CHAPTER

Plant extracts as coloring agents

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9.1 Introduction

Food, which is characterized by several sensory attributes like texture, taste, flavor, and color, is the basic need of everyone. Color is the most important viewable property, which acts as the focal point of costumer in foodstuffs. Color of any food material is an important sensory attribute, which affects consumer acceptance and around 62%−90% consumer assess the food by its color only (Singh, 2006). Basically, color is a molecule that absorbs certain wavelengths and transmits it or reflects it. As per the International Codex Alimentarius Commission (CAC), colorants are the substances that regulate the color of the food or are added to give color (Hastaoğlu, Can, & Vural, 2018). Food colorants are basically substances either natural or artificial, which retain the color of the food and cannot be eaten in the form of food as such (Batu & Molla, 2008). Color of food materials also imparts flavor, taste, and many nutraceutical properties and taken as an indicator for quality attribute. A strong cross linking of color with food makes it a profitable business and boosts the industries. Coloring of food has emerged during 1500 BCE and Egyptians also described colored drugs and wines (Burrows, 2009). It was reported that use of dyes by Egyptians in colored candy, and wine became popular during 400 BCE. According to historical background, the earliest record of using natural dyes was found in China in 2600 BCE. The process of dyeing was known in the Indus Valley period as early as 2500 BCE. There is a back record of saffron which has mentioned in the Bible and henna even before 2500 BCE (Gulrajani, 2001). In 1856 first organic colorant was discovered with the name of mauveine by William Henry Perkin. These colorants were named as "dye." In the beginning of 19th century, coal tar dye was produced from coal.

It has been reported by Burrows (2009) that food coloring substances were derived from some natural sources like saffron, paprika, turmeric, and flowers. Synthetic colorants gained popularity worldwide due to their long-term chemical stability in a small concentration, easy to use, and low cost (Borcakli, 1999). Synthetic colors like carmicin, amaranth, allura red, sunset yellow, tartrazine, ponceu 4R, indigo carmine, caramel etc. most common artificial food colors and are being used widely in food industry (Hastaoğlu et al., 2018). Wide application of these synthetic dyes raised serious health issues. The school going children are severely affected due to consuming processed food, ice creams and bakery products. In the changing scenario, use of natural healthy food becomes an excellent choice of costumer. Plant-based natural colors are considered to be safe and

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healthy. Therefore, in the recent past couple of years, consumer's mood has been driving a force to replace the synthetic dyes with natural plant-based colors.

9.2 Synthetic colors and health impact

Artificial colorants are used in foodstuffs having different structure and source producing specific color. Synthetic colors like carmicin, amaranth, allura red, sunset yellow, tartrazine, ponceu 4R, indigo carmine, caramel etc. are used in nonalcoholic beverages, cola and fruit flavored drinks and quite common in beverage industry (Borcakli, 1999; Özcan, Artık, & Üner, 1997). Similarly, confectionary and baking industry also usesuch colors abundantly without taking health concern into consideration. Dyes such as carmoicin, allura red, amaranth, sunset yellow, and tartrazine are most common in candies, dairy products, and baking industries (Hastaoğlu et al., 2018). These synthetic dyes have negative impacts on health. These may cause allergic reactions, hyperactivity, and skin burning and may cause cancer like disease in children (McCann et al., 2007). Many artificial colors cause behavioral disorders and chromosomal disorders especially when used in child nutrition (Omaye, 2004). It has been reported that some of synthetic dyes like triphenylmethane (malachite green and crystal violet) are illegally used in food materials for coloring and that is also available in fish tissues as a result of residual veterinary drug (Arnold, LeBizec, & Ellis, 2009). Hyperactivity, learning disabilities and behavioral disorders due to food additives in children was already reported (Bateman et al., 2004; Feingold, 1975; McCann et al., 2007). Besides, there are reports that six most common dyes viz. tartrazine(E102), quinolone yellow (E104), sunset yellow FCF (E110), carmoisine/azorubine (E122), ponceau 4R (E129), and allura Red AC (E129) are more common in sugary and beverages, which may cause negative effect on attention deficit, hyperactivity, cancer, chromosomal aberration, neurotoxicity, developmental toxicity, psycotoxicity, and DNA damage (Martins, Roriz, Morales, Barros, & Ferreiral, 2016; Sabnis, Pfizer, & Madison, 2010). The colorants are also associated with the allergy and asthma in human being and some recent evident suggested that Allura red increases urticaria and asthma (Amchova, Kotolova, & Ruda-Kucerova, 2015; Pandey & Upadhyay, 2012; Pollock & Warner, 1990). Impact of synthetic colorants on health is expressed and correlated in different ways. However, exact mechanism and linkage of such disorders are still unknown (Table 9.1).

