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Biochemical, Physiological and Horticultural Perspectives of Fruit Colour Pigmentation: A Review.

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Review Article

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ABSTRACT

Plant pigments are essential for the attractiveness of fruits, accumulating most often in the skin during ripening process. The most important pigments of fruit include carotenoids and anthocyanins. Beside their role in pigmentation, they are important for human health as a source of vitamin A and antioxidant compounds. Carotenoids comprise carotenes, derived from terpenoids are synthesized in fruit at a high rate during the transition from chloroplast to chromoplast. Anthocyanins, a class of flavonoids derived ultimately from phenylalanine, are water-soluble, synthesized in the cytosol, and localized in vacuoles. They provide a wide range of colors ranging from orange/red to violet/blue. They are widely distributed in the plant kingdom. Betalains, nitrogen-containing water-soluble compounds derived from tyrosine, also conferring yellow-to-red colors, found only in a limited number of plant lineages. All three classes of pigments act as visible signals to attract insects, birds and animals for pollination and seed dispersal. They also protect plants from damage caused by UV and visible light. A number of factors and signals influence the accumulation of pigments includes light, temperature, hormones etc. With regard to that of horticultural perspectives, several orchard management practices found to influence better coloration in fruits.

INTRODUCTION

Fruit ripening is a highly co-ordinated, irreversible phenomenon involving a series of physiological, biochemical, and organoleptic changes that leads to the development of a soft and edible ripe fruit with desirable quality attributes. The colour change during fruit ripening is due to the unmasking of previously present pigments by degradation of chlorophyll and dismantling of the photosynthetic apparatus and synthesis of different types of anthocyanins and their accumulation in vacuoles, and accumulation of carotenoids such as *β*-carotene, xanthophyll esters, xanthophylls, and lycopene [2]. The increase in flavor and aroma during fruit ripening is attributed to the production of a complex mixture of volatile compounds such as ocimene and myrcene, and degradation of bitter principles, flavanoids, tannins, and related compounds.

A number of factors and signals influence the accumulation of pigments of carotenoids and anthocyanins includes light, temperature, hormones etc. Apart, with regard to that of horticultural aspects, several orchard management practices like bagging, pruning and fertilization also known to strongly impact on fruit colour pigmentation.

Fruit colour

Colour is very important indicator of quality of fresh fruit. It also serves for estimating the stage of maturity of fruit and creates expectations of eating quality, advertises the fruit to consumers. Colour has three basic attributes: brightness, hue and colourfulness [10]. The term ‘brightness’ relates to extent of light absorption by a surface and is the achromatic aspect of colour. The brightness of an object’s surface decreases with a decrease in the light reflected from the surface. 'Hue' refers to terms such as red, green and blue; these colour tones differ from each other in the wavelengths of light that is reflected. For example, monochromatic lights at 530 nm and 680 nm appear green and red respectively. 'Colourfulness' relates to the purity of the colour. A vivid red fruit is highly reflective above 600 nm while wavelengths below 600 nm are poorly reflected.

Yousef *et al.,* [34] studied the influence of different harvest dates and ripening periods on fruit quality 'Fuerte' avocado and examined the skin colour parameters during ripening. Due to change of skin color of avocado fruits cv. Furete during different dates of harvest and storage at 20°C showed there were a slight significant differences between L*, a*, b* and C* and h* values at different harvest dates and storage at 20°C. Results showed a significant decrease in color parameters (L*, a*, b* and chroma and hue angle) of avocado fruits at different harvest dates and one week ripe at 20°C.

Fruit pigments

Pigments are essential for the attractiveness of fruits, accumulating most often in the skin during the ripening process, although many climacteric fruits accumulate pigments also in their pulp tissue. The most important pigments of fruit are carotenoids and anthocyanins. Beside their role in pigmentation, they are important for human health as a source of vitamin A and antioxidant compounds.

Carotenoids comprise carotenes, such as lycopene and ß-carotene, and xanthophylls, such as lutein. They are derived from terpenoids, and are synthesized in fruit at a high rate during the transition from chloroplast to chromoplast. Anthocyanins, a class of flavonoids derived ultimately from phenylalanine, are water-soluble, synthesized in the cytosol, and localized in vacuoles. They provide a wide range of colours ranging from orange/red to violet/blue. They are widely distributed in the plant kingdom. In grape, ethylene (or ethylene generator ethephon) stimulates berry colouration that found to be involved in the regulation of anthocyanin biosynthesis [17]. Betalains, also conferring yellow-to-red colours, are nitrogen-containing water-soluble compounds derived from tyrosine that are found only in a limited number of plant lineages. All three classes of pigments act as visible signals to attract insects, birds and animals for pollination and seed dispersal. They also protect plants from damage caused by UV and visible light.

