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REVIEW ARTICLE

Trends in biochemical, anatomical mechanisms and molecular aspects in enzymatic browning of apples: a review

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Abstract

Apples (*Malus domestica* Borkh), like fresh fruits and vegetables, are susceptible to enzymatic browning (EB) and especially during handling, storage and processing, mostly as a result of phenolic compounds being enzymatically oxidized, basically, by polyphenol oxidases (PPOs) enzymes. EB reduces consumer acceptance and marketability leading to postharvest losses of apples. This review focused on EB, addressing the trends in biochemical, anatomical mechanisms, molecular aspects and the future perspectives. At least 24 phenolic compounds were detected in apples, with the peel often retaining the highest concentrations. Phenolic compounds with low molecular weight, like catechin, *p*-coumaric acid, chlorogenic acid and caffeic acid seem to function as a more effective substrate for PPO. The first step in EB process is cell collapse either by cutting, peeling, bruising, senescence etc. The collapsed cells enable PPO to be in direct contact with the polyphenolic substrates localized either in cell vacuole or in the cell wall in the presence of oxygen to produce melanin, the brown polymer. A precise transformation of apple genomes is a major advance in gene-editing technology focused on improving crop quality, especially in post-harvest technology. The advancement of innovative genome editing techniques to attain precise and targeted alterations in apples for the mitigation of browning activity. Innovative genome editing methods are exploited to silence genes expressing PPOs and relative genes of oxidative enzymes, as well as underscoring notable differences in the constitutive expression pattern of critical phenylalanine ammonia lyase (PAL) enzyme, constituting the most promising strategy to avert undesired browning in plants. Food scientists are seeking to select cultivars that are less sensitive to EB, in addition to using genetic engineering tools to inhibit the browning enzymes. The Arctic® apple is a unique example of this, as researchers focused on producing an apple resistant to EB with the goal of reducing waste and limiting the use of chemicals in apple industry.

Keywords *Malus domestica* · Browning · Anatomy · Gene-editing · PPO · Phenols

Introduction

Enzymatic browning disorders in horticultural commodities are mainly due to the enzymatic oxidation of phenolic compounds leading to produce high molecular weight compounds (melanin); the brown substance. Both polyphenol oxidases (PPOs) and peroxidase (POD) enzymes are involved in this reaction in the presence of oxygen as a co-substrate substance [1–6]. This oxidation reaction often occurs in fresh horticultural commodities during harvest, handling, storage, and processing; leading to considered food losses [1, 4, 7, 8]. The enzymatic browning disorder appears in fruits and vegetables in several forms such as enzymatic flesh browning in most fleshy fruits and vegetables, external browning in dates [9], and litchi fruits [10], bitter pits in apples [11–13], blossom end rot in Anna apples

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[5, 6] and the browning of fresh-cut or sliced fruit and vegetables [14, 15].

Apple (*Malus domestica* Borkh) is grown in the temperate regions in an area of about 6.95 million hectares, producing about 143.41 million tons. The value of apples exports around the world amounts to about 7.93 billion USD, in addition to apple juice and apple juice concentrate exportations, the value of which is about 1.5842 and 1.5846 billion USD, respectively (FAO, 2022 avalible at: https://www.fao.org/faostat/en/#data/QCL). Apples like many kinds of fresh fruits and vegetables are prone to browning disorder during harvesting, handling, slicing and storage due to physiological or mechanical injuries. This disorder reduces the visual quality, nutritional value and flavor consequently leading to great economic losses [3, 7, 8, 16–18].

The intensity of melanin pigment formation depends mainly on PPOs and POD activities, which depend on several factors, the most important of which are phenolic concentration [7, 9] storage temperature [9], the presence of antioxidants [7, 19, 20], and the abundance of oxygen [10, 14, 21].

The expression profiles of phenylalanine ammonia lyase (PAL) and PPO, as specific candidate genes related to the polyphenolic pathway, clarified the physiology of fruit browning. Wang et al. [22] indicated that transcriptome analysis demonstrated significantly elevated expression levels of browning-related genes *PPO* and *POD* in the browning-sensitive cultivar relative to the browning-resistant cultivar in fresh-cut apples.

According to the available literatures, there are many reviews that addressed the causes and the control of EB-related disorders either in fruits and vegetables [4], or particularly in apples [3, 16], while none of them addressed the details of the individual phenolic compounds and their properties, localization and concentration in apple fruit. Also, no available review addressed the anatomical mechanisms and molecular aspects of EB. Well understanding biochemistry, mechanism and the anatomy of EB will help both postharvest physiologists and food scientists to reduce its incidence, reduce food loss, and achieve food sustainability in the future. Consequently, this review shed the light in some detail on EB in apples, addressing biochemistry, anatomical mechanisms, transcriptional regulation, gene editing and the future perspectives.

Biochemistry of enzymatic browning

Browning reaction and melanin forming are initiated mainly by the activities of the enzyme PPO on phenolic compounds and POD contributes to oxidative damage to cellular membranes. This reaction can occurs in fresh fruits and vegetables, due to the interaction of phenolic compounds (substrate), oxygen (co-substrate) and the enzymes [14, 21, 23]. The main factors involved in the process of browning are:

Apple phenolic compounds

Phenolic compounds are secondary metabolite produced naturally in plants, mainly from the shikimic acid pathway (Fig. 1) by the action of phenylalanine ammonia lyase (PAL) (E.C. 4.1.1.5) and other enzymes. The structure of phenolic compound contains an aromatic ring (C_6H_6), one or more hydroxyl (OH) group, and numerous other substituted components [24, 25]. Phenolic compounds are classified into five phenolic sub classes (Fig. 2), including flavanols, phenolic acids, flavonols, dihydrochalcones, and anthocyanins [25–31]. Anthocyanins compounds are mainly appearing in the peel of red apples and their concentration in the flesh is very low except for the red-fleshed varieties [31–33]. There are difficulties in comparing the phenolic content of apple fruits between different studies due to variation in genotypes [28, 34], sampling time, geographical regions, and the methods of extraction and measurement [25]. The fruit peel contains higher quantities of phenolics than the flesh tissues. Because fruit peels are directly exposed to sunlight, they respond more quickly than the fruit flesh. Protective secondary molecules, such as phenols, tend to accumulate in the surface tissues (peels) to protect them against biotic and abiotic stresses such as pathogens, temperature, and ultraviolet radiation [35].

There are several individual phenolic compounds present in apples (Table 1) which play a crucial role in determining the fruit color, taste and flavor. Moreover, some phenolic compounds have antioxidant activity [25, 26, 30, 36] and varieties containing greater amounts of phenolics show higher antioxidant activities [25, 30, 37]. The concentration of individual phenolic compounds in apple fruit (Table 1) varies according to the fruit tissue (i.e. peel and flesh), cultivar, rootstock, harvesting maturity, environmental conditions, storage, etc [25, 26, 29, 33, 34, 38–44]. Despite these variations, the cultivar remains the main source of variation, and the interaction with fruit tissue type (peel and flesh) was found to be significant [39]. This variation among cultivars might be due to variation in the biosynthetic pathway of phenols (Fig. 2) [25, 40, 44]. Red-fleshed cultivars showed higher contents of individual and total phenolic compounds in both the peel and flesh compared to white-fleshed cultivars [31]. The slandered cultivars like "Fuji", "Golden Delicious" and "Gala" showed lower phenolic profile compared to the autochthonous cultivars [31, 35]. Cultivars with high phenolic contents are prone to the browning reaction [34] and can be recommended for fresh consumption, while

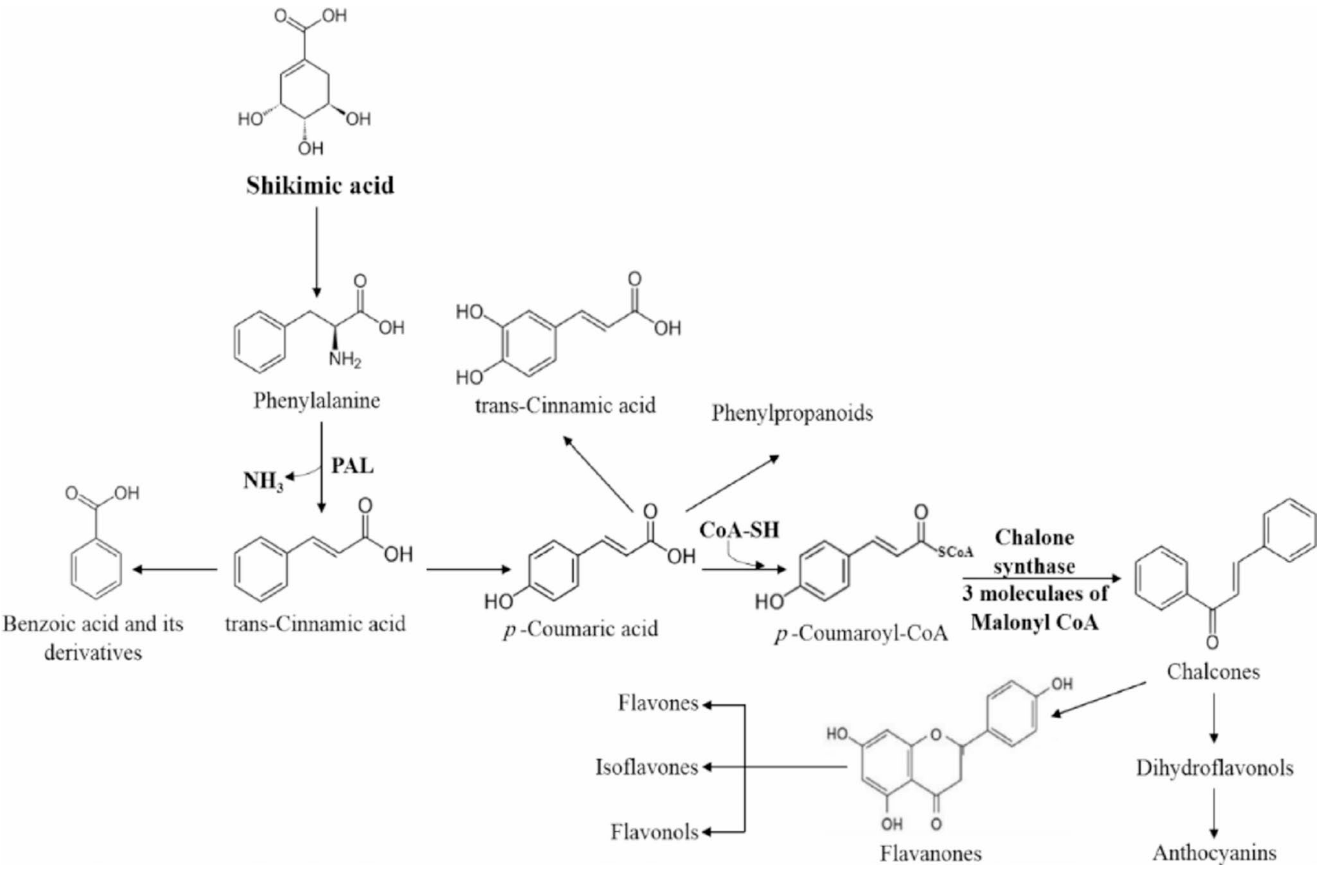


Fig. 1 Pathway of phenolics biosynthesis in apple fruit. Adapted from Kalinowska et al. [46] with some modifications

