol *via* interactions of ions and hydrophobic and intermolecular hydrogen bonds. Moreover, Hamazu *et al.* [2005] demonstrated apple phenolic compounds to be significant inhibitors of the influenza viral activity. Then, in later research, Pastene *et al.* [2009] observed suppressed *Helicobacter pylori* urease activity after incubation with apple peel extracts, which suggests that apple phenolic compounds have a strong gastro-protective effect. It was also proved that apple extracts had an antibacterial effect against Gram-positive bacteria [Pires *et al.*, 2018].

Flavonoids are metabolized *in vivo*, which suggests that they have a low impact on the antioxidant activity in humans. The significant importance of the higher overall antioxidant activity after the consumption of foods with a high flavonoid content (*e.g.*, fruits, vegetables, tea, wine, or chocolate) might be explained by the change in the concentration of antioxidant metabolites in human plasma (for example, uric acid) [Lotito & Frei, 2004a,b; 2006]. The addition of 'Red Delicious' apple extract to human plasma *in vitro* increased ferric reducing antioxidant power (FRAP). In this case, FRAP values of plasma increased from 454  $\mu\text{M}$  to 486  $\mu\text{M}$  (+7%) and up to 532  $\mu\text{M}$  (+17%), respectively, after the addition of 7 and 14  $\mu\text{g}$  of apple polyphenols to 1 mL of plasma [Lotito & Frei, 2006]. Such an increase in plasma FRAP is comparable to the results reported after the consumption of other polyphenol-rich foods [Lotito & Frei, 2006]. Further *in vivo* study of Wruss *et al.* [2015] has shown that the antioxidant activity of human plasma increased after apple consumption. The highest oxygen radical absorbance capacity (ORAC) of plasma was determined 1 h (120% of change) and 6 h (120% of change) post intake.

Moreover, the addition of apple polyphenols to plasma *in vitro* significantly increases its antioxidant resistance. Although apple polyphenols do not inhibit the peroxidation of ascorbate in plasma, the endogenous half-life of urate and  $\alpha$ -tocopherol and the delay in lipid oxidation were observed to be higher. Lotito & Frei [2004a] demonstrated a high content of polyphenols in extracts from 'Red Delicious' apples, as reflected by a high radical scavenging activity *in vitro*. To prove this *in vitro* observation, in a further *in vivo* study by Lotito & Frei [2004a], six volunteers consumed five apples per day to deliver a total of 1825 mg of polyphenols. Plasma was collected 6 h after consumption, and antioxidant activity was measured. The results of an *ex vivo* study did not prove the *in vitro* measurements; no significant impact of apple polyphenols on plasma antioxidant status was found. Furthermore, the antioxidant activity of plasma measured by the FRAP method significantly increased 1 h after apple consumption, to 60  $\mu\text{M}$  of TE [Lotito & Frei, 2004b]. In the next step, volunteers consumed 260 g of bagel, as a control without flavonoids and 750 mL of water; the amount was matched to the consumed amount of carbohydrates and weight of apples. Approx. 60 mg of vitamin C was delivered with apples, which led to a slightly higher amount of ascorbic acid in plasma. The removal of ascorbate from plasma by ascorbate oxidase did not increase the antioxidant capacity. The results indicated that vitamin C from apples did not significantly modulate the antioxidant activity. The bagel consumption resulted in a decreased level

of FRAP in plasma, which suggested the effect of food consumption *per se* [Lotito & Frei, 2004a,b]. This hypothesis was also tested on the same six volunteers. They drank a solution of 64 g of fructose in 1000 mL of water, and the fructose amount was estimated according to the weight of five apples. Plasma activity, expressed as FRAP, significantly increased after about 1 h, up to 40  $\mu\text{M}$ , since fructose administration. Moreover, the changes in antioxidant activity depending on time were comparable to those observed after apple consumption. The concentration of uric acid in plasma significantly increased in every patient after fructose administration; the same was observed after apple consumption, while the level of ascorbate in plasma remained unchanged [Lotito & Frei, 2004b]. This research is consistent with the findings reported by Wruss *et al.* [2015], who observed that an increase in the total phenolic content over time did not correlate with an observed, highly elevated antioxidant capacity in the blood plasma after apple juice consumption. In addition, they concluded that the antioxidant activity was rather due to a high fructose content of the apple juice.

#### ANTIOXIDANT ACTIVITY OF PHENOLIC COMPOUNDS

The antioxidant activity of different food products has been broadly addressed in recent investigations [Shahidi & Ambigaipalan, 2015]. For example, such products as coffee, tea, chocolate, wine, and beer possess high antioxidant activities that are linked to the high content of polyphenols. The total polyphenol content, including flavonoids, in fruits and juices is also correlated with the antioxidant activity of these products *in vitro* [Murillo *et al.*, 2012].

