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#### Chapter

# Anthocyanins in Apple Fruit and Their Regulation for Health Benefits

Kaori Matsuoka

### Abstract

In most industrialized countries, cancer and cardiovascular disease have been linked to lifestyle choices, the most important of which is diet. Antioxidants show protective effects against both of these diseases. Anthocyanins contribute greatly to the antioxidant properties of certain colorful foods, such as apples. Apples are rich in anthocyanins in the peel, followed by the whole fruit and then the flesh. From a nutritional point of view, therefore, regular consumption of apples with peel is recommended to enhance the dietary intake of antioxidant compounds. The anthocyanin concentration of apple peel changes according to internal plant conditions (e.g., fruit maturity and plant hormones), environmental factors (e.g., light, temperature, and nutrients), and the cultivar. The combination of cultivar variation and responsiveness to specific environmental conditions could create opportunities for the production and processing of apples with improved anthocyanins and antioxidant properties.

**Keywords:** anthocyanin, antioxidant activity, apple fruit, environmental factor, fruit maturation, global climate change, human health, plant hormone

#### 1. Introduction

The apple (*Malus domestica* Borkh.) is produced globally; about 89 million tonnes was produced in 2016, ranking it third in worldwide fruit production [1]. In Poland, most people across all age groups eat from one to six apples a week. Most Polish customers consider flavor, taste, and firmness at the top of the list and give less consideration to nutritional value, peel color, and size [2]. Nevertheless, Polish customers of all age groups prefer more redness over greenness [2]. In a study of Estonian customers from 2007 to 2012, the most important consumer preferences were apple taste, followed by appearance (e.g., color) and health benefits, which were rated of equal importance, and finally price; consumer preferences in taste and color remained unchanged, whereas preference for domestic and organic apples decreased, over the 5-year period survey [3]. In a UK study, consumers associated red apples with sweet sensory descriptors and green apples with grassy, astringent and drying, acidic, sour sensory, or unripe descriptors [4]. Therefore, the color of apple peel is important for market value, and well-reddened fruit has an increased price rating in Japan [5].

The redness of apple peel is due to the accumulation of anthocyanins, which are water-soluble plant pigments responsible for the blue, purple, and red in many plant tissues of fruits, flowers, and vegetables. Willett [6] showed that approximately 32% of cancers could be avoidable by changes in diet. People with low fruit and vegetable intake have about twice the risk of most cancers as those with high intake [7]. In addition, a case-control study of a group of 33 women recently diagnosed with breast cancer and a control group of 33 healthy women demonstrated that regular ingestion of apples, watermelons, and tomatoes was associated with protection against breast cancer [8]. Increased intake of apple fruits has been associated with reduced risk of disease, because apple fruits—especially their peel have strong antioxidant activity [9], which benefits human nutritional health.

This review provides a general overview of the nutritional impacts of polyphenols, flavonoids, and anthocyanins, which serve as antioxidants and counteract the prooxidant load of the human body [10]. The review focuses on anthocyanin in apple fruit peel and its health benefits and the means by which this anthocyanin concentration could be improved by regulating cultivation management and breeding cultivars with high anthocyanin levels. The literature on anthocyanin synthesis in red-fleshed apple fruits is not reviewed here; the details of genetic, environmental, and plant hormonal factors involved in anthocyanin synthesis have been reported by Honda and Moriya [11].

#### 2. Polyphenols, flavonoids, and anthocyanins

## 2.1 Concentrations of polyphenols, flavonoids, and anthocyanins and antioxidant activity in common foods

Polyphenols are among the most numerous products of the secondary metabolism of plants and are an integral part of the human diet [12]. They constitute more than 8000 phenolic structures and can be divided into at least ten different classes, depending on their basic chemical structure. The recent interest in food phenolics has increased greatly owing to their possible benefits to human health, such as prevention of cancer, cardiovascular disease, and other pathologies. The polyphenolic concentration of plant foods can vary by several orders of magnitude [12], with the cereals barley and sorghum having considerably higher levels than those found in some more commonly consumed foods (e.g., fruits, nuts, and vegetables). In the fruits mentioned in the review [12], blackcurrant has the highest polyphenolic concentration, followed by grape, raspberry, and apple. Therefore, apples can serve as an important source of polyphenols as dietary foods.