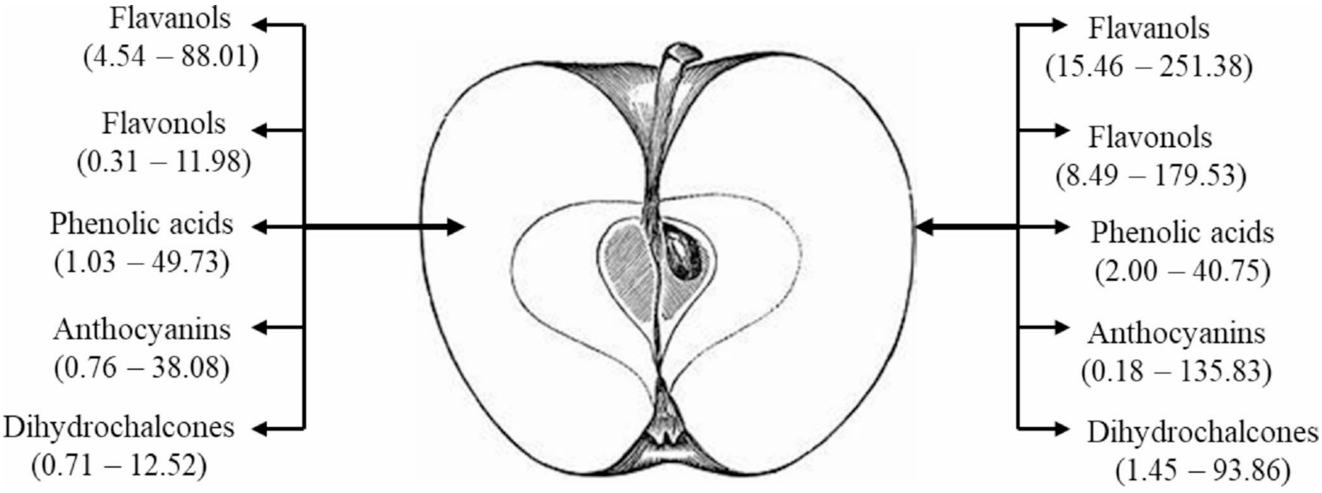


Fig. 2 Concentration of phenolic sub classes (mg/100 g FW) in apple fruit peel and flesh (data were re-elaborated from Table 1)

cultivars with lower phenolic content can be recommended for fresh cut and cider production because of their less susceptibility to enzymatic activity, and thus a more stable product [45].

The rootstock genotype also plays a critical role in phenolic profile. In Galaval' apples, it was found that total phenolic content and compounds varied in both peel (up to 90%) and flesh (2.9 times) according to the rootstock genotype. Moreover, total flavan-3-ols and flavonols contents also varied in the peel and the flesh by rootstock [38].

Fruit physiological age is an important factor in determining the phenolic content. For example, it was found that the level of dihydrochalcones in young apple fruit was very high and decreased rapidly in both the peel and the

Table 1 Individual phenolic compounds in apple fruit and their chemical properties and structure

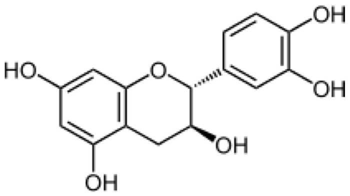
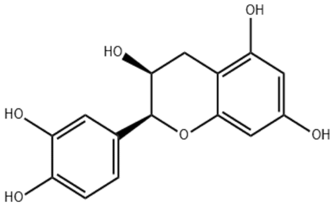
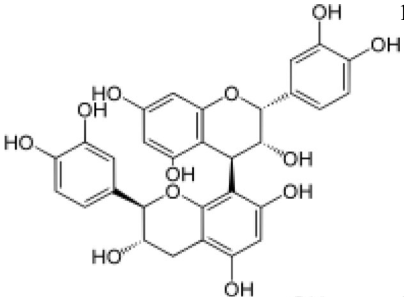
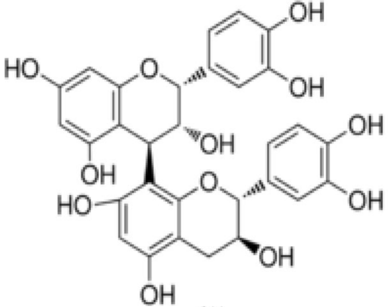
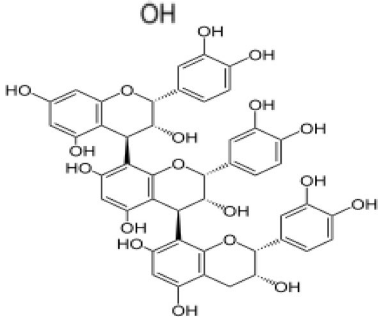
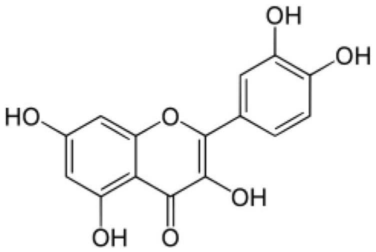
Polyphenolic molecule	Molecular weight (g/mol)*	Chemical formula*	Chemical structure*	Concentration (mg/100 g FW)	
				Peel	Flesh
Flavanols					
(+)-Catechin	290.27	$C_{15}H_{14}O_6$		0.42–48.60 [31, 39, 41, 48–53]	0.10–16.51 [31, 34, 39, 41, 48, 51, 53, 54]
(-)-Epicatechin	290.27	$C_{15}H_{14}O_6$		2.50–85.39 [31, 39, 41, 48–55]	0.17–32.50 [31, 34, 39, 41, 48, 51, 53, 54, 56]
Procyanidin B1	578.52	$C_{30}H_{26}O_{12}$		1.40–49.28 [31, 41, 48, 49, 57, 58]	0.59–19.69 [31, 41, 48]
Procyanidin B2	578.52	$C_{30}H_{26}O_{12}$		3.47–46.81 [31, 41, 48–50, 57, 59]	2.08–14.72 [31, 41, 48]
Procyanidin C1	866.8	$C_{45}H_{38}O_{18}$		7.67–21.30 [48, 58]	1.60–4.59 [48]
Flavonols					
Quercetin	302.23	$C_{15}H_{10}O_7$		0.06–14.29 [51–53]	0.10–0.69 [51]

Table 1 (continued)

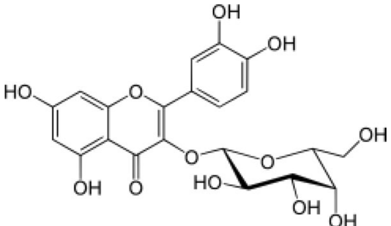
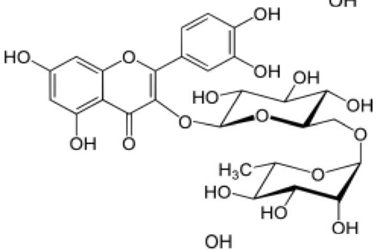
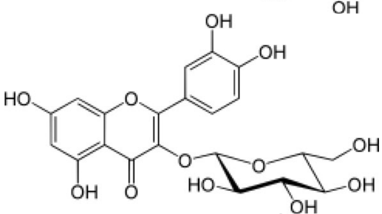
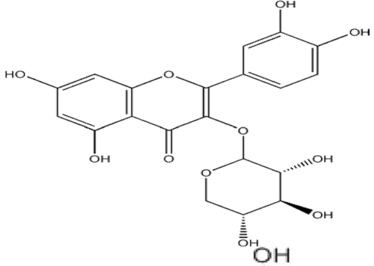
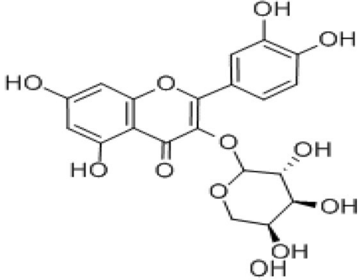
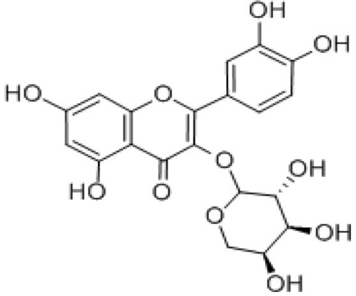
Polyphenolic molecule	Molecular weight (g/mol)*	Chemical formula*	Chemical structure*	Concentration (mg/100 g FW)	
				Peel	Flesh
Quercetin-3-galactoside (Hyperoside)	464.4	$C_{21}H_{20}O_{12}$		0.11–70.20 [31, 48, 56, 57]	0.02–7.00 [31, 48]
Quercetin-3-rutinoside (Rutin)	610.5	$C_{27}H_{30}O_{16}$		0.03–17.17 [31, 48, 51, 53, 56]	0.03–2.07 [31, 51, 53]
Quercetin-3-glucoside (Isoquercitrin)	464.4	$C_{21}H_{20}O_{12}$		1.52–23.15 [31, 48, 53, 57, 59, 60]	0.05–0.67 [31, 48, 53, 61]
Quercetin-3-xyloside (Reynoutrin)	434.3	$C_{20}H_{18}O_{11}$		2.22–10.68 [31, 48]	0.04–0.23 [31, 48]
Quercetin-3-arabinoside (Avicularin)	434.3	$C_{20}H_{18}O_{11}$		4.44–26.04 [31, 53]	0.04–0.68 [31, 53],
Quercetin-3-rhamnoside (Quercitrin)	448.4	$C_{21}H_{20}O_{11}$		0.11–18.00 [31, 48, 51, 53, 54, 56, 57]	0.03–0.64 [31, 48, 53]
Phenolic acids					