Flavonoids are strong antioxidants *in vitro*, mainly because of their low redox potential and ability to donate electrons and hydrogen atoms; however, their activities vary. For example, flavanols have a redox potential of +0.53–−0.57 V. From a thermodynamic point of view, they can protect urates (+0.59 V) but not ascorbate (+0.28 V) against oxidation by peroxide radicals (+1.06 V). Therefore, they are more effective than ascorbate in the antioxidant protection of  $\alpha$ -tocopherol in a micellar solution [Frank *et al.*, 2006]. However, high flavonoid contents are not always strictly correlated with strong antioxidant activity in the studied samples. For example, the highest antioxidant activity (ORAC assay) of blueberries was noted in their 'Northland' and 'North country' varieties, *i.e.*, 6747 and 6265  $\mu\text{mol TE}/100\text{ g}$  of fresh matter, respectively, whereas the highest total flavonoid content was determined in those of 'North country' and 'Blomidom' varieties [Wang *et al.*, 2017]. A weak correlation was also demonstrated between antioxidant activity and total phenolic compounds for different varieties of apple [Jelodarian *et al.*, 2012]. Despite the high antioxidant activity of flavonoids *in vitro*, their effectiveness *in vivo* is limited. In *in vitro* studies, the antioxidant activity of biologically active compounds of apples and their extracts were determined using the FRAP and ORAC methods [Lotito & Frei, 2006]. Water extracts of the apple variety 'Red Delicious' contained 176 mg of phenolic compounds per 100 g of apples. The FRAP and ORAC values were at



1421 and 1508  $\mu\text{mol TE}/100\text{ g}$  apples, respectively. However, polyphenols contribute 14–18% of FRAP and ORAC values. This could mean that other biologically active compounds also make a high contribution to the overall antioxidant activity measured *in vitro*.

The high antioxidant activity of apples might also be related to their procyanidin contents. Procyanidins are found in fruit and cocoa products and are deemed good antioxidants due to their multiple hydroxyl groups [Hellstrom *et al.*, 2009]. Their antioxidant potential depends on the chain length of oligomers and the type of oxygen they react with [Spranger *et al.*, 2008; Zhou *et al.*, 2014]. This also applies to apple procyanidins [Lu & Foo, 2000]. Procyanidins and their monomer, possess comparable antioxidant efficiency against peroxide radicals, whereas procyanidins with different degrees of oligomerization differ significantly in their lipid-protecting ability against oxidation induced by iron and ascorbate [Plumb *et al.*, 1998]. Procyanidins are stronger antioxidants than more absorbable (–)-epicatechin and (+)-catechin.

As it was mentioned above, the effectiveness of flavonoids *in vivo* is limited; however, some studies reported an increase in the overall antioxidant activity in plasma. This observation leads to the general conclusion that flavonoids play an important role as antioxidants in plasma and tissues [Arts & Hollman, 2005]. After the ingestion of food or a beverage, flavonoids in the ingested matrix must pass from the gut lumen into the circulatory system in order to be absorbed. Since *in planta* almost all flavonoids are in the form of glycosides, the attached sugar must be removed following consumption before absorption can take place [Williamson *et al.*, 2018]. Some studies have pointed out that flavonoids are poorly absorbed and that their plasma concentrations are low even after flavonoid-rich food consumption [Lotito & Frei, 2006].

### PHENOLIC COMPOUNDS – BIOAVAILABILITY AND ABSORPTION

A crucial aspect of research dedicated to molecules with antioxidant properties is their bioavailability from food. The list of factors that influence the bioavailability of antioxidants is quite long [Porrini & Riso, 2008]. They can be divided into four main categories: 1) related to the antioxidant (structure, molecular linkage, amount introduced, *etc.*); 2) related to food and its preparation (matrix characteristics, technological processing, presence of positive/negative effectors, *etc.*); 3) related to the host (disorders and/or pathologies, enzyme activity, gender, *etc.*); and 4) external (exposure to different environment and food availability). Also, the complex relationship between factors was discussed. In the mucosa of the intestine and liver, flavonoids are being modified *via* glucuronidation, methylation, and sulfating. Biotransformation influences their physical properties; therefore, they are more soluble in water and have better antioxidant properties. Some metabolites of phenolics have the same antioxidant activity as their precursors; however, in general, metabolites are weaker antioxidants because of the modifications of their catechol and phenol groups. Moreover, phenolics are degradable by digestive tract bacteria and their metabolites exhibit biolog-

ical activity *via* an antioxidant or non-antioxidant mechanism [Minatel *et al.*, 2017]. Phloretin was established as a urinary biomarker of apple intake by Saenger *et al.* [2017]. Phloretin content determined in urine 12 h after apple consumption allows discriminating low- and high apple consumers. Therefore, the study of Yuste *et al.* [2018] proved that glucuronide derivatives of phloretin, catechol, and hydroxyphenyl- $\gamma$ -valerolactone, and also sulfate derivatives of dihydroxyphenylpropionic acid and (methyl) catechol are the main apple phenolic metabolites detected in human blood and plasma. In a further study, Yuste *et al.* [2019] specified that phloretin glucuronide, and galactosides of cyanidin and peonidin were the intake markers of red-fleshed apples.