Health benefits of fruit colour pigments

- Carotenoids = Protection from photo oxidative damage, boosters immune system, inhibition of cardiovascular disease and cancer chemoprevention
- Anthocyanins = Treatment of vision disorders and neuro protection
- Betalains = Antiviral and antimicrobial properties and normal body metabolism

Carotenoids

Carotenoids give plant tissues a yellow – red colour, are either carotenes (hydrocarbons) or xanthophylls (derived from carotenes with additional oxidation). Fruit carotenoids show great diversity and exotic structures may be found. For example, in citrus fruits apocarotenoids (e.g., citraurin, citranaxanthin) are observed [12]. The typical colour of many fruits is derived from only a small number of carotenoids (e.g., lycopene in tomato, cryptoxanthin, and zeaxanthin in mango and persimmon, capsanthin and capsorubin in capsicum). Fruit peel contains higher amount of carotenoids than the pulp, and in some fruits (e.g., tomato and peach), the carotenoid concentration increases after harvest [15]. During the ripening of tomatoes, oranges, mangoes, and other fruits, carotenoids are synthesized in chromoplasts, derived from chloroplasts on chlorophyll breakdown. However, in some fruit (e.g., grapefruit) carotenoid synthesis may occur even before the initiation of chlorophyll disapearance, whereas in some early orange or mandarin varieties, fruit maturity at high temperatures is not associated with a higher carotenoid concentration because chlorophyll degradation is inhibited.

Carotenoid formation in ripening fruits is affected by environmental factors both before and after harvest. These include temperature, light, and the oxygen concentration within the atmosphere. For example, lycopene synthesis in tomato is inhibited by temperatures higher than 30°C. On the other hand β-carotene can be synthesized even at temperatures as high as 40°C.

Anthocyanins

Anthocyanins, the most important group of water-soluble pigments in plant tissues, are phenolic compounds with diverse chemical structure, localized within the vacuole of the plant cell, giving rise to colours from red to blue-purple or even black [29]. They are present mainly in mature epidermal cells (e.g., apple, apricot, fig, nectarine, peach, pear, plum, and pomegranate) as well as in the flesh (e.g., apple, blackberry, blueberry, cherry, cranberry, red- and black-currant, fig, grape, peach, plum, pomegranate, olive, 'Sanguine' orange, raspberry, strawberry).

The occurrence and accumulation of anthocyanins vary with the fruit species, cultivar, tissue structure, geographical location, position of the fruit on the tree, and cultivation conditions. Nasunin (delphinidin 3-*p*-coumarylrhamnoside) is the major anthocyanin within the peel of purple, but not white or green, eggplant varieties [21]. It has been isolated in crystalling form and shows strong antioxidant properties.

Li *et al.,* [16] examined the regulation of anthocyanin biosynthesis during fruit development in 'Nyoho' strawberry. In most strawberry cultivars, light conditions around the fruit surface affect fruit pigmentation. But 'Nyoho' produces uniformly colored fruits without any consideration of incident sunlight or shading.

Biochemical aspects on fruit pigmentation

Fruit colour pigmentation process in turn, associated with fruit maturity and ripening, where, ripening is a genetically programmed stage of development overlapping with senescence. Fruit is said to be ripe when it attains its full flavour and aroma and other characteristics of the best fruit of that particular cultivar. The words "mature" and "ripe" are essentially synonymous when used to describe these fruits that ripe on the plants known as non-climacteric.

However, in case of climacteric fruits a mature fruit will require a ripening period before attaining a desirable stage of edibility.

Chlorophyll degradation and enzymatic changes

With the approach of fruit maturation, the most obvious change which takes place is the degradation of chlorophyll and is accompanied by the synthesis of other pigments usually either anthocyanins or carotenoids. Phenylalanine ammonia lyase (PAL) and flavone synthase are key enzymes for the synthesis of anthocyanins. Based on the mechanism of colour change and the pigment composition at ripeness, fruits can be classified as follows,

- Fruits that lose all their chlorophylls, unmasking previously synthesized carotenes and xanthophylls and usually having a characteristic yellow colour (e.g., banana, plantain, lemon).
- Fruits with marked de novo biosynthesis of carotenoids, referred to as carotenogenic fruits (e.g., tomato, orange, persimmon, red pepper).
- Fruits with marked de novo biosynthesis of anthocyanins (e.g., grape, apple, olive, pomegranate, red cherry, raspberry, cranberry).
- Fruits that retain chlorophyll during ripening (e.g., some cultivars of kiwi and avocado).