Table 1 (continued)

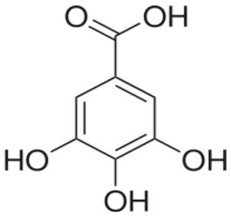
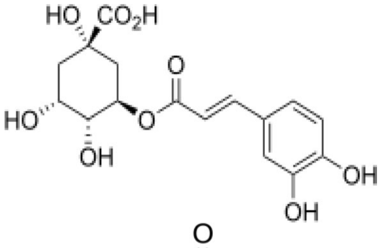
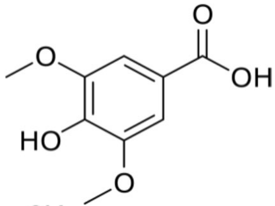
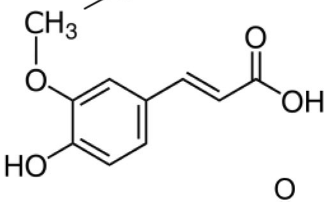
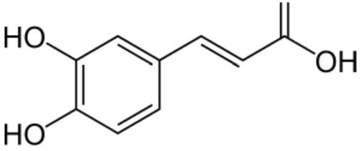
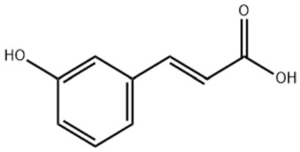
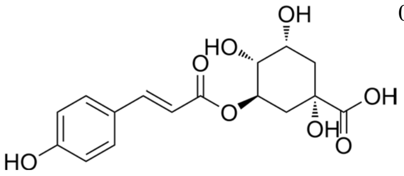
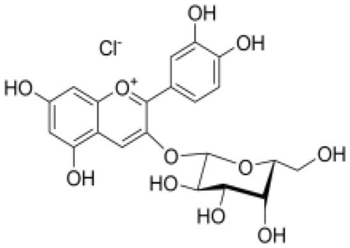
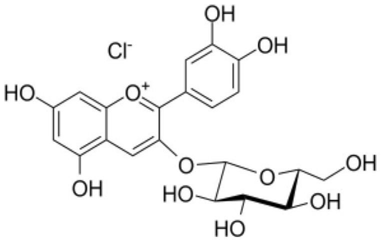
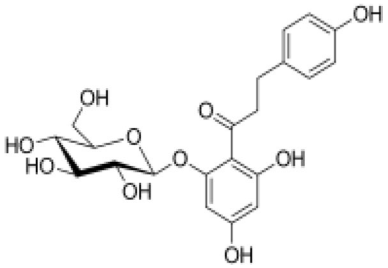
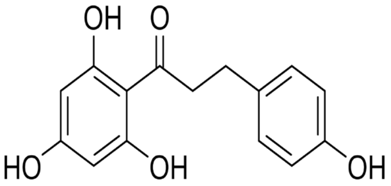
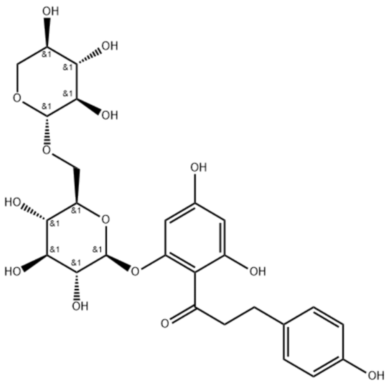
Polyphenolic molecule	Molecular weight (g/mol)*	Chemical formula*	Chemical structure*	Concentration (mg/100 g FW)	
				Peel	Flesh
Gallic acid	170.12	$C_7H_6O_5$		0.04–1.17 [31, 45, 51, 52, 60]	0.09–0.47 [31, 60]
Chlorogenic acid	354.31	$C_{16}H_{18}O_9$		0.15–28.97 [31, 39, 41, 48, 51–54, 62]	0.16–39.60 [31, 34, 39, 41, 48, 51, 53, 54, 56]
Syringic acid	198.17	$C_9H_{10}O_5$		1.38–4.81 [31]	0.35–2.34 [31]
Ferulic acid	194.18	$C_{10}H_{10}O_4$		0.02–0.11 [31]	0.02–0.05 [31]
Caffeic acid	180.16	$C_9H_8O_4$		0.05–2.52 [31, 56, 60]	0.03–1.44 [31, 49, 56]
p-Coumaric acid	164.16	$C_9H_8O_3$		0.06–0.29 [31, 54, 59]	0.09–0.80 [31, 34, 54]
p-Coumaroyl quinic acid	338.31	$C_{16}H_{18}O_8$		0.30–2.88 [48, 59, 63]	0.29–5.03 [41, 50, 59, 63]
Anthocyanins					
Cyanidin-3-galactoside (Ideain)	484.83 (chloride) 449.38$^+$ (cation)	$C_{21}H_{21}ClO_{11}$ (chlorid) $C_{21}H_{21}O_{11}^+$ (cation)		0.11–119.85 [31, 53, 57, 63]	0.74–37.95 [31, 53],

Table 1 (continued)

Polyphenolic molecule	Molecular weight (g/mol)*	Chemical formula*	Chemical structure*	Concentration (mg/100 g FW)	
				Peel	Flesh
Cyanidin-3-glucoside (Glucocyanidin)	484.83 (chloride) 449.38^+ (cation)	$C_{21}H_{21}ClO_{11}$ (chlorid) $C_{21}H_{21}O_{11}^+$ (cation)		0.07–15.98 [31, 52, 59]	0.02–0.13 [31]
Dihydrochalcones					
Phloridzin	436.4	$C_{21}H_{24}O_{10}$		0.62–86.81 [31, 41, 48–53, 55, 57]	0.09–5.76 [31, 34, 41, 48, 51, 53, 54]
Phloretin	274.27	$C_{15}H_{14}O_5$		0.06–6.00 [51, 53]	0.04–1.64 [34, 41, 51, 53]
Phloretin 2-xyloglucoside [a]	568.5	$C_{26}H_{32}O_{14}$		0.77–1.05 [39]	0.58–5.12 [39, 41]

[*] Source: National Center for Biotechnology Information. Retrieved January 29, 2024 available at: https://www.ncbi.nlm.nih.gov/

[a] Tentatively identified

flesh after 14 weeks of fruit set [46, 47]. Organically grown apples are supposed to have a higher phenolic content compared to integrated farming apples [25, 46].

The geographical origin and altitude of the orchard also affected the chemical components of Fuji apples including amino acids, organic acids, phenolics, etc [33, 64]. The results of Li et al. [27] showed that apples grown at medium altitudes (800–1000 m) contained higher concentrations of procyanidin B2, epicatechin (-), phlorizin, and chlorogenic acid than those grown at high (1000–1200 m) or low (500–700 m) altitudes. Conversely, quercetin glycosides and flavonol glycosides were positively correlated with high altitude in some cultivars and negatively correlated with other cultivars. Exposure of the fruits to sunlight is an important factor affecting phenolic biosynthesis, and shading caused low phenolic concentration, but the negative effect was strongly dependent on cultivar genotype. This suggests a complex interaction between the genotype and environmental factors [27, 52]. Growing apple tree under gray photoselective netting enhances the production of some phenolic compounds by allowing better passage of blue and ultraviolet light to the tree canopy, which enhances the biosynthesis of flavonols compounds, including quercetin [52].

Storage conditions also affect the phenolic content. The results of Ferreira et al. [40] have shown that although total phenolic decreased but there was an increase in some phenolic compounds, such as flavan-3-ols, total dihydrochalcones and total hydroxycinnamic acids in the flesh of ’Red Delicious’ apple fruits when stored in a modified atmosphere after treatment with 1-methylcyclopropene. Also Jin et al. [65] found that phenolic content increased when Golden Delicious apples were exposed to 4 w/m^2 white light for 12 h daily during storage at 25 °C.

Browning enzymes

PPOs are a group of oxidoreductase enzymes linked with four copper atoms. Plant-derived PPOs contain a highly conserved catalytic center (the active site). This center contains two copper ions which called CuA and CuB, each of which is coordinated by three histidine leftovers [66]. The structure of copper site of PPOs is present in three isoforms, called; oxy-PPO [Cu(II) Cu(II) O_2], met-PPO [Cu(II) Cu(II)], and deoxy-PPO [Cu(I) Cu(I)] [67]. The activity of PPO depends on the change in active sites in its protein [68]. The results of Di Guardo et al. [69] showed that Golden Delicious apple has 10 genes encoding PPO and distributed on three chromosomes. PPOs are divided into three types by Enzyme Commission (EC) depending on their optimum substrate and oxidation mechanisms (Table 2). PPO is present in the chloroplasts thylakoid lumen in two forms which are: a membrane-bound (mPPO) form with molecular mass of 65–67 kilo Daltons (kDa) and a soluble (sPPO) form with molecular mass of 30–31 kDa [70, 71]. mPPO was more active (19.17 and 34.12 times) than sPPO in both Granny Smith [70] and Red Fuji [71] apples respectively.