Quercetin is one of the most studied phenolic compounds because of its wide distribution in food and high antioxidant potential *in vivo*. Its main sources include onions, apples, tea, and wine. Quercetin occurs in a conjugated form as a quercetin glycoside. It is transferred to enterocytes *via* a sodium-dependent transporter of glucose 1. Some other studies pointed out that, before absorption in the intestine, enzymatic or bacterial deglycosylation takes place. Quercetin glycosides can persist in the bloodstream for more than 10 h, which is longer than other flavonoids like anthocyanidins and flavanols. After the intake of foods rich in quercetin, its blood plasma level was at 0.7–7.6  $\mu\text{M}$  [Lotito & Frei, 2006]. In contrast to quercetin, flavanols are the main compounds of tea and chocolate, but are also found in apples (the amounts of (+)-catechin in apples and apple products are presented in Tables 1 and 2). Usually, flavanols occur in plants as aglycones and can be directly absorbed into the bloodstream. However, the concentration of free flavanols in the plasma is low were they are found in glucuronated and methylated forms. Anthocyanidins from berries and wines are absorbed intact, as glycosides. It was shown that their ethylated and glucuronated conjugates can be found in blood plasma in the nanomolar ranges [Porrini & Riso, 2008]. Other important phenolics in apples, phloretin and phloridzin, were investigated in the plasma of rats by Crespy *et al.* [2001]. They showed that the highest concentration of phloretin, in both conjugated and unconjugated forms (conjugated forms of phloretin were dominant at 85–95% of the total amount), was found in plasma after 10 h and rapidly decreased after 24 h. In turn, the research conducted by Rios *et al.* [2003] has shown that procyanidins are degraded by bacterial intestinal microflora to more absorbable phenolic acids with low molecular weights. While the bioavailability of quercetin, flavanols, and anthocyanidins in organisms is limited, the absorption of non-degradable procyanidins seems to be even lower, or almost null [Serra *et al.*, 2010].

The absorption of flavonoids in humans is rather low in comparison to other antioxidants, such as vitamins C and E. Their maximum concentration in human plasma is observed between 1 and 3 h after flavonoid-rich food consumption. Levels of flavanols, flavanols, and flavanones are established at 0.006–7.6  $\mu\text{M}$ , while these of anthocyanidins are lower than 0.15  $\mu\text{M}$  [Hollman *et al.*, 1997]. Moreover, the half-life of flavonoids in human plasma is short, reaching only a few hours. These limitations influence the higher activity of diet-derived flavonoids as antioxidants *in vivo*, in comparison to other active compounds present in higher amounts,

TABLE 2. Phenolic compounds determined in products from apples according to apples' variety, country of origin and special pre-treatment.