Plant flavonoids constitute one of the largest groups of naturally occurring phenolics, and they possess an ideal chemical structure for antioxidant activity. They commonly contain diphenylpropanes with a 15-carbon skeleton ( $C_6$ — $C_3$ — $C_6$ ), which comprises two aromatic rings linked through three carbons that usually form an oxygenated heterocycle. The flavonoids in foods comprise 13 types of basic structure, one of which is anthocyanidin [12]. The glycosides of anthocyanidin are referred to as anthocyanins.

Anthocyanins vary in the number and position of hydroxyl and methoxyl groups on the basic anthocyanidin skeleton. There are over 600 naturally occurring anthocyanins. Approximately 17 anthocyanidins are found in nature, and 6 of them—cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin are ubiquitously distributed in nature.

Antioxidants are present at high levels in apples [13], and the antioxidant activity of apples has significant positive relationships with total phenolic and flavonoid concentrations [10]. When compared with many other commonly consumed fruits, apples have the second-highest level of antioxidant activity [13]. Antioxidant activity provides a measure of protection that slows the process of oxidative damage, which is an important cause of disease initiation and progression in the human body [14].

# 2.2 Concentrations of polyphenols, flavonoids, and anthocyanins and antioxid ant activity in apple fruits

Many studies have shown that polyphenol, flavonoid, and anthocyanin concentrations and antioxidant activity in apples differ between the edible parts of the fruit. In a study of four cultivars, "Rome Beauty," "Idared," and "Cortland" (all three red-skinned), and "Golden Delicious" (golden-skinned), total phenolic and flavonoid concentrations were highest in the peel, followed by the whole fruit and then the flesh [9]. The peels of these apple cultivars also had significantly higher total antioxidant activities than the whole fruit or the flesh. In a study of ten different cultivars in New Zealand, on average, 46% of polyphenols in whole apples were in the peel, and essentially all of the flavonols (quercetin derivatives) were found to be present in the peel [15]. In a more recent study of "Fuji" (bright red skin with green patches) and "Epagri COOP24" and "Epagri F5P283" (both deep red skin) cultivars, the total phenolic and total flavanol concentrations and total antioxidant activity were also highest in the peel, followed by the whole fruit and then the flesh [16]. The peel and whole fruit possessed 1.1–4.1 times the total phenolic concentration, 1.2–4.9 times the total flavanol concentration, and 1.1–3.9 times the total antioxidant activity of the flesh. The total phenolic concentrations of the flesh, the whole fruit, and the peel were all positively associated with total antioxidant activity [16]. From a nutritional point of view, the above findings suggest that regular consumption of apples with peel is recommended to enhance the dietary intake of antioxidant compounds.

## 2.3 Anthocyanin concentration in apple peel and its relation to colorimetric values

In apples, pigmentation is controlled by the relative concentrations of anthocyanin pigments; the main pigment responsible for the red is cyanidin-3-galactoside (idaein). The anthocyanin concentration of the peels of mature apples of eight "Gala" strains (bright red over yellow background) provided regression coefficients of determination ( $R^2$ ) of 0.73 and 0.74 and  $P \leq 0.05$  for the colorimetric values of a\*/b\* (greater value means higher redness) and hue angle (lower value means higher redness), respectively, as measured with a tristimulus colorimeter [17]. This strong relationship between anthocyanin concentration and chromaticity parameters in apple peel shows good prediction of anthocyanin concentration based on chromaticity values and offers the possibility of using a portable colorimeter for nondestructive estimations of fruit anthocyanin concentration in situ.