9.3 Natural food colors

Natural food colors are mostly produced from plant-based sources like leaves, fruits, vegetables, roots, etc. Plant contains an array of natural colorants, which have nutritional and health protective potentials. Coloring of the food with natural plant based colorants is gaining popularity and remarked in trend line worldwide due to health concern and safety point of view. Msagati (2013) mentioned type of food colorants on the basis of their synthesizing source such as natural colorants, natural like colorants and synthetic colorants. Food colorants may be based on their source of origin like plants, bacteria, fungi or animals; as per their hue such as red, blue, green, purple etc. and their chemical derivatives like anthocyanins responsible for blue-purple color (flavonoids derivatives),

Table 9.1 Major permissible synthetic colors approved by the Food Safety Standard Authority of India.

S. No.	Color	Common color name	Color index	Chemical class
1.	Red	Ponceau 4R	16,255 ×	Azo
		Carmoisine	14,720	Azo
		Erythrosine	45,430	Xanthene
2.	Yellow	Tartrazine	19,140	Pyrazolone
		Sunset Yellow	15,985	Azo
3.	Blue	Indigo	73,015	Indigold
		Carmine Brilliant Blue FCF	42,090	Triarylmethane
4.	Green	Fast green FCF	42,053	Triarylmethane

Adopted from Gazette of India, Food Safety Standard Authority of India (FSSAI), GoI, India released on August 1, 2011. https://fssai.gov.in/upload/uploadfiles/files/FSSAI-regulations.pdf (retrieved on 08.06.2021).

carotenoids for red, yellow/orange color (isoprenoid derivatives) and betalains red—pink (nitrogen—heterocyclic derivatives) (Mittal, Sharma, & Singh, 2007; Viera et al., 2019). Moreover, these plants based colorants are called as pigments which have several medicinal, nutritional and cosmetic values (Frick & Meggos, 1988; Hari, Patel, & Martin, 1994; Shamina, Shiva, & Parthasarathy, 2007a, 2007b). Plant pigments are popularizing globally as a potential source of food color due to safe nature (Shamina et al., 2007a, 2007b). Major drawback of using natural colors in the food is their low stability and off odor to food. It has been reported that color could be used after removal of strong aroma and flavor from red cabbage and radish (Giusti & Wrolstad, 2003). The natural color of foods due to occurrence of natural pigments like carotenoids, chlorophylls, myoglobins, anthocyanins and their modification during processing such as caramel and color additives (Parkinson & Brown, 1981). Plant based colorants posses certain chemicals which can be used directly as such or modified into other form to impart hue. Certain processes like collection of raw materials, extraction, purification and their stabilization followed by formulation are used. As a result of these natural pigments a range of hues can be obtained from green to yellow, orange, blue, violet, red, purple and many more depending upon the source (Shameena et al., 2007). Some of the most common pigments are and their source are depicted in Table 9.2 and described underneath.

9.4 Anthocyanins

The word "anthocyanin" is derived from two Greek words "Anthos," which means flower and "kyanos" means dark blue (Delgado-Vargas & Paredes-Lopez, 2003). Anthocyanins are most spectacular water soluble plant pigments, which are responsible for red, blue, magenta, and purple color of plant-based food. Anthocyanin imparts different colors to the food and makes it attractive. Apart from this, it has health-promoting potential. Several studies suggested that consumption of anthocyanins reduce the risk of some chronic diseases. It also possesses antioxidant properties and helps in boosting of immunity. It is broad group of polyphenolic compounds that is, flavonoids which is

Table 9.2 Major color and pigments obtained from different plant extracts.