Material	Country of origin	Apple variety	Content of dominant phenolic compounds	Main remarks	Reference
Juice	Germany	'Elstar Elshof' (variety susceptible to scab) 'Rubinola' (scab-resistant variety)	procyanidin B <sub>2</sub> : <b>74.6</b> mg/L (-)-epicatechin: <b>61.8</b> mg/L procyanidin C <sub>1</sub> : <b>46.6</b> mg/L procyanidin B <sub>2</sub> : <b>106.6</b> mg/L (-)-epicatechin: <b>89.9</b> mg/L procyanidin C <sub>1</sub> : <b>53.3</b> mg/L	Higher content of flavonoids was determined in varieties resistant to scab	Schempp et al. [2016]
Cloudy juice	Poland	'Shampion'	procyanidin B <sub>2</sub> : <b>169.5</b> mg/L (-)-epicatechin: <b>95.1</b> mg/L	Addition of ascorbic acid preserved the content of flavonoids in juice during storage	Kolniak-Ostek et al. [2013]
Cloudy juice after 3-year storage			procyanidin B <sub>2</sub> : <b>157.6</b> mg/L (-)-epicatechin: <b>95.1</b> mg/L		
Cloudy juice with ascorbic acid (AA) addition			procyanidin B <sub>2</sub> : <b>202.1</b> mg/L (-)-epicatechin: <b>73.7</b> mg/L		
Cloudy juice with AA addition after 3-year storage			procyanidin B <sub>2</sub> : <b>165.9</b> mg/L (-)-epicatechin: <b>82.3</b> mg/L		
Cider after ethanol fermentation	Turkey	'Red Delicious'	(+)-catechin: <b>1.46</b> mg/L (-)-epicatechin: <b>3.33</b> mg/L caffeic acid: <b>0.75</b> mg/L chlorogenic acid: <b>16.50</b> mg/L <i>p</i> -coumaric acid: <b>0.04</b> mg/L	Pulp maceration increased the content of phenolic compounds in cider	Budak et al. [2015]
Cider after maceration with pulp and ethanol fermentation			(+)-catechin: <b>2.13</b> mg/L (-)-epicatechin: <b>4.63</b> mg/L caffeic acid: <b>0.96</b> mg/L chlorogenic acid: <b>24.13</b> mg/L <i>p</i> -coumaric acid: <b>0.03</b> mg/L		
Vinegar (Zi Lin)	China	no information, commercial samples	protocatechuic acid: <b>0.82</b> µg/mL chlorogenic acid: <b>10.91</b> µg/mL <i>p</i> -coumaric acid: <b>0.17</b> µg/mL	The content of phenolic compounds differed among vinegars from various producers	Liu et al. [2019]
Vinegar (Heng Shun)			protocatechuic acid: <b>1.54</b> µg/mL chlorogenic acid: <b>2.99</b> µg/mL		
Cider vinegar (Heinz)			protocatechuic acid: <b>1.00</b> µg/mL chlorogenic acid: <b>0.23</b> µg/mL		
Cider vinegar (Xin He)			chlorogenic acid: <b>4.67</b> µg/mL		
Vinegar	Turkey	'Malus domesticus'	gallic acid: <b>0.8</b> mg/100 mL <i>p</i> -hydroxybenzoic acid: <b>0.2</b> mg/100 mL (+)-catechin: <b>2.4</b> mg/100 mL syringic acid: <b>0.12</b> mg/100 mL caffeic acid: <b>0.4</b> mg/100 mL <i>p</i> -coumaric acid: <b>0.08</b> mg/100 mL	Significant changes appeared in phenolic compounds during vinegar making	Bakir et al. [2016]
Pomace	Cameroon	'Malus sylvestris'	(-)-epicatechin, caffeic acid and quercetin Total amount: <b>141.30</b> mg/100 g DW	The content of phenolic compounds increased after blanching	Dibanda et al. [2020]
Pomace after microwave blanching; 50°C, 5 min			Gallic acid, (-)-epicatechin, caffeic acid, <i>p</i> -coumaric acid, ferulic acid, rosmarinic acid, quercetin. Total amount: <b>93.13</b> mg/100 g DW		

*e.g.*, 30–150 µM of vitamin C, 160–450 µM of urinate, or 15–40 µM of vitamin E. Long-term consumption of flavonoid-rich food does not lead to the accumulation of their high amounts in plasma. Concentrations of quercetin in human plasma are lower than 1 µM [Loito & Frei, 2006]. Data about the concentration of flavonoids in human tissues are not available, but it is unlikely that flavonoids have a significant impact on the antioxidant activity in cells, where ascorbate and reduced glutathione can be found in mM concentrations [Loito & Frei, 2006]. Poorly absorbed phenolic compounds are metabolized in intestines and liver. Moreover, they are good substrates and indicators of phase II enzymes [Williamson et al., 1996]. This suggests that they are recognized by organisms

as xenobiotics, potentially toxic compounds, because of their polyhydroxy structure and strong redox potential [Dunnick & Hailey, 1992]. Therefore, by inducing the detoxification of enzymes, flavonoids protect the organism against mutagenic and carcinogenic factors, thus preventing cancer development [Knekt et al., 2002]. Probably, the absorbed flavonoids (in low concentrations) and their metabolites exert other biological activities, including *e.g.*, changing cell signaling and gene expression, which contributes to the health benefits. Hence, flavonoids after intensive metabolic transformations and chemical forms of flavonoids present in fruits and vegetables (mainly glycosides, except for flavanols in aglycon forms), differ from the metabolites formed *in vivo*.