Table 2 Types of PPO and their substrate and oxidation mechanisms

PPO types	Substrate specificity and oxidation mechanisms
EC1.14.18.1 (monophenol monooxygenase, cresolase, or tyrosinase).	Has the cresolase (hydroxylation of monophenols to *o*-diphenols) plus catecholase activities [8, 16, 17, 72].
EC1.10.3.1 (diphenol oxidase, catecholase, catechol oxidase, or *o*-diphenol oxygen oxidoreductase).	Catalyzes the oxidation from *o*-diphenols to *o*-quinones [8, 72, 73].
EC1.10.3.2 (laccase, benzenediol, or oxygen oxidoreductases).	Oxidize various aromatic compounds through a radical-catalyzed reactions mechanism. Has the ability to oxidize o- and *p*-diphenols to produce *o*- and *p*-quinones [16, 74].

POD (EC1.11.1.7) is a heat-resistant and heme-containing oxidoreductase enzyme that catalyzes the oxidation of phenolics in the presence of hydrogen peroxide (H_2O_2) to produce the melanin pigment [73, 75]. The molecular mass of POD ranges between 26 and 40 kDa [76].

Since the endogenous level of H_2O_2 in plants controls the activity of POD; thus, the activity of the POD is linked to the activity of the PPO, meaning that the PPO is activated first and produces H_2O_2 through which the POD acts [2, 23, 77]. Therefore, delaying the activity of the PPO would hinder the undesirable activity of the POD [4]. EB associated with POD activity can be distinguished by adding a H_2O_2 quenching agent like catalase, which will prevent browning caused by POD-mediated reactions [2].

In addition to its role in color and nutritional value changes, POD enzyme causes changes in the consistency and flavor of the fruit, all of which are unacceptable changes to the consumer [34, 78]. Both PPO and POD contents in fruit may vary depending on the developmental stage and the ratio between bound and soluble enzymes [4].

PAL enzyme plays indirect role in browning reaction. An increase in PAL activity leads to an increase in phenolic production, the main substrates for PPO and POD activities. Some authors believe that the activity of the PAL may be more effective than PPO enzyme in enhancing browning reaction and the browning can be controlled by suppressing PAL activity [18, 73].

Browning reactions

Browning reactions and melanin formation are a direct consequence of PPO action on phenolics [18, 21, 23], however, some believe that POD is also involved, even partially, in this reaction [23]. Some evidence indicates that POD could enhance browning reactions that coincided with ongoing PPO oxidation reactions [23, 77] as mentioned above in this review.

The enzymatic oxidation is catalyzed by PPOs (Table 2), which catalyzes two basic reactions (Fig. 3a):

i) Oxidation of monophenol such as phenol, tyrosine to *o*-diphenols (monophenol oxidase activity).
ii) The oxidation of diphenol, such as caffeic acid, chlorogenic acid and catechol to *o*-quinones (diphenol oxidase activity), which are highly reactive compounds. Molecular oxygen participates in both reactions (an aerobic oxidative reactions) as a co-substrate [14, 21, 68]. Then, polymerization of *o*-quinones to form the brown pigment (melanin), high molecular weight compounds (Fig. 3b) [4, 68].

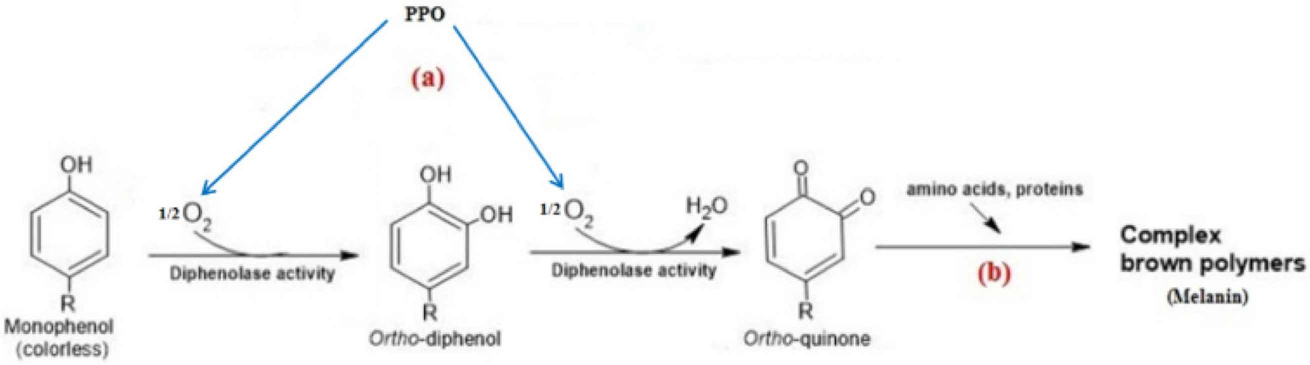


Fig. 3 The simplified processes of phenols oxidation by polyphenol oxidase (PPO) enzyme and the formation of melanin pigment. Adapted from Singh et al. [4] with some modifications

The two reactions mentioned above is not limited to mono phenols only, but the common is the oxidation of di-phenolic compounds such as catechol, caffeic acid and chlorogenic which makes such compounds the most common in the browning reaction [16, 79]. The amino acid tyrosine is the easiest and most common model for the oxidation of monophenols by PPO, especially in fungi, bacteria and mammals [4].

Many apple phenolic compounds (Table 1) have an *o*-diphenolic group; hence they are considered suitable substrates for PPO activity. However, oxidation of diphenols such as caffeic acid and chlorogenic acid remains the most common substrate for PPO in apples [16]. It was reported that PPO is often has high specificity for substrates with negatively charged side chains like caffeic acid and chlorogenic acid in contrast to catechol substrate [80]. The advanced studies of Zou et al. [81] showed that catechin, chlorogenic acid, gallic acid, syringic acid, procyanidin, phloridzin, and caffeic acid act as its catalytic substrates and involved in browning reaction.

PPO and POD activities and browning rate

It was found that browning rate was influenced by the variety and various endogenous factors, such as PPO activity, phenolics concentration, cell wall integrity and endogenous antioxidants [18, 20, 34, 70]. In the case of Red Delicious and Granny Smith apples, PPO activity was the best criterion to express the rate of browning. In contrast, in Gala and Fuji apples, the higher phenolics concentration was more suitable than PPO activity to express the browning rate. While in other cultivars like Honey Crisp apples, it was observed that the slow browning rate was coincided with both a low concentration of phenolics and a slow PPO activity [17]. The abundance of oxygen to the fruit tissue affects the enzymatic browning rate (Fig. 3) [73].

The activities of both PPO and POD enzymes are mainly affected by temperature, pH and the presence of antioxidants [19, 20, 82]. PPO activity is significantly reduced at temperature below 7 °C, although not deactivated. Therefore, cooling and chilling are recommended treatments to reduce PPO activity. Thermal shock is also effective to retard PPO activity. Hot water treatment of the fruit at a temperature of 45–70 °C for less than 5 min is sufficient to inhibit PPO activity [83].

pH value plays a critical role in the browning reaction, and PPO activity is inhibited in the presence of acids. A pH range of 5–7 is considered as the optimal for the browning reaction, and it decreases below pH 3.0 [70]. Consequently, high acidic fruits like citrus are less prone to browning reaction [4].

The presence of antioxidants like L-ascorbic acid can convert *o*-quinones back to diphenols and consequently delays the EB incidence [1, 19–21, 77, 82].

According to Wang et al. [83], exposure of apple fruits to heat stress during the maturation stage accelerates PPO activity. There were increases in H_2O_2, malonaldehyde, and superoxide anion (O^{2-}) in the peel during the exposure apple fruit to heat stress during the maturity stage. They also found that exposure to heat stress during the cell elongation phase did not affect the activity of the PPO.

Mechanisms of enzymatic browning

Understanding the mechanism of EB is critical to delaying fruit deterioration and reducing fruit and vegetable postharvest losses. So, it will be reasonable to throw the light on the mechanisms, by which the EB of apple fruit tissues takes place. As shown in Fig. 4, phenolics are mainly located in the vacuole (about 97%), followed by the cell wall (about 3%). Trace amounts of phenolic compounds can be found in chromoplasts, cytoplasm, and mitochondria [14, 84, 85]. In sound cells, phenols in either vacuole or cell walls are separated (isolated) from PPO and POD enzymes by membranes, thus preventing the oxidation reaction [14,