So, colour is the most obvious change that occurs in many fruits. The most important common change is the loss of green colour. The loss of green colour is due to degradation of chlorophyll structure. The principle agents responsible for this degradation are pH changes (due to leakage of organic acids from the vacuole), oxidative systems and chlorophyllases. The disappearance of chlorophyll is associated with the synthesis and or revelation of pigment ranges from yellow to red (Fig 1).

Apart, many of the chemical and physical effects during pigmentation attributed to enzyme action. Softening of fruits during ripening is closely associated with an increase in pectic esterase and poly galacto uronase activities. Besides, oxidative enzymes, glycolytic enzymes, hydrolytic enzymes, invertase, transaminase, and chlorophyllase increased in activity during ripening of most of the fruits.

Enzymatic changes in Sweetsop (*Annona squamosa* L.) and Golden Apple (*Spondias citherea* Sonner) was reported by Guadarrama and Andrade [11]. Pectin methyl esterase and polygalacturonase were the key enzymes in fruit softening. The results revealed that the trends of pectin methyl esterase and polygalcturonase enzyme activities follow the same pattern in both fruit species for all stages of ripening, but is always higher activities in sweetsop fruits.

As the fruit advances in ripening reduces the strength of them due to structural and compositional changes in the cell walls by enzymatic hydrolysis of cellulosic substances, pectic acid and polygalacturonic.

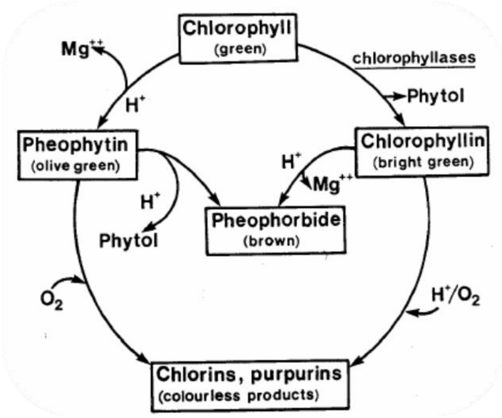

Figure 1: Pathway for chlorophyll degradation

Organic acids and flavouring compounds

The non volatile organic acids are among the major cellular constituents undergoing changes during fruit colour pigmentation. There is a considerable decrease in the acidity during the ripening of fruit due to their conversion in to sugar. Acids could be consider as reserve source of energy to the fruit and would therefore, be expected to decline during the greater metabolic activity that occurs on ripening. Banana and pine apple are exceptions where the highest level of acid are attained at the full ripe stage. The decline in the content of organic acids during fruit ripening might be the result of an increase in membrane permeability which allows acid to be stored in the respiring cells.

Aroma plays an important part in the development of optimal eating quality of fruits. The major chemical compounds found are esters of aliphatic alcohols and short chain fatty acids. In general, alcohol and esters are formed when fermentation develops in the ripening fruit. Branched chain alcohols may arise from reductive deamination of amino acids. Aldehydes and ketones are believed to have been derived from alcohols by oxidation.

Carbohydrates

Carbohydrate break down is the largest quantitative change associated with fruit ripening, particularly near total conversion of starch to sugars. The increase in sugar renders the fruit much sweeter and therefore, more acceptable. Even with the non climacteric fruits, the accumulation of sugar is associated with the development of optimum eating quality, although the sugar is derived from the sap imported in to fruit than break down of starch reserves of the fruit. During ripening, protopetin, insoluble form of pectin is gradually breaks down to lower molecular weight fractions, which are soluble in water. The rate of degradation of pectic substances is directly correlated with the rate of softening of fruit.