### FACTORS INFLUENCING THE CONTENT OF PHENOLIC COMPOUNDS IN APPLES

There are a lot of factors influencing the phenolic content in apples. In the West Himalayan apple varieties, the phenolic content was significantly differentiated among cultivars and locations [Bahukhandi *et al.*, 2018]. It was also confirmed that the variety and maturity of apples significantly affected their chemical composition, content of phenolics, and antioxidant activity [Oszmiański *et al.*, 2008]. Oszmiański *et al.* [2008] compared 120 apple varieties and found they were highly diversified in terms of phenolics content. According to this research, the highest phenolic content was found in apples of 'Ozark Gold' variety (~2116.03 mg/kg), whereas the lowest one in these of 'Ligol' variety (~814.17 mg/kg). The quality and quantity of phenolics in apples directly influences their antioxidant activity [Wojdyło *et al.*, 2008]. Orchard cultivation system has an impact on the chemical composition of apples as well. Apples from organic farming have a higher content of phenolics in comparison to those from conventional cultivation [Wojdyło *et al.*, 2010]. Sut *et al.* [2019] noted that the content of phloretin 2-*O*-xyloglucoside and quercetin 3-*O*-arabinoside was significantly higher in ancient apple varieties ('Friuli Venezia Giulia', northern Italy) than in the commercial ones (e.g., 'Golden Delicious', 'Red Delicious', 'Granny Smith', and 'Royal Gala'). Also, ancient Croatian apple varieties were observed to be an important source of phenolics, especially proanthocyanidins, phenolic acids, and dihydrochalcones, compared to the commercial ones [Jakobek *et al.*, 2013]. Also, Kschonsek *et al.* [2018] noticed the significant difference in phenolics content between old (29 mg/100 g) and new (13 mg/100 g) cultivars of apples from Germany. It might be said that even the ancient cultivars can be a good source of bioactive compounds in the formulation of novel products. However, the results are not unambiguous in comparison to other research data. For example, the peel, pomace, and juice made of the old Spanish cultivar 'Verde Doncella' had lower contents of flavanols and quercetin derivatives compared to 'Red Delicious' cultivar. However, this study pointed out that 'Verde Doncella' had a higher content of *p*-coumaric acid and procyanidin B<sub>2</sub> in the peel [Krawitzky *et al.*, 2014]. Interesting results were described by Skłodowska *et al.* [2018], who observed that apple leaves might be a rich source of bioactive compounds due to the variety and geographical origin, which significantly differentiated the apple leaf phenolics. The methods and conditions of apple processing also have a major impact on polyphenols.

### FACTORS INFLUENCING THE CONTENT OF PHENOLIC COMPOUNDS IN APPLE PRODUCTS

In Poland, clarified beverages and wines are the most popular products made from apple juice concentrate. This type of manufacturing leads to large differences in the chemical composition between the raw material and the final product, such as an almost total lack of pectin and vitamin C, and 90% loss of phenolics. The lowest content of valuable procyanidin is found in apple juice. Procyanidins bind to the cell walls of apples and are removed during the clarification process

of juice. This leads to a significant decrease in the polyphenols in juices [Kolniak-Ostek *et al.*, 2013]. Oszmiański & Wojdyło [2009] noted a 6-fold lower antioxidant activity of apple juice compared to blackcurrant juice. Cloudy and puree juices are good alternatives to clarified apple juices. The content of phenolic compounds depends on the apple variety and type of manufactured juice. Clarified juice made of 'Idared' apples contained only 250.1 mg/L of phenolics, whereas the content in cloudy juice made from 'Szampion' was 5-fold higher reaching 1044.4 mg/L of juice [Oszmiański *et al.*, 2007]. Cloudy juice made of 'Szampion' apples had up to 31% higher antioxidant potential, measured by the DPPH method, and that made of 'Idared' apples had about 45% higher antioxidant potential than the respective clarified juices [Oszmiański *et al.*, 2007]. In other research, cloudy juices produced without the enzymatic treatment, clarification, or filtration contained more biologically active compounds [Oszmiański & Wojdyło, 2009]. The juice clarification process removes by-products of phenolic compounds oxidation. Omitting this step in cloudy juice manufacture leads to their unattractive brown color. Cloudy juices should be produced from high-quality, fresh, and matured apples. Their cloudiness can range from 15% (var. 'Elstar') to 47% (var. 'Gostar') [Wojdyło *et al.*, 2008]. Schempp *et al.* [2016] noted that 'Elstar' variety was a rich source of flavanols, e.g., (-)-epicatechin, and their oligomers, e.g., procyanidin B<sub>2</sub> and C<sub>1</sub>, with their content approximating 240.0 mg/L. Considering the oxidation and browning of juices, they should be manufactured in an inert gas environment, after ascorbic acid addition to the pulp. The quality of cloudy juices can be improved by employing decanting instead of pressing. Juice production using decanters takes only a few seconds, which inhibits the oxidation of crushed fruits. The addition of ascorbic acid or rhubarb to apple pulp during fruit crushing reduces the effects of oxidation processes, including color change. The addition of ascorbic acid proved necessary in all types of juices except for those made of var. 'Szampion' apples. Juices with ascorbic acid contain significantly more phenolic compounds (approx. 1.2-fold higher) and have higher antioxidant activity (3.5-fold higher) compared to juices without its addition [Kolniak-Ostek *et al.*, 2013]. Promising results in terms of decreasing the browning of juices were achieved by using high-pressure carbon dioxide treatment [Murtaza *et al.*, 2020]. This processing of juices also increased (-)-epicatechin and (+)-catechin concentrations; however, more studies need to be done to establish the appropriate temperature and pressure conditions to achieve the highest content of phenolics. In another study, an attempt to increase the content of phenolic compounds and antioxidant activity in apple juices and beverages by the enzymatic treatment of apple pulp and the addition of apple leaves proved successful [Zielinski *et al.*, 2014]. The phenolic compound contents increased by 2 to 5 times, whereas the antioxidant potential by about 1.29 times. Moreover, the addition of apple puree produced from different enzymatic formulations increased the phenolic content in the studied juices from 441 mg/L to 612 mg/L, and antioxidant activity by 5–21% [Zielinski *et al.*, 2014]. The obtained data show that enriching natural cloudy juices with ascorbic acid, apple pulp or leaves is useful. If enriched with this technology, juices can be an