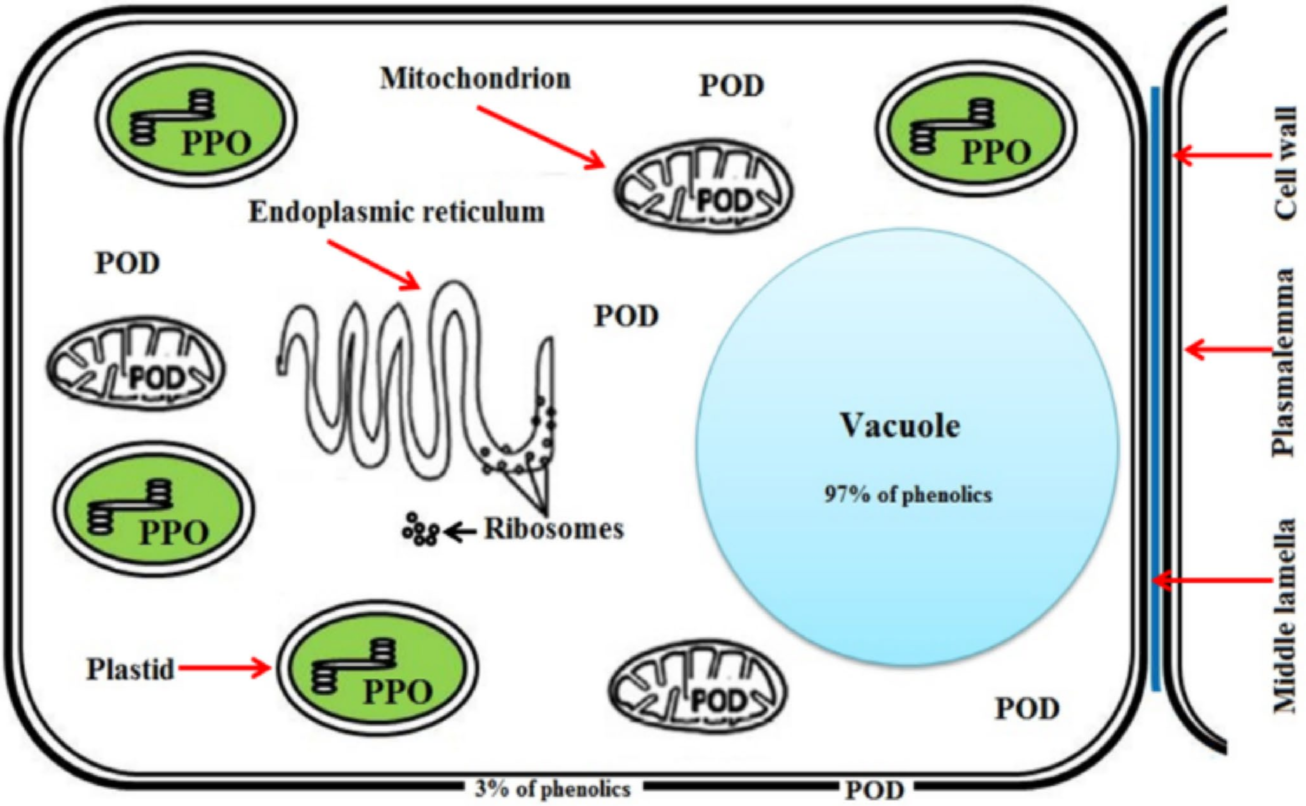


Fig. 4 A schematic drawing showing the localization of phenolic compounds and phenolic oxidizing enzymes (PPO and POD) in a typical plant cell. Adapted from Toivonen and Brummell [14], with some modifications. PPO: polyphenol oxidase, POD: phenol peroxidase

86]. Consequently, the first step in the oxidative browning process must be the collapse of the membranes (the barriers) inside the cells either by cutting, peeling, bruising, senescence etc [5]. The collapsed cells enable the oxidizing enzymes PPO and POD to be in direct contact with the polyphenolic substrates [1, 21], localized either in cell vacuole or in the cell wall in the presence of oxygen (an aerobic oxidation) to produce quinones [5, 14]. Thus, the oxidation of phenolic compounds into quinones and their derivatives will be completed to produce melanin, the brown, high molecular weight polymer. Therefore, it is important to maintain the integrity of the fruit's tissues and cells during harvesting and handling to avoid browning incidence [1, 5].

Anatomical features of enzymatic browning

The anatomical changes in the EB-affected tissues of Anna apples were tracked by Sultan et al. [5] during 10 days at room temperature (26 °C ± 2). On the third day of storage, it was found that the epidermal cells appeared normal; whereas some expanded cells appeared. Some cells of the cortex tissue appeared with very thin walls and some others appeared with deformed cell walls (Fig. 5A).

Two days later, the malformed cells in EB-affected tissues increased and extended in two axis; namely towards the fruit core as well as towards the basal fruit part, and some cell walls inside the cortex tissue were completely ruptured (Fig. 5B). Then, scattered groups of completely collapsed cells appeared in the cortex layer. The individuality of the cells disappeared due to the deformation of the cells, so some grooves and elliptical cavities appeared in the cortex layer. Cell components began to disappear (Fig. 5C). On the 8th day, the cell walls appeared torn and scattered, and cell components completely disappeared (Fig. 5D). Similar changes were observed by De Freitas et al. [87] who reported that the first sign of bitter pit is the collapse and plasmolysis of cell walls and by the time that pitting becomes visible to the naked eye. The pits cavities resulted from the collapse of several cells.

The previous anatomical changes were coincided with a drastic decrease in flesh firmness, high respiration rate and the imbalance between calcium (Ca) and other nutrients especially magnesium (Mg), potassium (K) and nitrogen (N) in EB-fruit compared to the EB-free one [3, 6, 87]. The role of Ca in reducing browning disorders in plant tissues is mainly attributed to its effect on maintaining cell wall rigidity. Moreover, the degradation of pectates is mediated by

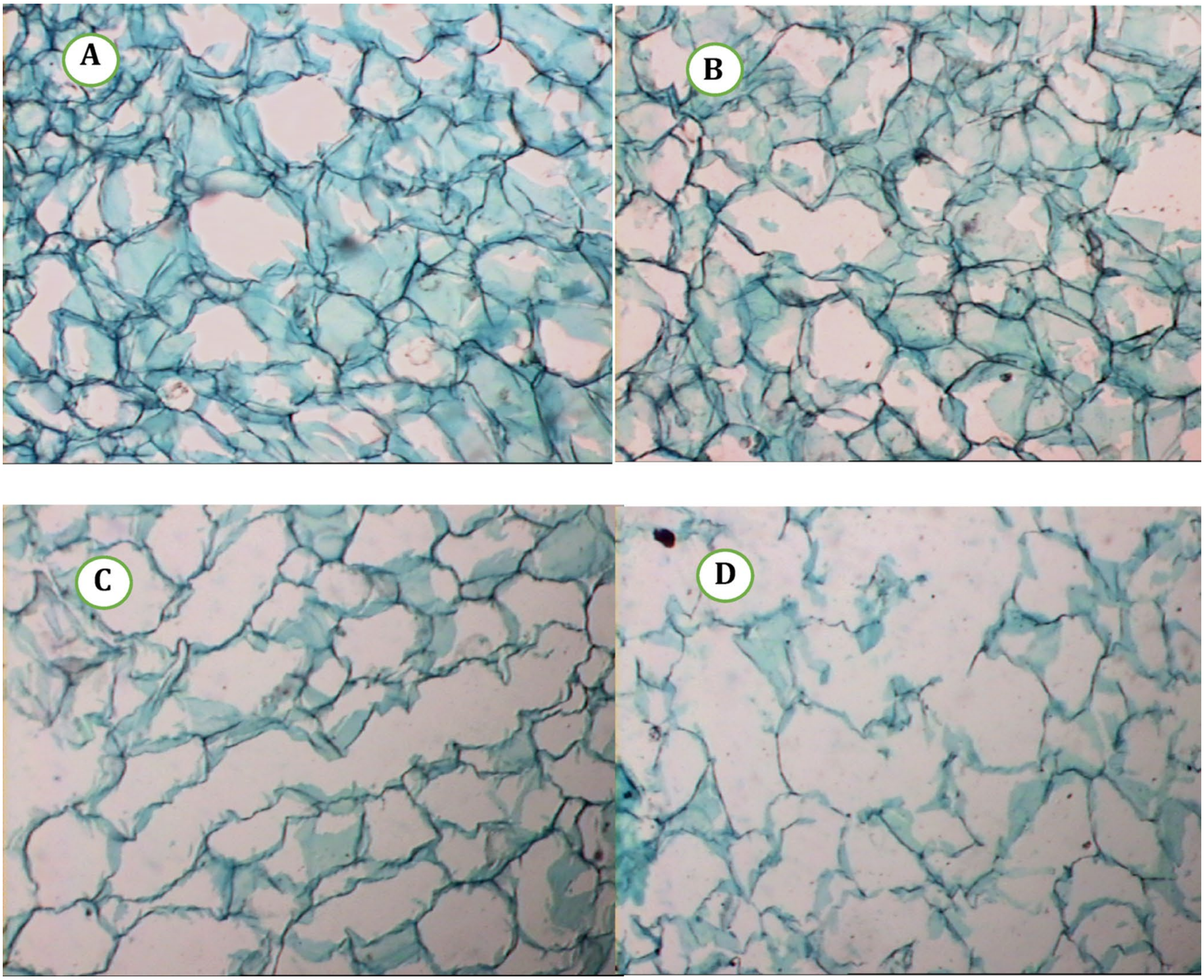


Fig. 5 Transversal microscopic sections in the cortex tissues of EB-disordered Anna apple fruit showing the developmental stages of EB incidence (x: 20 × 10) (cited from Sultan et al. [5])

polygalacturonase, which is significantly inhibited by high Ca concentration. Accordingly, in calcium-deficient tissue polygalacturonase activity is increased hence, disintegration of cell walls should be enhanced [3, 88].

Genomic and transcriptional regulation of enzymatic browning

Genome editing in mitigating enzymatic browning

The precise alteration of plant genomes is a breakthrough in gene-editing technology aimed at enhancing crop quality, particularly in post-harvest management. Over the decades, comprehensive research has resulted in the development of novel genome editing methods to achieve precise and targeted modifications in plants. Oligonucleotide Directed Mutagenesis (ONM) is one of the oldest gene-editing techniques created in plant science research over the decades, while Engineered Nucleases (ENs) have been predominantly utilized previously. Examples of gene-editing tools that have been effectively utilized to generate desirable traits in various crop species include endonucleases/meganucleases (EMNs), zinc-finger nucleases (ZFNs), TAL effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR) [7, 89]. Furthermore, the genetic manipulation through the utilization of small or micro RNA (miRNAs) that target PPO genes has been thoroughly investigated to identify a viable candidate for genetic engineering aimed at preventing or mitigating browning in fruits and vegetables. TALENs and the CRISPR system are the predominant strategies employed in plant genome editing among engineered nuclease systems. The CRISPR/Cas9 system has emerged as the preferred method for plant genome editing due to its straightforward applicability to a majority of common crop species. Furthermore, the use is anticipated not only for utilization in plant breeding programs and post-harvest management to improve storage and shelf life, but it is also capable of preserving the quality of fruits and vegetables. Importantly, CRISPR/Cas9 is a novel technology utilized to deactivate genes encoding PPOs, representing the most promising approach to prevent undesirable browning in plant-derived products. It has effectively converted research potential into reality by inducing precise modifications in target genes through a straightforward mutation at the DNA level [7, 89]. Multiple studies have documented the application of RNA silencing and genome editing to down-regulate *PPO* genes, hence mitigating enzymatic browning in apples, exemplified by the browning-resistant Arctic® Apples [90, 91]. Subsequent to the release of the genomes of apple, banana, and grapevine, molecular investigations in these fruits got more attainable through the application of next-generation sequencing (NGS), high-quality de novo assembly, transgenics, and ultimately, genome editing utilizing CRISPR-Cas9. Presently, the utilization of modern genome-editing approaches, such as CRISPR-Cas systems, facilitates both fundamental and applied research on apple, banana, and grapevine with speed and precision. With the advancement of CRISPR technologies, including base editing, prime editing, and several Cas protein types [92]. Similarly, CRISPR/Cas9-mediated genome editing has been effectively implemented in several fruit crops, including apple, kiwi, orange, and banana, demonstrating its applicability and utility [93]; therefore, the utilization of CRISPR technology in enhancing fruit crops and the potential strategies for developing transgene-free fruit cultivars. Furthermore, a CRISPR/Cas9-based mutagenesis approach could be utilized to knock-out various *PPO* genes in apples, which exhibit elevated transcript levels in fruit.

