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Review

Peel colour in apple (*Malus* × *domestica* Borkh.): An economic quality parameter in fruit market

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ABSTRACT

Apple is one of the most important and popular fruits in the world. Among the various fruit quality traits, skin color is the preferred one which determines a cultivar's market acceptance. Fruit color in apple is dogged primarily by the ground color of the skin and secondarily by the imposed anthocyanin pigmentation, if present. The prime anthocyanin pigment in apple is Cyanidin 3- galactoside commonly named idaein. However, Cyanidin 3-pentoside was also identified in two species. Cyanidin arabinosylgalactoside has also been mentioned from the flesh of Komsomeletes apples but has not been reported elsewhere and is either specific to this cultivar having arisen from a mutation or is a mis-identification. Various factors like light, temperature, mineral nutrition, growth regulators, and carbohydrate availability etc. effect anthocyanin accumulation. The intensity of light and low temperature is crucial for the accumulation of anthocyanins in the skin of apple fruit. The light intensity especially UV-B, could activate anthocyanin synthesis pathway through photoreceptor and finally promote the accumulation of anthocyanins. In apple, low temperature could promote anthocyanin synthesis and high temperature could inhibit accumulation of the same. MdMYB1, the light responsive regulatory factor was found to control the transcription of apple flavonoid genes. Transcription of MdMYBA, MdDFR, MdCHS, MdLDOX and MdUFGluT in apple peel has also been found to be regulated by light particularly UV-B radiation. Optimum temperature for anthocyanin accumulation was found to be 25 °C. Application of Urea increases the Chlorophyll and Carotenoid concentration in fruit skin and reduces anthocyanins concentration in the blush side of fruit at maturity. Ethylene is a key factor in the regulation of anthocyanins biosynthesis and color development in apples. It has been found that the accumulation of anthocyanins is clearly stimulated by ethephon (an ethylene releasing agent) and delayed by ABG-3168 (a likely inhibitor of ethylene production). Anthocyanin reddening in apple fruit skin has been a topic of genetic studies since the 1930's. Several studies have indicated a monogenic approach of inheritance and a single dominant gene *Rf* was proposed to control anthocyanin reddening on apple fruit skin. Recent molecular genetic studies strongly support the hypothesis of a major gene inheritance as has been reported in the earlier literature. However, there has been much controversy among researchers and many of the studies have been questionable. It therefore requires a thorough analysis and understanding of the development of over color in apple so as to increase the apple trade in national and international market. In this view, an updated summary of published reports on the peel color in apple has been attempted but the experimental groundwork still needs to be done to overcome the misconception.

1. Introduction

Apple peel color is one of the most important factors determining apple market acceptance. In general, red cultivars are the most preferred, and within a cultivar, better colored fruits are in higher demand (Saure, 1990). However, consumer preferences vary from country to country and region to region (Cliff et al., 2002). New Zealand consumers prefer striped apples; consumers from Nova Scotia, Canada favor blushed apples, while consumers in British Columbia, Canada were more accepting of a range of apple types. Panelists in Lleida, Spain, on the contrary, did not show a preference for peel appearance when presented with eight 'Gala' strains with varying pigmentation

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(Iglesias et al., 2008). In addition, peel color is one of the main traits enabling cultivar discrimination, and there is increasing interest in breeding materials with altered color. In New Zealand, for instance, researchers are working towards the development of a red fleshed apple cultivar (Chagne et al., 2007).

The accumulation of anthocyanin pigments in apple fruit is an important determinant of fruit quality. Usually restricted to the skin of apples, these pigments provide essential cultivar differentiation for consumers and are implicated in the health attributes of apple fruit (Boyer and Liu, 2004). Anthocyanins belong to the diverse group of ubiquitous secondary metabolites collectively known as flavonoids. In plants, flavonoids are believed to have a variety of functions, including defence and protection against light stress, and the pigmented anthocyanin compounds play an important physiological role as attractants in plant/animal interactions (Harborne and Grayer, 1994; Koes et al., 1994). Differences in colour attributed to anthocyanins may be due to a number of factors including the number of hydroxyl groups on the B-ring, the sugars and acyl side groups (Harborne, 1967), the environment of the vacuole including its pH or the accumulation of specific metal ions (Brouillard, 1988), or cellular ultrastructure (Noda et al., 1994). One of the most common anthocyanin pigments are cyanidin, which, in the form of cyanidin 3-O-galactoside, is the pigment primarily responsible for red colouration in apple skin (Tsao et al., 2003). The enzymes operating in this biosynthetic pathway in apple have been well characterized (Honda et al., 2002; Kim et al., 2003).

Cyanidin which is the immediate precursor for the red pigment idaein (cyanidin-3-galactoside) is formed from phenylpyruvate. Cyanidin is produced under the following conditions: First, the enzyme which catalyzes the reaction (from precursor to cyanidin) is temperature sensitive 25-18 °C. Temperatures lower than 25 °C, promote this reaction. The temperature threshold may be different depending upon the cultivar. Fuji apples, for instance, require 18 °C. Second, the precursor formation reaction requires light of 650 nm (red), 350 nm (ultraviolet) and 450 nm (violet) wavelengths (Downs et al., 1965). Both high light interception and photosynthetic activity are necessary to induce red color formation on apples. Third, if there are high nitrogen levels in the plant cells, the phenylpyruvate will be converted to proteins which will be used in growth and not in pigment precursor formation (Ruiz et al., 1986; Ludders and Bunemann, 1969; Faust, 1965).