attractive product, with antioxidants that will have a beneficial influence on human health. Oszmiański *et al.* [2008] demonstrated a high content of polyphenolic compounds and antioxidant activity in juices made of 'Idared' apples. Furthermore, the addition of blackcurrants not only enriched apple juice with phenolic compounds and improved its color, but also protected it from enzymatic oxidation. Ascorbic acid from blackcurrants, used in this reaction, also effectively protected the polyphenols of 'Idared' apples from oxidation. Antioxidant activity is measured as the ability to scavenge DPPH radicals; therefore, the antioxidant activity of blackcurrant juice was about 40-fold higher than that of apple juice. The addition of 20% blackcurrant juice to apple juice increased the antioxidant activity about 8-fold in comparison to apple juice without any additives [Oszmiański *et al.*, 2008]. However, Średnicka-Tober *et al.* [2017] demonstrated that the addition of chokeberry juice to apple juice led to a decrease in quercetin and kaempferol contents. Whereas green tea extract addition resulted in a significant enrichment of apple juice with phenolic acids (~2- times) and flavonoids (~10- times), with molecules characteristic for tea: (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, and (-)-epigallocatechin 3-*O*-gallate. Bat *et al.* [2018] successfully used phytochemicals as markers to prove the origin and variety of apple juices. Moreover, Adamenko *et al.* [2019] established a new product, *i.e.*, fermented apple beverage with Cornelian cherry addition (10%) having a strong antioxidant properties (ABTS, DPPH, and FRAP tests) and enriched with phenolic compounds and iridoids. This treatment caused the highest content of 5-*O*-caffeoylquinic acid (from 70.0 to 96.8 mg/L). In turn, Verdu *et al.* [2014] presented that the apple progeny significantly influenced the phenolic compounds content of another type of apple beverage, namely cider. The content of 12 phenolics varied in apples as a genetic effect and between harvest years.

Kahle *et al.* [2005] pointed out that the phenolic compounds content was significantly different in juices made from dessert or cider apples. Moreover, there was a difference in phenolic contents between apple cultivars, while freshly squeezed juices were richer in phenolic compounds than commercially available juices. The influence of storage on phenolic stability in apple juices was also studied by Maragó *et al.* [2015]. They demonstrated that the contents of total phenolics and flavanols were almost 5-fold and 8-fold higher, respectively, in 'Panaia-red' juice than in juice made of 'Golden Delicious' apples. Higher stability of phenolics was achieved in 'Panaia-red' juice. Pavun *et al.* [2018] highlighted that the use of fruit nectar definitely lowered the phenolic content in commercial juices, which was ultimately correlated with their antioxidant activity. It was also demonstrated that not pasteurization but pre-pasteurization had a significantly stronger impact on phenolics content during processing of not from concentrate (NFC) juices [Tian *et al.*, 2018].

Another important aspect affecting contents of antioxidants is the technological preparation of raw fruits. The microwave-treatment of apple peels [Dibanda *et al.*, 2020] and their storage method affected phenolics content and antioxidant activity [Santarelli *et al.*, 2020]. According to Santarelli *et al.* [2020], there is a difference between the response

of tissues of organically and conventionally farmed apples to freezing. The antioxidant activity of organic apples after freezing was reduced by only 13%, whereas in the conventional ones a decrease of up to 25% was noted. This was related to the higher resistance of organic apple tissue to thermal stress, which caused a decrease in free and conjugated phenolics related to antioxidant activity. Fernández-Jalao *et al.* [2019] increased extractability of flavonoids from apple pulp by up to 54% using high-pressure processing at 600 MPa. In another study, Ferrentino *et al.* [2018] successfully used supercritical fluid extraction to improve the recovery of extracted phenolics from apple pomace. The mechanism of these two methods might be related to the flavonoid compounds from cell walls being trapped in an insoluble matrix.