## 3. Apple fruits and their health benefits for humans

Regular consumption of apples is inversely associated with breast cancer occurrence and affords some degree of protection against the development of the disease [8]. Wolfe et al. [9] investigated the effects of the apple peel, the whole fruit, and the flesh in inhibiting the growth of HepG<sub>2</sub> human liver cancer cells in vitro by estimating the median effective concentration (EC<sub>50</sub>; expressed as concentration of apple component (mg mL<sup>-1</sup>)) as an indicator of antiproliferative activity. Phytochemical extracts of the apple peels of each cultivar tested ("Rome Beauty," "Idared," "Cortland," and "Golden Delicious") inhibited the growth of HepG<sub>2</sub> cells more effectively than extracts of the flesh or the whole fruit. The peels also had a lower EC<sub>50</sub> than the flesh and the whole fruit components, representing higher antiproliferative activity. The inhibitory effects of the cancer cell proliferation varied widely depending on the apple cultivar, with "Rome Beauty" apple peels showing the greatest bioactivity.

#### 4. Anthocyanin accumulation during fruit maturation

Anthocyanin production depends mainly on carbohydrates (especially galactose), which are formed during photosynthesis and glucose metabolism in apple leaves and are later transported to the fruit [18]. Therefore, anthocyanin synthesis is closely associated with plant metabolism and depends on the stage of fruit development. For instance, during fruit growth, anthocyanin concentrations in "Gala" apple peel are low and fluctuating, and they then increase markedly on the blush side near maturation [19]. Similarly, Chalmers et al. [20] found that anthocyanin accumulation in red apple cultivars occurs rapidly during the transition from the immature to mature stages and precedes the normal harvest date by about 2–3 weeks. Honda et al. [21] also reported that anthocyanin production proceeded rapidly in the fruit peel of potted trees of red-skinned cultivar "Misuzu Tsugaru" during the last week before harvest, not only under control conditions (25°C 12 h/15°C 12 h) but also under hotter temperature conditions (29°C 12 h/19°C 12 h) for 5 weeks. A continuous increase in anthocyanin concentration, especially in the last 2 weeks before harvest, on both the blushed and shaded sides of the apple fruit was also found in a superior redness sport of "Shotwell Delicious" called "Topred Delicious" [18]; later, the authors reported that the largest increase in anthocyanin concentration and color development in the eight "Gala" apple strains occurred from 2 weeks before the commercial harvest date, and the increase continued for up to 1 week after the harvest date [17]. These findings show the importance of the ripening period for anthocyanin accumulation in apple fruit peel.

#### 5. Regulation of anthocyanin synthesis in apple fruits

Anthocyanin synthesis in apple fruits is affected by environmental factors and internal plant conditions, including biotic and abiotic factors such as light, temperature, nutrients, and plant hormones, and by the type of cultivar. The use of special cultivation methods and cultivars that produce high levels of anthocyanin has been proposed for improving anthocyanin concentration and redness of apple peel.

## 5.1 Light irradiation and its effective use for enhancing anthocyanin accumulation

Development of redness in apple fruit peel requires light [22, 23], and the amount of anthocyanin synthesized is correlated with the light intensity received [24]. Therefore, redness development in apple fruit peel is higher on the side that receives more exposure to light (the blushed side) than on the shaded side (**Figure 1**). Hamadziripi et al. [25] revealed the effects of microclimate at different positions in the apple tree canopy "Starking" on peel redness and anthocyanin concentration. They found that the outer-canopy fruits were redder and contained more anthocyanins than the inner-canopy fruits. This seemed to be related to microclimatic differences in irradiance, because outer-canopy fruits were exposed to almost 30 times the irradiance



# ht(

#### Figure 1.

Occurrence of anthocyanins in "Fuji" apple peel on the blushed side (the right side in this photo), which receives more exposure to sunlight.

as fruits in the innermost portion of the canopy, and the average peel temperature of outer-canopy fruits was about 10°C higher than that of the inner-canopy fruits. In addition, among the eight "Gala" strains mentioned in Sections 2.3 and 4, the anthocyanin concentration on the blushed side was about three to five times that on the opposite shaded side at commercial harvest and after the harvest in low- and mediumhigh-coloring "Gala" strains [17]. Apparent differences in anthocyanin concentrations between the blushed and shaded sides of "Gala" apple peel occur near maturation (125 days from flowering), when the anthocyanin concentration increases markedly only on the blushed side [19].