Physiological functions

Non foliar photosynthesis can make a significant contribution to the carbon cost of the fruit. This additional photosynthetic benefit may be the reason why some fruits retain chlorophyll and remain green when ripe at the sacrifice of conspicuousness and visual information about maturity [8]. Immature apples accumulate anthocyanins shortly after they have set. Immature apple and pear also accumulate anthocyanins during sudden periods of cold weather. It is possible that the anthocyanins provide protection against photo-oxidative stress when adverse environmental conditions increase excitation pressure [26]. Anthocyanins in immature apple and pear fruits have been shown to decrease excitation pressure on the photosynthetic apparatus within the peel when environmental stressors such as low temperatures decrease the rates of photochemical reactions.

Functions and biosynthesis of carotenoids

Carotenoids are isoprenoid compounds (mostly C40) with polyene chains that may contain up to 15 conjugated double bonds. More than 700 naturally occurring carotenoids have been identified [6].

The color of compounds in nature does not necessarily correspond to the colors of purified compounds in solution due to interactions with other components of chromoplast membranes and to concentration effects. The first step in the pathway is condensation reaction that result in the formation of geranylgeranyl diphosphate (geranylgeranyl pyrophosphate, GGPP). Phytoene synthase (PSY) catalyzes the condensation of two molecules of GGPP into prephytoene pyrophosphate (not shown) and then into phytoene (Fig 2.). A series of desaturation reactions results in the synthesis of lycopene, which is then cyclized into ß-carotene. The circular arrow shows the interconversion of zeaxanthin and violaxanthin, the two main compounds of the xanthophyll cycle. The phytohormone ABA is derived from xanthophylls.

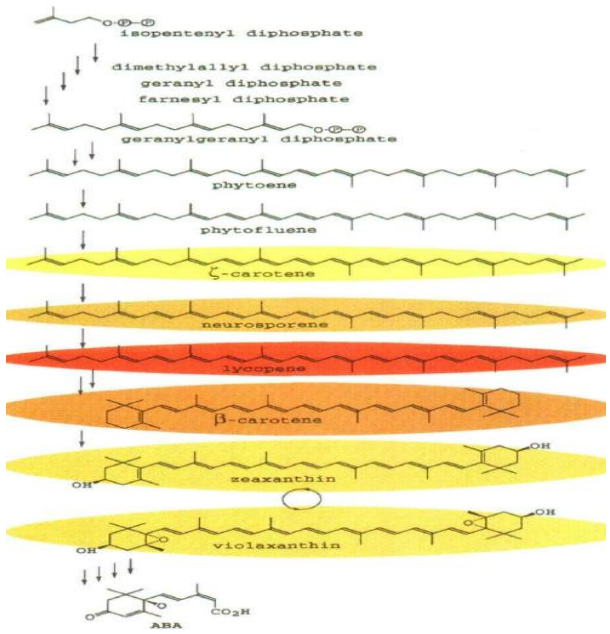

Figure 2: Biosynthesis pathway of carotenoids

Evident to it, a study was made by Charles *et al.,* [7] on carotenoid accumulation in kiwifruit. Post-harvest accumulation of carotenoids in *A. macrosperma* was examined at two different temperatures (20°C and 4°C). Fruit ripening at 20°C was associated with an increased accumulation of carotenoids, resulting in an orange-coloured fruit within 27 days. The ripening process was delayed in fruit kept at 4°C until they were transferred to 20°C.

Functions and biosynthesis of anthocyanins

Anthocyanins / flavonoids are among the best-characterized plant secondary metabolites, synthesized by the phenylpropanoid metabolic pathway, where the amino acid phenylalanine is used to produce 4-coumaroyl- CoA (Fig. 3). The conjugate ring-closure of chalcones results in the familiar form of flavonoids, the three-ringed structure of a flavone. The metabolic pathway continues through a series of enzymatic modifications to yield flavanones and anthocyanins. Along this pathway, many products can be formed, including flavonols, flavan-3-ols, and proanthocyanidins or tannins [30].

Relationship among colour, maturity and quality

The correlation between colour change and fruit maturity is strong enough for external colour to serve as maturity index in various fruits [22]. The use of peel colour as a maturity index usually entails the establishment of the relationship among colour change, storage life and eating quality. In some fruits, peel colour is manipulated in order to ensure that internal maturity and quality is reflected in the appearance of the peel colour (eg. Limes, lemons and oranges). In litchi and longan, browning of pericarp within a couple of days after being harvested due to degradation of anthocyanin and accumulation of polymeric polyphenol oxidation tends to decreases the marketability of fruits [20].