Transcriptional regulation of enzymatic browning

The expression profiles of PAL and PPO, as two particular candidate genes associated with the polyphenolic pathway, elucidated the physiology of flesh browning. The transcriptome analysis revealed that the expression levels of browning-related genes *PPO* and *POD* were considerably higher in the browning-sensitive cultivar compared to the browning-resistant cultivar in freshly cut apples [22]. The expression of *PPO* genes related to browning is intricate, with variations observed among plant species, within various regions of the same plant, and even within identical plant parts [94]. Plants are believed to have two types of *PPO* genes. Standard conditions trigger the expression of one type of *PPO* genes, while environmental pressures, pathogen incursions, and mechanical damage trigger the expression of the second type. The PPO expressed by these genes can augment melanin synthesis, thereby improving the resilience of plants to detrimental exogenous influences. A previous investigation of the three-dimensional structure of the Fuji apple *MPPO* found 6 α-helices, 2 short β-chains, and 10 random coils [95]. The apple PPOs associated with genes *MDP0000500159* and *MDP0000744636* were detected in mitochondria. The three theories about the mechanism of browning are predicated on the spatial distribution of phenolase, free radical damage, and the protective enzymatic system. The free radical injury hypothesis posits that, under typical conditions, free radical metabolism is regulated by an inherent defense system within the body. Nevertheless, during drought, elevated salt, CO_2, O_2, low temperatures, or water scarcity, the equilibrium of active oxygen production and removal is disrupted due to excessive free radical generation, resulting in cellular damage and browning in plants [69, 94]. Thus far, genetic control of flesh browning has been only partially evaluated using the QTL mapping approach, identifying many genetic regions associated with flesh browning [90]. As the browning process is contingent upon the enzymatic activity of PPO, ten *PPO* genes were found and mapped to three primary chromosomes (2, 5, and 10) in the 'Golden Delicious' apple genome. Among these genes, a specific element designated as *Md-PPO*, situated on chromosome 10, was subjected to further examination and genetic mapping in two apple progenies ('Fuji x Pink Lady' and 'Golden Delicious x Braeburn'). Both linkage maps, consisting of 481 and 608 markers respectively, were utilized to identify QTL areas associated with fruit flesh browning, resulting in the finding of 25 QTLs linked to various browning parameters. Furthermore, *Md-PAL* is recognized for its ability to activate the complete polyphenolic cascade. Similarly, *Md-PPO* was subsequently considered to be more activated in order to regulate the polyphenolic signaling pathway. This gene exhibited two different haplotypes, which may account for the initial enzymatic quantity stored in the cell (before wounding) and influence the degree of ultimate browning [69].

PPO plays a key role in the initial browning phase; nevertheless, the expression of the PPO gene was not elevated compared to that of the WRKY, AP2, and MYB transcription factors [94]. Transcriptome analysis revealed that *MdPPO7* expression contributes to flesh browning following cutting. The injection of fruit and stable genetic transformation of the *MdPPO7* gene into apple fruit and calli revealed that syringic acid, procyanidin, phloridzin, chlorogenic acid, gallic acid, catechin, and caffeic serve as catalytic substrates in the browning process. Moreover, transcriptome analysis revealed that the WRKY transcription factor (MdWRKY3) interacts with the promoter area of the polyphenol oxidase gene (*MdPPO7*) and positively influences its expression, hence facilitating apple flesh browning [81]. MYB transcription factors are essential for the development of eukaryotic meristems, secondary metabolism, cell cycle regulation, stomatal dynamics, and responses to biotic and abiotic stressors. In apple, MYB10 modulates anthocyanin production. The apple bHLH3, recognized as a collaborator with MYB1/10 in the MBW complex, has also been associated with the activation of *ACO*, *ACS1*, and *ACS5* genes to promote ethylene synthesis. This activation may occur through the up-regulation of *ERF* genes, establishing a connection between anthocyanin concentrations and ethylene, alongside increased levels of PPO and substrates for the browning reaction [96, 97]. Bielsa et al. [90] demonstrated the utilization of three apple cultivars exhibiting varying EB phenotypes, from minimal to extreme browning in apple pulp, to investigate transcriptome alterations across time following fresh cutting (0, 30, and 60 min). At 60 min (T60 vs. T0), 77 differentially expressed genes (DEGs) were common across all genotypes, indicating a conserved regulatory network. This network comprised genes that encode protein families, including calcium-binding proteins, heat-shock proteins, redox-responsive transcription factors, WRKY family transcription factors, zinc finger family proteins, and disease resistance proteins, among others. A co-expressed gene cluster, discovered via Weighted Gene Co-Expression Network Analysis (WGCNA), was found to correlate with EB and comprised 323 genes enriched in various biological categories as per Gene Ontology analysis. Furthermore, a comprehensive examination of the identified WGCNA gene cluster regulatory sequences facilitated the identification of cis-regulatory regions associated with the *CAMTA*, *WRKY*, and *WUSCHEL* transcription factor families. The finding of these sequences, coupled with a substantial and diversified array of overexpressed transcription factors from multiple families (*WRKY*, *ERF*, *GRAS*, *GATA*, etc.), indicates a highly controlled stress response intricately linked to innate plant immunity. In comparison to the gene expression of various principal transcription factor families during the browning process, *PPO* expression was usually up-regulated and then down-regulated, while *PAL* expression was up-regulated in the later stages of browning. PAL would initiate the initial reaction in phenylpropanoid metabolism, leading to the production of diverse phenolic compounds in subsequent reactions. Additionally, the findings demonstrated that tyrosine aminotransferase (TAT) interacts with PPO, whereas *PAL1* does not [94].

Tang et al. [79] utilized metabolomics to demonstrate that the concentrations of hyperoside, POD, superoxide dismutase (SOD), and ascorbic acid, together with the expression levels of associated genes (*MdPAL*, *MdCHS*, *MdCHI*, *MdANS*, and *MdFLS*), were diminished in apples susceptible to browning. Four genes encoding PAL and one gene encoding 4-coumarate-CoA ligase (4CL) were identified in the phenylpropanoid biosynthetic pathway, all exhibiting elevated expression levels relative to the control group. Additionally, two *PAL* genes demonstrated heightened expression levels at T0 minutes and T60 minutes, respectively. In the flavonoid biosynthetic pathway, the cinnamate-4-hydroxylase gene (*C4H*), flavonol synthase (*FLS*) gene, and chalcone-flavanone isomerase gene (*CHI*) were upregulated in response to exogenous Se treatment at 60 min post-cutting compared to 0 min, whereas in the control group, these genes exhibited an opposite trend [22]. Similarity, Xu et al. [98] indicated that the expression levels of polyphenols, including catechin, quercetin, and phloretin, diminished in the browned tissues of apples, leading to a reduction in intracellular antioxidant compounds responsible for scavenging reactive oxygen species (ROS). This conclusion was corroborated by transcriptional analysis [22], which revealed that the expression levels of the *PAL*, *4CL*, *C4H*, *FLS*, and *CHI* genes associated with the phenylpropanoid, and flavonoid biosynthetic pathways were elevated.

The future perspectives on enzymatic browning

The Earth's population is growing year after year, and their food requirements are increasing, which necessitates reducing food waste to achieve a sustainable healthy food. Therefore, controlling EB has become a priority for those interested in the food industry. In this regard, various attempts have been made to suppress browning enzymes either by synthetic chemicals or plant extracts, which may not be acceptable to some people or are less effective in other cases. Therefore, those interested in the apple industry are looking to select varieties that are less sensitive to enzymatic browning, in addition to using genetic engineering tools to inhibit the browning enzymes. There are several methods to control EB in apples and apple products, such as the application of edible coating and chemicals like ascorbic acid and sodium bisulfate, as well as lowering the pH value, etc [7, 8, 16, 18]. Unfortunately, some consumers do not accept these chemicals, and they are also inappropriate for use in whole fruit. Therefore, selection of genotypes (cultivar and rootstock) and modern plant breeding techniques using genetic engineering by gene editing and controlling the relative gene expression pattern of the PPO enzyme may be more effective [16, 18].

Genotype selection

Cultivar selection

There is a great diversity in apple genotypes, with over 7,000 cultivars are registered worldwide including autochthonous and slandered cultivars. However, global apple production relies on a very limited number of standard varieties, such as ‘Fuji’, ‘Gala’, ‘Golden Delicious’, ‘Red Delicious’ and ‘Granny Smith’ [99, 100]. Autochthonous cultivars have higher contents of secondary compounds, such as phenols [27, 28, 35, 41, 42, 63], making them more resistant to diseases and pests and more adaptable to climate change than standard varieties [35]. The phenol content of standard cultivars has been reduced through breeding programs to reduce their susceptibility to enzymatic browning [42]. Genetic diversity in autochthonous and standard apple cultivars can be leveraged to further advance the apple industry. The chemical composition of the fruit plays a key role in classifying apple cultivars into those for fresh consumption and those for processing [28, 35]. While those interested in human diet and the pharmaceutical industry are looking for apples with high phenolics [25, 26, 30], food processors are looking for the opposite [45]. Plant breeders are challenged to meet the needs of stakeholders in the apple industry from growers to consumers and processors. While keeping in mind that disease and pest resistance and climate change adaptation are strategic objectives for reducing pesticide use, protecting the environment and public health, and achieving food sustainability.