Also, the consumption of dried apples is quite popular among consumers nowadays. It is known that food product stability over storage period is an important aspect to be considered during its production process. In this context, the addition of a green tea extract to dried apples might be a perfect solution, providing a new type of product that would combine the preservation of apples and an increased phenolic content in the final product [Corey *et al.*, 2011]. Pająk *et al.* [2017] proposed biofilms made of white or green tea extracts to prolong the freshness of sliced apples. The proposed application of white tea allowed them to obtain a stored product with a similarly high content of total phenolics to that in the fresh material. Tappi *et al.* [2018] used cold atmospheric plasma to study the effect of phenolic behavior in fresh-sliced apples. They noticed that phenolic content significantly decreased in slices after 2 h (1.774 mM/kg fresh weight); however, it was not significantly lower after half an hour (from 2.142 to 2.092 774 mM/kg fresh weight). The high content of phenolic compounds and health-promoting properties of food products play an important role in consumer acceptance. From the sensory point of view, in the study of Wang *et al.* [2019a], the most preferred by the panelists were the dried apple slices pretreated with steam blanching and osmotic dehydration of the apple slices. Freeze-dried apple powders were also found to be good sources of antioxidants [Raudone *et al.*, 2016], like chlorogenic acid and procyanidin C<sub>1</sub> which were responsible for their high antioxidant activity.

Apples are eagerly consumed mostly because of their pleasant taste and the fact that they are widely available worldwide. FAO statistics reported that, in 2016, the global production of apples exceeded 80 million tons, and over 3 million were converted into juice [Matsuoka, 2019]. According to these statistics, a large amount of waste is simultaneously produced. Therefore, growing attention has been paid to the reuse or limitation of the amount of waste produced. The benefits of apple pomace consumption were described in a publication prepared by Skinner *et al.* [2018]. The positive antioxidant effect on human health might be related to the phenolics in this apple waste. Therefore, quercetin (5.72 µg/kg), phloretin (2.01 µg/kg), and phloridzin (1.23 µg/kg) were determined to be the main phenolics in apple pomace [Rana *et al.*, 2015]. Apple peel is also a rich source of bioactive compounds [Raudone *et al.*, 2017]. Its main flavonoids are (-)-epicatechin and quercetin, at 0.28 and 0.19 mg/g, respectively [Bhagwat *et al.*, 2011]. This is in agreement

with Escarpa & González [2001], who found high amounts of phenolic compounds (in the order: rutin, chlorogenic acid, and (+)-catechin) in “Golden” and “Reinette” apples (both peel and pulp). A possible innovation is the use of apple peels after microwaving, which is a good method for releasing phenolic compounds bound in the cell wall [Dibanda *et al.*, 2020]. Furthermore, apple peels can be a good component to formulate functional foods, because of the high total phenolic content (1.1 g GAE/g of dry weight). Du *et al.* [2020] proposed “cold-pressing technology” as an effective method for peeling and deseeding apple fruits, with a positive effect on phenolic compounds retention in pomace. A 3- to 18-fold higher content of gallic and vanillic acids, (–)-epicatechin 3-*O*-gallate, and phloridzin was detected in apple pulp than in juice, cider, and/or vinegar. Xu *et al.* [2016] pointed out that seeds could also be a rich source of phenolic compounds. However, the content of phenolics differs in seeds from different apple cultivars. The dominant phenolics determined in seeds were phloridzin > hyperin > chlorogenic acid. Other examples of apple products and the main conclusions about phenolics variation during apple processing are summarized in Table 2.

#### METHODS OF PHENOLICS DETERMINATION AND ANTIOXIDANT ACTIVITY MEASUREMENT IN APPLES

A broad review of phenolic compounds’ determination was prepared by Naczka & Shahidi [2004]. They gathered the main existing knowledge about phenolic compound extraction and analysis of their profile using spectrophotometric and chromatographic methods; however, since then, some new insights have been presented in this respect. The Folin–Ciocalteu’s reagent is still commonly used to spectrophotometrically characterize the total content of phenolic compounds in apples and their products [Azlan *et al.*, 2018; Bakir *et al.*, 2016; Dhyani *et al.*, 2018; Xu *et al.* 2016]. Total phenolic content is one of the most frequently analyzed factors, even after it was shown that the spectrophotometric method can lead to inaccurate results because of the strong interference of sugars and proteins in the assay results [Escarpa & Gonzalez, 2001]. In this context, HPLC-UV-VIS was used to determine the phenolic compound profiles in apple samples, preceded by an appropriate extraction procedure. Solvent extraction, sample fragmentation and its pretreatment, and compound-of-interest isolation are also important steps [Starowicz, 2019]. For example, Stan *et al.* [2017] proposed conventional extraction as a more efficient method to extract phenolics from dried apples than microwave- or ultrasound-assisted extraction. However, they noted that the content of compounds extracted can be different upon using different extraction procedures. Chlorogenic acid, (+)-catechin, and quercetin contents were higher after conventional than microwave-assisted extraction. The microstructure analysis conducted by Wang *et al.* [2019b] revealed that ultrasound treatment released phytochemicals from fruit cell walls, proving to represent a more efficient technique to increase phenolic compounds extractability. An interesting procedure was presented by Lončarić *et al.* [2020], who extracted flavonoids from apple peels