Detachment of immature and non climacteric fruits (eg. raspberry) arrest pigment accumulation, thereby results in poor appearance that make them undesirable to consumers. When climacteric fruits harvested immature, will ripe off the tree and attain full colour during storage. In certain fruits, where pigment synthesis is affected by environmental conditions, most notably light and temperature, the relationship between maturity and colour may vary according to position on the tree and at different localities [5]. In these instances, fruit colour may be unreliable as a maturity indicator.

Horticultural perspectives

A number of factors and signals influence the accumulation of pigments of carotenoids and anthocyanins includes light, temperature, hormones etc. Apart, several orchard management practices like bagging, pruning and fertilization also known to strongly impact on fruit colouration. Each one of the aspect were clearly discussed as follows,

Light and temperature

Elevated sunlight promotes ripening associated pigment changes in apple peel, including more profound breakdown of chlorophyll, induction of carotenoid synthesis, and specific changes in carotenoid patterns [25]. In case of apple, anthocyanin production tightly depends not only the light intensity but also the quality, which influences anthocyanin formation. Both phytochrome and specific UV-B photoreceptors appear to be involved in a synergistic activation of anthocyanin synthesis.

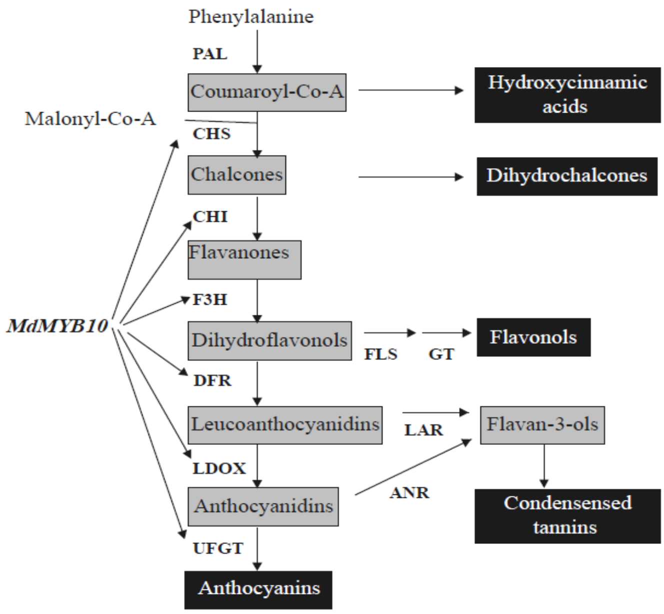

Figure 3: Biosynthetic pathway of anthocyanins

In different studies, transcription of MdMYBA, MdCHS, MdDFR, MdLDOX, and MdUFGluT in apple peels was also found to be regulated by light, particularly UV-B radiation [3]. When fruits are unbagged and exposed to light, MdCHI transcription increases by 240-fold, followed by MdCHS and MdLDOX at 80- and 60-fold, respectively [28], in agreement with results obtained by Ben Yehudah *et al*. [4]. MdMYB1 was identified as the lightresponsive regulatory factor controlling transcription of apple flavonoid genes. Flavonols accumulate in apple peel during acclimation to strong sunlight. They can serve as an efficient UV-B screen, playing an important role in the resistance of the photosynthetic apparatus to the UV-B component of solar radiation. Reay and Lancaster [21] studied the potential of detached fruit to accumulate anthocyanins and quercetin glycosides and found that the shaded side of the fruit has a much greater potential than the exposed side. They concluded that the fruit peel's previous exposure to light is a modifying factor in the potential for accumulation of anthocyanins by 'Gala' and 'Royal Gala'. Moreover, position of fruit on the tree can affect the pattern of anthocyanin deposition in 'Honey crisp' apple. More striped fruit are produced on southwest- facing branches; these fruits were additionally more strongly striped than those on the least sun exposed northeast branches. These results suggest that in this cultivar, higher light incidence or temperature on the bud or the fruit correlates with an increase in the occurrence and strength of stripes.

Shahak *et al.* [24] studied the effect of colouration with use of colour nets in peach orchard. Color net approach aims at combining physical protection of the crop, together with specific light filtration that promotes desired physiological responses for commercial value of crop, including yield, quality and rate of maturation. As they were applied over the peach orchard only 6 weeks prior harvest, most net treatments seemed to advance fruit sizing and red coloration.