To enhance peel color, cultivation techniques that are effective in promoting anthocyanin synthesis and improving color in apple peels have been developed. In red-skinned cultivars, to allow the apple skin to receive more exposure to light and thereby promote redness, the leaves covering fruits are removed before harvest [5]. Rotating the fruit and branch management such as pruning and putting support posts beneath the canopy can also improve the redness of apple peel [5]. Placing reflective films on the ground also effectively increases the intensity of light entering the apple tree canopy (**Figure 2**) [26, 27]. Ju et al. [27] reported that, after film application, the light intensity inside the tree canopy "Fuji" increased from 30% of daylight to 50–68% of daylight for foil film (crinkled aluminum foil bonded onto cloth) and metalized film (aluminum metalized polypropylene film); the anthocyanin concentration and percent redness of the fruit peel at harvest were also increased.

Anthocyanin synthesis in apple fruit is highly dependent on light quality, such as ultraviolet-B (UVB) and visible light (e.g., red, blue, and white fluorescent light). UVB and white fluorescent light are essential for anthocyanin accumulation, regardless of the temperature, but white fluorescent light alone does not increase anthocyanin concentration [23]. Anthocyanin synthesis is stimulated more by irradiation with UVB with an emission peak at a wavelength of 312 nm (UVB312) than by UVB353 [28]. UVB312 is more effective in stimulating anthocyanin production



Figure 2.

Reflective material laid on the soil surface between tree rows in a "Fuji" apple orchard to enhance the trees' interception of sunlight.

than red or blue light. Simultaneous irradiation with UVB312 and red light [28] or UVB312 and white fluorescent light [29] produces much higher anthocyanin than irradiation with either light alone. This synergism seems to have an important role in development of the desirable red peel under field light conditions.

#### 5.2 Effect of temperature on anthocyanin synthesis

Temperature has a major effect on anthocyanin synthesis in apple plants. As a result of recent global warming, there are increasing concerns that global warming may be detrimental to fruit reddening.

In general, apples redden best in climates with clear bright days and cool nights during the preharvest period [26]. Night temperatures below 18°C enhance reddening in "Fuji" apples [26]. Reay [23] reported that a cool temperature of 4°C in the dark period followed by 20°C with UVB-visible irradiation was the most effective condition for anthocyanin accumulation in apple peel of "Granny Smith." This is the equivalent of cool nights followed by warm clear days. With continuous 20°C temperature in both dark and UVB-visible irradiation periods, the anthocyanin accumulated was less than half that with the former temperature condition. This result shows the importance of a low-temperature period in the development of the red blush on apple peel.

Generally, high temperatures increase the respiration rate and carbohydrate consumption of apple trees, thus reducing anthocyanin synthesis in the fruit. Wang et al. [30] reported that anthocyanin accumulation during the ripening period in the peel of "Royal Gala" red-skinned cultivar grown in hot climates (17–35°C, using artificial heating of fruit on the tree over two 7-day periods) caused a dramatic reduction in peel anthocyanin concentration and decrease in redness compared with those in unheated control fruit (7–22°C for unheated apples). Proctor [24] had earlier found that redness cultivars of "McIntosh," "Northern Spy," and "Cortland" did not color with supplementary light when the 48-h average temperatures were greater than 20°C, suggesting that anthocyanin accumulation requires temperatures lower than 20°C. Consistent with this notion, Honda et al. [21] showed that 15°C was generally the optimal temperature for anthocyanin accumulation during fruit ripening of redness cultivars, "Tsugaru," "Tsugaru Hime," and "Akibae," whereas anthocyanin accumulation was repressed under 30°C.