As mentioned above in this review, apple cultivars vary in their phenolic contents, and the phenolic composition plays an important role in their browning tendency and PPO activity in various cultivars. Tables (3 & 4) summarize the results of recent studies conducted on the phenolic content of some commercial apple cultivars. The variation in phenolic content of fruits of the same variety (Tables 3 and 4) from one study to another reflects the interaction of genotype with the climate factors and the response to agricultural practices from one region to another [27].

Many authors are of the view that low molecular weight phenolic compounds like catechin, *p*-coumaric acid, and chlorogenic acid may be more effective substrates for PPO [34, 80, 81]. The concentration of those substrates varies according to the cultivar genotype (Tables 3 and 4). This fact suggests that the evaluation of the cultivars for browning susceptibility should be based on the fruit content of individual phenolic compounds, especially those favored substrate for PPO.

The relation between cultivar genotype, enzymatic browning activities, individual phenolic compounds, and browning intensity in apple cultivars (Fig. 6) has been studied by Serra et al. [34]. Fourteen cultivars were subjected to the study, divided into three groups according to flesh browning sensitivity, which are: lower sensitive (Granny smith, Cripps Pink, Red Rome, and Durello), moderately sensitive (Fuji, Florina, Braeburn, Golden Delicious, Delorina, and Gloster), and highly sensitive (Topaz, Fiesta, Jonathan, and Mondial Gala) cultivars. In most cultivars, they found a positive correlation between quantitative phenolic content, PPO activity, and browning intensity. However, this relationship cannot be taken for granted, as the ‘Topaz’ cultivar exhibited low PPO activity despite its high total phenolic content and at the same time showed a deeply brown appearance. Therefore, it appears that additional oxidation pathways accelerate the browning reaction. It was reported that phenolic compounds with low molecular weight, such as catechin, *p*-coumaric acid, and chlorogenic acid, seem to function as a more effective substrate for PPO [34, 80, 81]. This may elucidate why cultivars like ‘Granny Smith’ and ‘Cripps Pink,’ which exhibited the minimal concentrations of efficient substrates for PPO, including *p*-coumaric acid, catechin, and chlorogenic acid (Fig. 6), exhibited the least browning [34]. The EB-sensitive cultivars can be devoted to fresh consumption, while cultivars with lower sensitivity can be devoted to fresh cut and cider production [34, 45]. The sensitivity of apple cultivars to EB depends not only on the phenolic content but also on the antioxidant contents, particularly ascorbic acid (AsA). It is known that plants protect themselves against oxidative stress by naturally producing antioxidants such as AsA. AsA can bind to free radicals and reduce oxidative stress, including the oxidation of phenols. Furthermore, AsA has the ability to reduce *o*-quinones to diphenols, thereby inhibiting melanin formation [16, 19, 20, 101]. In general, cultivars cannot be classified as susceptible or less susceptible to EB based on their phenolic content only, as other factors can influence the phenolic content of a cultivar, such as rootstocks, cultivation area, agricultural practices, etc. Further in-depth studies are needed on this matter.

Rootstock selection

There are no available studies that address the effect of rootstock on the activity of browning enzymes in fresh fruit, and particularly in apples. Several studies have examined the effect of rootstock on the phenolic content of apples, which is expected to influence PPO activity and browning susceptibility. Rootstock genotype can affect the amount of phenolic through the control of PAL activities [103, 104]. The perfect compatibility between apple scion and rootstock can enhance phenolic compound synthesis and improve the phenolic profiles [38]. The effect of eleven rootstocks on the phenolic content of ‘Ligol’ apples was

Table 3 The summary of individual phenolic compounds concentration in fruit peel of some commercial apple cultivars

Phenolic compound concentration (mg/100 g FW)	Cultivars						
	Golden Delicious	Red Delicious	Granny Smith	Fuji	Gala	Cortland	Jonagold
(+)-Catechin	3.81–10.27 [51, 59]	3.53–8.19 [51, 59]	0.369–37.40 [49, 52]	0.15–10.68 [51, 52]	0.60–10.05 [51, 52]	3.06–12.39 [41, 59, 63]	5.167 [56]
(+)-Epicatechin	20.72–31.00 [51, 59]	45.11–59.16 [51, 59]	2.21–24.60 [49, 52]	2.50–44.80 [51, 52, 54]	2.40–34.73 [51, 52, 54]	2.93–16.47 [41, 59, 63]	7.995 [56]
Procyanidin B1	3.20–15.91 [31, 49, 59]	12.70–18.38 [49, 59]	17.30 [49]	NA	8.05–15.42 [31, 57]	4.20–15.30 [58, 59, 63]	1.40 [58]
Procyanidin B2	6.90–27.68 [31, 49, 50, 59]	43.30–46.81 [49, 59]	24.10–55.80 [49, 59]	NA	15.65–27.72 [31, 57]	2.52–25.11 [58, 59, 63]	20.40 [58]
Quercetin	3.20 [51]	0.12 [51]	9.07 [52]	0.17–14.29 [51, 52]	4.34 [52]	1.76 [41]	22.4** [42]
Quercetin-3-glucoside (Isoquercitrin)	13.25 [31]	1.52–23.15 [59, 60]	5.70 [56]	0.41* [102]	5.33–5.60 [31, 57]	7.74–8.92 [41, 59, 63]	12.67 [56]
Quercetin-3-galactoside (Hyperoside)	70.20 [31]	9.01–320.51 [59, 60]	6.342 [56]	1.85* [102]	31.10–33.98 [31, 57]	10.11–15.07 [41, 59, 63]	10.383 [56]
Quercetin-3-rutinoside (Rutin)	1.16** [42]	1.76 [51]	1.88–39.00 [49, 52]	0.80–1.89 [51, 52]	0.04–1.37 [51]; Teixeira et al., [52]	NA	10.02 [56]
Quercetin-3-rhamnoside (Quercitrin)	6.62 [31]	3.23–17.17 [59, 60]	1.425 [56]	4.51–18.00 [52, 54]	2.10–19.10 [31, 52, 54, 57]	3.16–4.20 [41, 59, 63]	2.744 [56]
Gallic acid	0.49 [31]	0.716 [60]	0.28 [52]	0.04–0.43 [51, 52]	0.26–0.38 [31, 52]	NA	4.2** [42]
Chlorogenic acid	4.10–6.39 [31, 51]	4.46–11.98 [51, 59]	0.15–0.60 [49, 52]	1.55–17.40 [51, 52, 54]	2.42–22.40 [31, 51, 52, 54, 57]	1.10–2.48 [41, 59, 63]	10.575 [56]
Caffeic acid	0.08–0.63 [31, 60]	0.891 [60]	1.00 [49]	NA	0.05–0.55 [31, 60]	NA	2.520 [56]
p-Coumaric acid	0.09 [31]	NA	ND [52]	0.06 [54]	0.08–0.29 54, 31]	ND [52]	2.00** [42]
p-Coumaroyl quinic acid	0.90–0.96 [50, 59]	0.55 [59]	0.30 [50]	NA	NA	1.42–2.88 [59, 63]	NA
Cyanidin-3-galactoside (Ideain)	0.12 [31]	447** [40]	NA	NA	19.77–21.01 [31, 57]	14.35 [63]	NA
Cyanidin-3-glucoside (Glucocyanidin)	ND [31, 59]	14.89 [59]	ND [52]	4.49 [52]	0.09–6.95 [31, 52]	15.98 [59]	NA
Phloridzin	0.80–14.31 [31, 51]	0.99 [51]	0.62–1.20 [49, 52]	1.62–11.10 [51, 52, 54]	1.41–13.00 [31, 51, 52, 54]	3.08–3.97 [41, 59, 63]	8.8** [42]
Phloretin	1.02 [51]	0.55 [51]	2.90 [49]	0.86 [51]	0.68 [63]	0.36 [63]	NA

ND: not detected, NA: no available data, *: values per 100 gm fresh weight whole fruit, **: values per 100 gm dry weight

studied under Lithuanian conditions [103]. The results indicated that the highest phenolic content was recorded in the fruits of trees grafted onto super-dwarfed rootstocks (P 61 and P 22). Dwarfed (M.9 and P 62) and semi-dwarfed (M.26) rootstocks achieved the lowest content of the tested phenolic compounds. The fruit contents of (+)-catechin, procyanidin B1, and total procyanidins were affected by the rootstock genotype, while quercitrin, (-)-epicatechin, total catechin, phloridzin, and chlorogenic acid were affected to a lesser extent by the rootstock type. On the other hand, Butkeviciute et al. [38] reported that the effect of rootstock on fruit phenolic content depends solely on rootstock genotype, regardless of the effect of rootstock on the degree of scion vigor. The rootstocks produced in New Zealand (PFR series) showed lower amounts of phenolic compounds than those produced in the UK (EM series).

Genetic engineering

Plant breeders via genetic engineering have been able to manipulate the plant genome using molecular methods to obtain the desired phenotype according to the grower

Table 4 The summary of individual phenolic compounds concentration in fruit flesh of some commercial apple cultivars