2. Anthocyanins

The term anthocyanin was first used by Marquart in 1835 to denote the blue pigment of cornflower and later was used to define the whole group of related pigments (Hayashi, 1962). The anthocyanins (glycosylated anthocyanidins) are particularly characteristic of the angiosperms and, apart from a few reports in ferns and mosses, are not found elsewhere in nature (Harborne, 1967). Anthocyanins are the pigments that give most flowers and fruits their pink, red, violet or blue colours although in some cases, such as the tomato, anthocyanins are absent and the red colour is due to the presence of certain types of carotenoids (Macheix et al., 1990). There are six anthocyanidins that are widely distributed in plants: pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin (Fig. 1).

Anthocyanidins other than these six common ones are few in number and very rare in occurrence (Harborne, 1967). These six anthocyanins differ in the number of hydroxyl groups on the B-ring and the presence or absence of methyl groups that affect the colour (The colour of anthocyanins is also affected by the nature and number of sugars attached to the molecule, the position of the attachment, the nature and number of the aliphatic or aromatic acids attached to the sugar and the physiochemical medium in which they occur (Mazza and Brouillard, 1990).

The anthocyanin responsible for the red colour of apple skin was tentatively identified as long ago as 1931 by Robinson and Robinson as a 3-monoside of cyanidin. Then Duncan and Dustman (1936) and Sando (1937) identified the predominant anthocyanin pigment in apples as cyanidin-3-galactoside, commonly named idaein (Fig. 2). Since then, a number of workers have confirmed this as the major pigment in a number of cultivars and *Malus* species from around the world (Sun and Francis, 1967; Pais and Gombkoto, 1967; Durkee and Jones, 1969). Harborne (1967) surveyed 15 *Malus* species and found that they all contained cyanidin-3-galactoside, and in two species a cyanidin-3-pentoside was also identified. However, Samorodova-Bianki and Bazarova (1970) mentioned only one pigment, reported to be cyanidin arabinosylgalactoside, from the skin and flesh of Komsomolets apples. This has not been reported elsewhere and is either specific to this cultivar, having arisen from a mutation, or is a misidentification. There have also been several reports on the composition of the minor anthocyanins present in different apple cultivars. These are all cyanidin derivatives but of varying glycoside composition. Cyanidin-3-gentiobioside has been reported by Kolesnik and Putintseva (1963) but it has not been reported elsewhere. Sun and Francis (1967) reported two minor pigments, which they identified as cyanidin-3-arabinoside and cyanidin-7-arabinoside, as well as confirming the identity of cyanidin-3- galactoside as the main pigment. They surveyed 83 cultivars, and of these 74 contained all three pigments, six had only two, two cultivars contained only the major anthocyanin and one cultivar contained no anthocyanins. These three anthocyanins were also reported from Jonathan and Wagner apples grown in the USSR (Posokhlyarova, 1976). The overall biosynthetic pathway of anthocyanin is represented in Fig. 5 and the genes and transcription factors that regulate the anthocyanin biosynthetic pathway genes are presented in Table 1.

3. Hunter colour lab values

The surface colour of apple in terms of Hunter L, a, b values is determined by Hunter lab colorimeter. 'L*' denotes the lightness or darkness, 'a*' green or redness and 'b*', blue or yellowness of the samples. L* measures lightness and varies from 100 for perfect white to zero for black, a* measures redness when positive and greenness when negative and b* measures yellowness when positive and blueness when negative. According to Jha et al. (2012), Hunter colour values L, a, b were 48.7–56.1, 11.0–19.4, 18.8–20.2 respectively. The L* value decreased initially, but later it increased due to appearance of redness and later on it decreased in the last days of storage period (Jha et al., 2006). The a* value, indicating green colour of the skin, increased from 11.0 to 19.4 during storage. The presence of yellowness i.e. b* values on the surface of the fruit remained stable during initial stages, followed by a decrease in the middle and finally increased in the last stages.

According to Ganai et al. (2015), early harvested apples (H_1) recorded the maximum L* and b* values (40.60 and 16.90) and the minimum values for a* (30.50). After the storage period of 100 days fruits harvested at late maturity (H_2) received the minimum values for L* and b* whereas maximum values for a* was recorded by fruits harvested at late stage of maturity (H_3). The reason behind the higher a* values in harvest date third (H_3) might be the full pigment development upto late stages of maturity. As it is evident that there was continuous increase in L* and b* values and decrease in a* values during the storage period irrespective of treatment and harvest dates. The reason behind this increase in L* and b* values and decrease in a* values might be the pigment degradation during the storage. Colour changes are pronounced in ambient storage than in refrigerated storage. This is because all the degradation reactions including those responsible for color get slowed down under low temperature conditions.

4. Anthocyanin content (mg/100 g)

The level of red anthocyanin pigments in apple skin is determined mainly by the cultivar, light exposure, ripening and temperature (Creasy, 1968; Chalmers et al., 1973; Faragher, 1983). During ripening of some cultivars, the concentration of anthocyanin in the skin may