Iglesias et al. [18] have noted that poor fruit coloration has already become a problem owing to frequent periods of high temperatures (>30°C) with very low rainfall in summer in many apple-producing areas in Spain. Seasonal differences in temperature and rainfall in the period before harvest often cause harvesting to be delayed in order

to attain a certain degree of color, a practice that has negative effects on quality—especially firmness and flavor. This lack of color is an important cause of reduction in grade and is generally associated with poor consumer acceptance. To overcome this problem, Iglesias et al. [18] applied overtree microsprinkler irrigation for 25–30 days preceding the harvest to improve fruit redness and increase anthocyanin concentration in "Topred Delicious" cultivars. Similarly, the warm climate of California can delay harvest owing to inadequate reddening of "Fuji" apples; this has been associated with physiological problems such as skin cracking and internal browning [26].

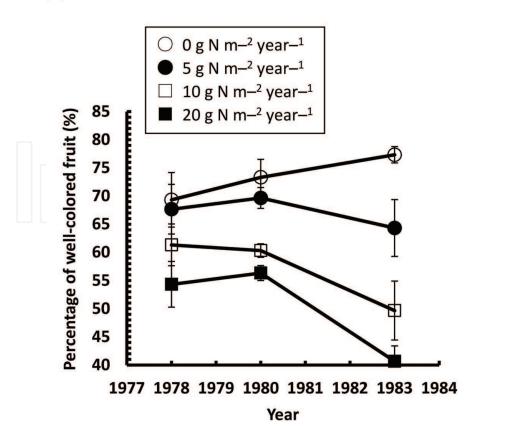
On the basis of 30–40 years of climate records, Sugiura et al. [31] pointed out that the annual mean air temperature in Japan has risen by 0.31–0.34°C per decade. They proposed that since such climate warming brings earlier blooming and higher temperatures during the maturation period, it is likely to change the taste (acidity and soluble solids) and texture (fruit firmness and watercore development) of apple fruits. They suggest that the slower advance toward the benchmark blush rate caused by warming during the maturation period will offset the advance in fruit maturity induced by the earlier blooming. Honda and Moriya [11] demonstrated that the temperature during fruit ripening of apple trees is an important factor in anthocyanin synthesis in both apple fruit peel and fruit flesh. They observed that the anthocyanin concentrations in the flesh of mature "Pink Pearl" (normal fruit flesh color, pale pink or red; fruit color, red and orange flush) harvested in 2016 were considerably lower than the typical level, probably because of the high temperatures recorded during the ripening period in that year. This suggests that the higher temperatures caused during fruit ripening by recent climate warming may be detrimental to fruit reddening.

Many reports about the effects of treatment-induced changes (e.g., light, temperature) on the characteristics of fruit peel color in apples indicate that production areas for apple fruits will likely increasingly suffer from inadequate reddening owing to ongoing climate warming. Therefore, further research is needed to clarify the effects of ongoing climate warming on the rate of anthocyanin synthesis in apple peel at each growth stage.