Phenolic compound concentration (mg/100 g FW)	Cultivars						
	Golden Delicious	Red Delicious	Granny Smith	Fuji	Gala	Cortland	Jonagold
(+)-Catechin	0.40–4.21 [31, 34, 49, 51, 59]	2.43–4.40 [49, 51, 59]	2.60–13.60 [34, 49]	5.38–10.00 [34, 51]	0.7–8.98 [51, 54, 57, 63]	0.44–4.17 [41, 59]	0.5** [42]
(+)-Epicatechin	1.86–7.49 [31, 34, 49, 51, 59]	3.60–12.26 [49, 51, 59]	6.00–7.10 [34, 49, 56]	5.04–7.00 [34, 51]	0.37–9.54 [51, 54, 57, 63]	1.14–13.38 [41, 59]	1.405 [56]
Procyanidin B1	1.00–3.19 [31, 49, 59]	1.10–9.60 [49, 59]	6.20 [49]	3.38 [61]	1.67–2.34 [31, 57, 63]	3.79–5.10 [58, 59]	1.30 [58]
Procyanidin B2	2.30–12.14 [31, 49, 59]	9.70–12.23 [49, 59]	9.70 [49]	10.44 [61]	2.08–8.33 [31, 57, 63]	6.65–14.01 [59, 63]	2.00** [42]
Quercetin-3-glucoside (Isoquercitrin)	0.28 [31]	0.13* [99]	0.27* [99]	0.67 [61]	0.17* [99]	NA	NA
Quercetin-3-galactoside (Hyperoside)	0.70 [31]	0.82* [99]	0.80* [99]	0.31* [99]	0.69* [99]	NA	NA
Quercetin-3-rutinoside (Rutin)	0.28* [99]	0.59* [99]	0.58* [99]	0.31* [99]	ND [28, 51]	NA	NA
Quercetin-3-rhamnoside (Quercitrin)	0.20–0.64 [31, 59]	0.37 [59]	0.819* [99]	0.44* [99]	0.15 [57]	ND [59, 63]	1.7** [42]
Gallic acid	0.201 [60]	0.217 [60]	0.01* [99]	0.01* [99]	0.215 [60]	NA	2.1** [42]
Chlorogenic acid	1.45–15.36 [31, 34, 49, 51, 59]	3.57–12.50 [49, 51, 59]	2.8–6.34 [34, 49, 56]	4.07–20.00 [34, 51]	3.44–18.31 [51, 54, 57, 63]	0.36–10.30 [41, 59, 63]	11.21 [56]
Caffeic acid	0.04–0.60 [31, 49]	0.20 [49]	0.4–1.44 [49, 56]	NA	0.03 [60]	NA	0.60 [56]
p-Coumaric acid	0.09–0.80 [31]; [34]	NA	0.20 [34]	0.60 [34]	0.01–0.20 [31, 54]	NA	1.70** [42]
p-Coumaroyl quinic acid	1.10–1.40 [50, 59]	1.17 [59]	0.30 [50]	NA	1.94–2.88 [57, 63]	0.29–5.03 [41, 59, 63]	NA
Cyanidin-3-galactoside (Ideain)	NA	ND [40]	NA	0.50 [61]	NA	NA	NA
Cyanidin-3-glucoside (Glucocyanidin)	ND [31]	ND [40]	NA	NA	ND [28, 31]	NA	NA
Phloridzin	0.29–1.95 [31, 34, 49, 51]	0.25 [51]	0.40–0.50 [34, 49]	1.40 [34, 51]	0.20–1.18 [54, 57, 63]	0.8–1.64 [41, 59, 63]	0.7 [42]
Phloretin	0.11–0.30 [34, 51]	0.30 [51]	NA	0.20 [51]	ND [51]	1.64 [41]	NA

ND: not detected, NA: no available data, *: values per 100 gm fresh weight whole fruit, **: values per 100 gm dry weight

requirements, which are called genetically modified (GM) plants [89, 105]. While most researchers were interested in producing apple genotypes that are resistant or tolerant to biotic and abiotic stress, some were interested in producing apples that are resistant to enzymatic browning, which later became known as the Arctic® apple. The Arctic® apple was developed by Okanagan Specialty Fruits Inc., (OSF) Summerland, British Columbia, Canada at the request of a producer based on a consumer survey. The Arctic® apple remains a unique case in point, as researchers focused on producing an apple that is resistant to the enzymatic browning [106]. The non-browning trait was induced by inactivation of PPOs encoded genes into 'Golden Delicious', 'Granny Smith', and 'Fuji' cultivars via the Agrobacterium-mediated transformation method [72, 106]. According to Zhao at al. [107] and Stowe and Dhingra [106], conserved zones of four apple genes (*MdPPO2*, *MdGPO3*, *MdAPO5*, *and MdpSR7*) were ligated and put in a touch orientation under the control of the CaMV35S promoter and nopaline synthase terminator to produce a PPO inhibitor transgene. The use of transgenic sense suppression of PPO activity has been registered by OSF via the US patent No. 8,563,805 for the year of 2013 and Arctic® Apple has subsequently become a distinctive trademark within the apple industry [106]. Arctic® Apple is a product towards reducing the waste and limiting the use of chemicals in the apple industry. There are some reservations about GM crops in general, perhaps due to a lack of trust in their developers or for unspecified ethical reasons. Genetic engineering opponents fear that consuming the Arctic® apple will transfer this resistance to

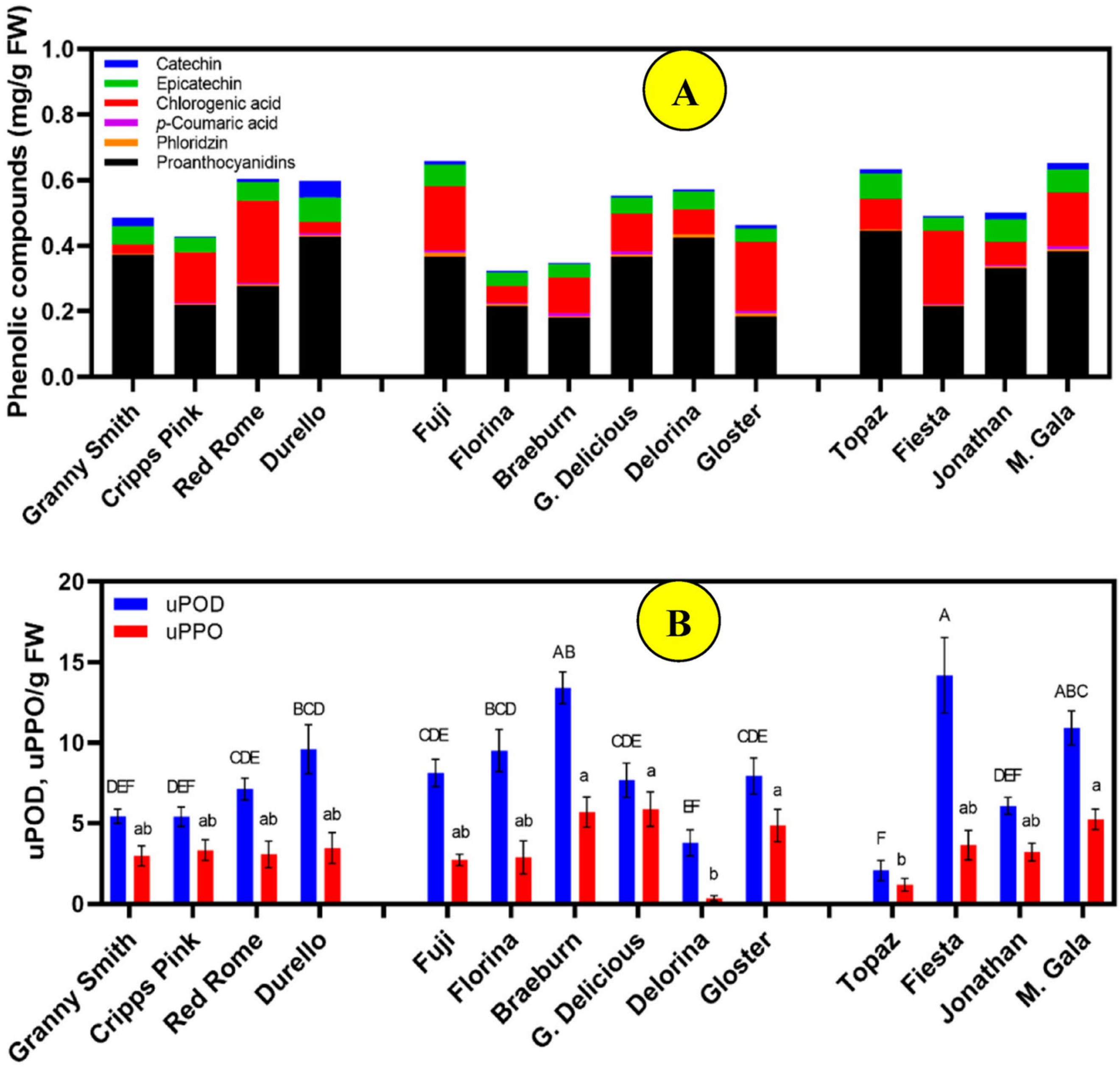


Fig. 6 The relationship between cultivar genotype, phenolic compounds concentration (mg/g flesh fresh weight (A) and enzymatic browning activities (uPOD, uPPO/g flesh fresh weight (B) in 14 fresh-cut apples cultivars (cited from Serra et al. [34] with permission)

humans [108]. It should be noted that the trait of resistance in the Arctic® Apple to the EB is only transmitted through seeds once they have been planted. Thus, the Arctic® Apple pollens can pollinate non-GM cultivars, but the resulting fruit will not carry the EB resistance gene [106].

Conclusion

Apples, like many fresh fruits and vegetables, are susceptible to enzymatic browning caused by PPOs and POD enzymes. Phenolic compounds (substrate), are mainly localized in the vacuole and cell wall, while PPO and POD enzymes are mainly in some cell organelles like plastids and mitochondria. Collapsing or cell wall degradation enables the oxidizing enzymes PPO and POD to be in direct contact with their substrates in the presence of oxygen to complete the browning reaction. Phenolics with low molecular weight, like catechin, *p*-coumaric acid, chlorogenic acid and caffeic acid seem to function as a more effective substrate for PPO. Numerous studies indicate that the gene pool related to the apple pulp's response to EB encompasses a varied array of differentially expressed genes that encode calcium-binding proteins, heat shock proteins, redox-responsive transcription factors, WRKY transcription factors, zinc finger proteins, and disease resistance proteins, among others. Moreover, distinct genotype responses in redox homeostasis, cellular reorganization, and lipid metabolism were observed between low and high browning genotypes, underscoring notable differences in the constitutive expression of critical enzymes such as PPO and PAL, thereby affirming the intricacy of this condition. Further investigation is required to corroborate the principal differentially expressed genes and transcription factors found (e.g., knockout, RNA silencing, or overexpression studies). EB control has become a priority for those interested in the food industry. Therefore, those interested in the apple industry are looking to select cultivars that are less sensitive to EB, in addition to using genetic engineering tools to inhibit the browning enzymes. The Arctic® apple is a unique example of this, as researchers focused on producing an apple resistant to EB with the goal of reducing waste and limiting the use of chemicals in apple industry. The Arctic® apple was developed by OSF at the request of a producer based on a consumer survey.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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