using micro-matrix solid-phase dispersion (micro-MSPD) with sea sand and a solvent mixture of methanol:water:formic acid. Also, different instrumentation is used to increase the efficiency of compound determination. Both qualitative and quantitative methods are in matter of interest, and can be used with internal/external standards to determine phenolic compounds content. The HPLC with a diode array detector (DAD) is the most common method applied for phenolic compound determination in apples [Duda-Chodak *et al.*, 2010]. However, its modifications were proposed in further studies. Gomes *et al.* [2016] used the electrochemical properties of flavonoids to measure their content in fruit powders by RP-HPLC-EC. This method was appropriate to determine low content of selected polyphenols in either fresh or dried samples. The LC-MS/MS was also adapted to phenolic compound determination in samples of apples because of its low sensitivity and good repeatability [Lončarić *et al.*, 2020]. In the study of Jakobek *et al.* [2013], two different detectors were used to determine contents of specific phenolic compounds in apples, *i.e.*, UV-Vis and MS/MS. Moreover, using various HPLC columns and conditions in the inter-laboratory study, Hollands *et al.* [2017] developed, validated, and assessed analytical method for the separation, identification and quantification of procyanidins in an apple extract. Additionally, many researchers have tried to establish a relationship between bioactive compounds and antioxidant activity, to determine which phenolic compound has the greatest influence on antioxidant activity.

There are several mechanisms of the antioxidant action of phenolic compounds and therefore different methods must be used to determine their antioxidant activity. Alam *et al.* [2013] found that the most frequent methods used to determine antioxidant activity *in vitro* in plant material are:  $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazyl (DPPH) radical scavenging assay, hydroxyl radical scavenging assay, and superoxide radical scavenging activity determination. According to these authors, the *in vivo* methods frequently used to determine antioxidant activity included lipid peroxidation assay (LPO) in tissues and determination of superoxide dismutase (SOD) and catalase (CAT) activities in erythrocytes of experimental animals. The antioxidant activity is often measured by using a few methods. The DPPH $\cdot$  and ABTS $^{*+}$  scavenging activities of apple extracts were measured by Duda-Chodak *et al.* [2010]. A significant difference was found between the analyzed ABTS $^{*+}$  scavenging activities of apple extracts, whereas no significant differences were noted in the DPPH method. Also, the FRAP method is usually introduced to evaluate antioxidant capacity. Pająk *et al.* [2017] found a high correlation between the antioxidant activities measured by FRAP, DPPH, and ABTS assays, and total phenolic content (with correlation coefficients of 0.914, 0.950, and 0.965, respectively). Furthermore, ethanol, water, and methanol are the solvents most commonly used to extract phenolic compounds before antioxidant activity determination usually conducted with spectrophotometric methods using cuvettes. As an innovation, Horszwald & Andlauer [2011] proposed 96-well plates (measurement done on a microplate reader) instead of cuvettes. The main advantages of this method are the low amounts of chemicals and extracts needed, and the very good repeat-

ability of the results obtained. Gökmen *et al.* [2009] presented a novel approach, “QUENCHER,” to the analysis of antioxidant activity. A direct QUENCHER procedure allows measuring the antioxidant activity in the samples in which “a great part of antioxidants is bound to insoluble matrices” [Gökmen *et al.*, 2009]. An interesting method was presented by Raudone *et al.* [2017], in which a combination of HPLC-DAD and FRAP assay was applied to measure the flavonoid content and antioxidant activity in apples and apple peels. Based on the HPLC-DAD–FRAP post-column analysis, it was established that among 12 flavanol compounds, flavanols and phenolic acids exhibited antioxidant activity, whereas no ferric ion reducing activity was observed for phloridzin. Therefore, it was observed that phloridzin cannot be used as a reference compound in antioxidant activity determination.

## CONCLUSIONS

Apples are an important source of phenolics in the human diet, and their consumption has been implicated in the prevention of degenerative diseases. The polyphenols that can be found in apple peel and pulp are flavonols (mainly quercetin and its glycosides), flavanols ((–)-epicatechin and (+)-catechin), procyanidins, anthocyanidins, and dihydrochalcones (phloridzin and its derivatives). Their content was strictly related to the geographical origin, variety, and processing techniques of the apple products. Information about cultivar-typical apple polyphenol content and profile is important for bioactivity studies and, consequently, essential for the development of consumer-relevant products with particular nutritional functionalities. As to what the future holds, much work should be put into determining the bioavailability, transport, and bioactivity of dietary flavonoids. Moreover, a strong emphasis should be put in further studies on the reuse of apple by-products, *e.g.*, peels and seeds, as novel food components.

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