## 5.3 Enhancing anthocyanin accumulation through the choice of the nutrient and nutrient level

Fruit nutrient composition has a strong association with fruit color. In general, nitrogen (N) negatively affects apple fruit redness [32, 33]. The percentages of well-colored fruit of "Jonathan" apples were higher in trees given ammonium nitrate fertilizer at 0–5 g N m<sup>-2</sup> year<sup>-1</sup> than in trees given the same fertilizer at higher rates of up to 10-20 g N m<sup>-2</sup> year<sup>-1</sup> from 1978 to 1983 (Figure 3) [32, 34]. The excessive nitrogen application decreased the redness of the apple peel. Similarly, in "Elshof" (bright red with yellow background color), the anthocyanin concentration and percentage of blush are generally decreased by increasing the amount of N fertilizer [35]. Foliar application of nitrogen by using urea spray lessens the increase in anthocyanin concentration in the blush-side peel of "Gala" apples at maturity [19]. Furthermore, Awad and Jager [35] reported that the anthocyanin concentration in the peel of "Elshof" and "Elstar" (mostly red with yellow showing) apples at maturity is negatively correlated with the concentrations of N and magnesium (Mg) and with the N to calcium (Ca) ratio in the fruit at maturity, but the most important nutrient factor associated with anthocyanin concentration at maturity is the N concentration in the fruit during growth and at maturity. The result obtained from Awad and Jager [35] suggests that the maximum level of anthocyanin concentration in apple fruit peel could be achieved if the N concentration could be maintained at marginal N fertility levels to minimize the N concentration in the fruit.

(A)



(B)



#### Figure 3.

Effects of N application as ammonium nitrate on the percentage of well-colored "Jonathan" fruit. (A) Percentages of well-colored fruits from 1978 to 1983 in a long-term N fertilization field, Japan. A well-colored fruit is designated as one in which over 80% of the peel is colored. Data are running averages from 3 years  $\pm$ SD, n = 3. Data are from [34]. (B) Representative fruit from an unfertilized tree (o g N m<sup>-2</sup> year<sup>-1</sup>; left picture) and a fertilized tree (up to 20 g N m<sup>-2</sup> year<sup>-1</sup> as application of ammonium nitrate fertilizer; right picture); the peel of apples from the unfertilized tree is a superior red.

Anthocyanin synthesis in apple fruit is associated with not only N but also phosphorus (P) and Ca nutrients. P-Ca mixtures with mineral ions, such as Phostrade and Seniphos, have been used widely to enhance fruit coloration. Superior redness

of fruit and high anthocyanin concentrations could be achieved in "Braeburn" apple fruits with foliar application of Phostrade Ca (23.6% P<sub>2</sub>O<sub>5</sub>, 4.3% CaO, and 3% N); this effect was due to the P and Ca, not the N [36]. They showed that the anthocyanin concentration in the peel of treated apples at harvest was 20 times, whereas the concentration in the untreated apple peels (control) at harvest was only 9 times those at 5 weeks earlier of the harvest. Another P-Ca mixture, Seniphos (310 g L<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, 56 g L<sup>-1</sup> CaO, and 30 g L<sup>-1</sup> total N: 1% NO<sub>3</sub> and 2% NH<sub>3</sub>, the Phosyn Ltd. Company (Phosyn PLC, York, UK)) is also used commercially to improve peel color in apples. The positive influence of Seniphos on apple redness has been reported in various cultivars, such as "Starking Delicious" [37, 38] and "Fuji" [39], but it is not effective in "Jonagold" [40]. Gómez-Cordovés [37] showed that application of Seniphos increased the anthocyanin concentration in "Starking Delicious" apple peel over the ripening period and also altered the percentage composition of anthocyanins.

Sucrose can promote reddening of apple peel. Anthocyanin concentrations of peel disks of wax apple fruit (light to dark red, sometimes green) increase in a linear fashion with concentration of sucrose in the culture solution [41].

## 5.4 Plant hormones and their application to enhance anthocyanin accumulation

Ethylene, a hormone involved in maturation processes, plays an important role in regulating anthocyanin synthesis in apples. A close relationship between internal ethylene accumulation and anthocyanin accumulation in "Fuji" apple fruits [27] and in "Jonathan" apple fruits [42] has been reported. Therefore, ethylene can be used commercially to increase anthocyanin accumulation in red apples. Ethephon (2-chloroethyl phosphonic acid), an ethylene releasing compound that acts as a growth regulator, effectively promotes anthocyanin synthesis in "Starking Delicious" [37, 38] and "Fuji" [39] when light and temperature requirements for reddening are met [26]. Awad and Jager [40] compared the effects of the following seven growth regulators on the accumulation of anthocyanin in "Jonagold" apple peel: ethephon, CCC (2-chloroethyltrimethyl ammonium chloride), prohexadione-Ca (3-oxido-4-propionyl-5-oxo-3-cyclohexane-carboxylate), GA<sub>4 + 7</sub>, plantacur-E (a vitamin E formulation containing 25% alpha-tocopherol), shikimic acid, and galactose. Among these growth regulators, ethephon resulted in the highest anthocyanin concentration in fruit peel at commercial harvest.