Mineral Nutrition

Generally, surplus nitrogen fertilization is associated with a reduction in the percentage of well-coloured fruits at harvest time, although the total yield of well-coloured fruit may be higher. Reay and Lancaster [21] found that the application of urea increases the chlorophyll and carotenoid concentrations in the fruit peel and reduces anthocyanin concentrations in the blush side of the fruit at maturity. Nitrogen may inhibit flavonoid synthesis by enhancing the channeling of L-phenylalanine toward protein synthesis, or, alternatively, it might negatively influence the enzyme system involved in the biosynthesis of phenolics. Strissel *et al.* [27] studied the effect of nitrogen on the activities of anthocyanin biosynthetic enzymes and found that PAL activity seems to be down regulated by high nitrogen levels.

Yao *et al.* [32] reported the effect of optimal fertilization on pigment content in peel of banana cv. Baxi. Fruits from the optimum treatment had higher contents of chlorophyll and the lowest cyanin and flavonoid contents, all of which contributed to delayed post-harvest ripening and longer shelf life. In contrast, the OPT-N, OPT-P, and OPT-K treatments prompted the degradation of chlorophyll and formation of cyanin and flavonoid in the peels of fruits.

Growth Regulators

Ethylene is a key factor in the regulation of anthocyanin biosynthesis and colour development, with positive correlations being found between ethylene and total anthocyanin but not with other flavonoid compounds [31]. Ethylene initiates rapid anthocyanin accumulation during apple ripening by increasing the level of PAL in the peel.

The application of ethephon and a seniphos-like substance (a phosphorus- calcium mixture) produces an enhancement of red peel colour and an increase in concentration of flavonoid compounds [18]. Ethylene is not involved in the colouration responses due to sunlight, temperature or bagging. Gibberellic acid reduces anthocyanin accumulation, without influencing fruit maturation [1]. However, application of the gibberellin inhibitors cycocel and prohexadione-calcium does not significantly influence the formation of anthocyanin or fruit maturation. Khorshidi and Dhavarynejad [14] examined the effect of pre harvest ethephon spray on sour cherry and reported that there is a strong correlation between total anthocyanin and SSC of ‘Cigany’ sour cherry.

Other plant growth regulators like Jasmonic acid found to increases anthocyanin concentration in apples and synergistic or additive responses were found between ethylene and methyl jasmonate in apple peel pigment synthesis pathways [23]. Abscisic acid, auxins, and cytokinins previously have been implicated in anthocyanin regulation.

Orchard Management Practices

Bagging is widely applied as an effective practice for inducing some colour development even in fruit cultivars that do not usually show any red colour upon ripening [33]. Once fruits are bagged, anthocyanin accumulation is inhibited, consistent with the requirement of light for anthocyanin accumulation. By removal of bags, fruit rapidly develop red colour. Liu *et al.* [19] studied the effect of bagging on fruit colouration in non red (Granny Smith, Golden Delicious) and red apples (Starkrimon, Pink Lady). Young fruits were bagged 40 days after flowering with two-layer paper bag. Bagged fruits exposed to light (‘Golden Delicious’ and ‘Starkrimon’, 120 days after fruiting, while ‘Granny Smith’ and ‘Pink Lady’ 160 days after fruiting). Fruits were harvested after 0, 2, 4, 6, 8, 10 and 15 day of bag removal. The results revealed that L* values decreased and a/b values increased with the apple colouring in non red (Granny Smith, Golden Delicious) culivars.

Another practice that increases anthocyanin concentration is to cover the orchard floor with reflecting films that stimulates internal ethylene synthesis. The use of a white polypropylene ground cover, an aluminized plastic film, and a reflective foil all increase peel red colour in apple [13]. Fruit thinning can increase total red colour in ‘Honeycrisp’ fruit peels, as demonstrated in experiments by Delong [9]. Crop loads can additionally affect the pattern of pigment accumulation in this cultivar, with higher crop loads being associated with a lower percentage of blushed fruit.

Leaf pruning involves the removal of leaves blocking light incidence on the fruit, while fruit turning seeks to expose the least coloured side of the fruit by turning the branch and securing it in its new position [33].

CONCLUSION

Fruit colour would be appreciated solely for its aesthetic appeal. In the endeavor to improve fruit colour, it may prove worthwhile to be mindful of the physiological and ecological factors that underlie colour development. Due to the importance of colour in promoting purchasing decision of the consumers, the fine-tuned cultural manipulations would promote the increased proportion of the crop that fetches better returns to the growers.

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