The above results differ from reports that did not find a positive relationship between ethylene synthesis and anthocyanin accumulation in apple fruits that were irradiated with UV for "Starking Delicious," "Jonathan," "McIntosh," "Fuji," "Ralls Janet," "Tsugaru," "Jonagold," "Mutsu," and "Golden Delicious" cultivars [29] or subjected to hot climatic conditions [21]. For instance, apple trees of "Misuzu Tsugaru" subjected to hot climatic conditions (29°C 12 h/19°C 12 h) had lower anthocyanin accumulation in fruit at harvest but 9 times the ethylene production of those grown under the control (25°C 12 h/15°C 12 h) conditions [21]. These findings suggest that ethylene does not always play a role in regulating anthocyanin synthesis in apple fruits when the trees were grown under such light and hot temperature conditions.

In addition to ethephon, the plant hormone methyl jasmonate can alter anthocyanin accumulation. Treatment of apple peel disks of "Tsugaru" (yellow-green background covered in red-pink blush with occasional striations) with methyl jasmonate stimulates anthocyanin accumulation regardless of the fruit growth stage [43]. "Fuji" apple fruit after harvest that was treated with increasing concentrations of methyl jasmonate shows decreasing hue angles ( $\Delta$ h°) (redness) of the peel [44], and field application of methyl jasmonate to apple fruit can also decrease the hue angle [45]. Therefore, the application of methyl jasmonate can enhance apple fruit anthocyanin formation and peel reddening. Daminozide, paclobutrazol, auxins, and auxin analogs, which are growth regulators, can also promote reddening [44].

#### 5.5 Cultivars

Fruit color development of apple peel varies greatly with the cultivar. The most colored strains of "Royal Beaut" and "Buckeye Gala" (both semistriped) and "Ruby Gala" (blushed) redden on both fruit sides, with a greater average fruit surface colored, whereas the less colored strains of "Galaxy" and "Mondial Gala" (both striped) exhibit different colorations between sides, more bicolor fruits, and lower average fruit surface colored [17]. Furthermore, the reddest strains ("Royal Beaut," "Buckeye Gala," "Ruby Gala," etc.) have high coloring potential even at the early stages of fruit development or under environmental conditions associated with hot temperatures or with low-light conditions such as the shaded parts of tree canopy. However, in medium or poorly colored strains ("Galaxy," "Mondial Gala," etc.), color development depends mainly on fruit maturity. The genotype is therefore one of the main factors determining the degree of redness in apple fruits, and this provides important information on how to make the best use of apple in breeding.

#### 6. Conclusions and perspectives

Consumption of apple contributes to improved health and well-being by helping protection from diseases, including cancer and cardiovascular disease. Increasing the consumption of apple antioxidants requires the development of apples with high antioxidant content without sacrificing taste or storability. One of the most effective means of improving the antioxidant content of the diet is by apple cultivar selection. In addition to genetic factors, environmental factors before and after harvest can influence antioxidant content. Cultivar selection and the manipulation of cultivation could lead to specific conditions for optimal antioxidant enhancement. This would offer the consumer a wide selection of health-promoting cultivars and would result in premium prices for high antioxidant produce. Continued research will require integrated studies such as metabolomic and ionomic approaches to factor analyses that will identify specific conditions for enhancing the antioxidant content of apple products.

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#### **Conflict of interest**

No conflict of interest.

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