S. No.	Color	Type of pigment	Name of plant	Parts used
1.	Red	Anthocyanins	Elderberry	Fruit
2.	Purple/black		Black currant	Fruit
3.	Red, purple, blue		Grape	Fruit
4.	Red		Strawberry	Fruit
5.	Purple		Plum	Fruit
6.	Red, pink, purple		Raspberry	Fruit
7.	Blue		Blueberry	Fruit
8.	Black/dark purple		Black carrot	Root
9.	Red		Red radish	Root
10.	Blue		Blackberry	Fruit
11.	Red, violet	Betanins	Beet root	Root
12.	Yellow	Carotenoids	Yellow carrot	Root
13.	Yellow-orange, red	Bixin, carotenoids	Annatto	Seeds
14.	Orange-red	Crocin	Saffron	Stigma
15.	Pink, purple	Anthocyanin	Karonda	Fruit
16.	Pink	Betalins	Cactus pear	Fruit
17.	Yellow-orange	Lutein	Marigold	Flower
18.	Yellow	Curcumin	Turmeric	Rhizome
19.	Red-orange	Lawsone	Lawsonia	Leaves
20.	Red	Anthocyanins	Roselle	Calyces
21.	Blue	Indigo compounds	True indigo	Leaves
22.	Yellow−orange	Butein	Dhak	Flower
23.	Yellow	Barberine/Anthocyanins	Barberry	Fruits

a secondary metabolite synthesized by plants. These polyphenolic substances are mainly glycosides, polyhydroxy and polymethoxy derivative of 2-phenylbenzopyrylium or flavylium salts (Jackman et al., 1987). Anthocyanins are glycosides of anthocyanidins and sugars (Mortensen, 2006). Anthocyanidins are mostly glycosylated at 3rd position and many times at other positions. A number of anthocyanins are known due to their diverse glycosylation and acylation (Mortensen, 2006). Presence of double bond and carbonyl group on C ring differentiates them to each other. Aglycones have long chromophore with eight conjugated double bonds carrying cation charge fall between 465 to 550 nm visible range. The hydroxyl group on the aglycone is substituted by sugars with glycosidic bond or acylated with aromatic or aliphatic acids (He & Giusti, 2010). Anthocyanins are highly water soluble pigments therefore usually extracted with water and lower concentration of alcohol. To date, more than 635 anthocyanins have been identified but only few have been reported as most important and abundant (Andersen & Jordheim, 2008). Pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin are most common type of anthocyanins present in plants parts. There are several sources of anthocyanins depending on availability. The most common sources for the production of anthocyanins are grape, black currant, red cabbage, elderberry, rose, strawberry, jamun, karonda, black and purple carrot, and red radish. Besides, many flowers, leaves, and plants species are used as source of color. Anthocyanins are mostly used as natural colorant in beverage industry like wines and fruit juices.

Anthocyanins are highly stable in acidic medium but instable and prone to degradation under normal condition and may form insoluble brown product. Anthocyanins produce a diverse range of colors depending on chemical structure. It has been well established that more number of hydroxyl group produces blue color whereas more methoxy group yields red color. Pelargonidin is associated with orange color while delphinidin and malvidin are purple (Mortensen, 2006). Anthocyanins act on the basis of pH of medium. Four major anthocyanin forms exist in equilibria: the red flavylium cation, the blue quinonoidal base, the colorless carbinol pseudobase, and the colorless chalcone (Brouillard & Delaporte, 1977). When pH goes below 2, anthocyanins exist in red flavylium cation form.

Anthocyanin is the chief component of human diet and chiefly present in many colored plant tissues (Naczk & Shahidi, 2004). Human intake anthocyanins from several fruits like grape, pomegranate, plums, berries etc. The composition of anthocyanin varies across the plant species and even variety to variety. However, it is stated that berries provide most anthocyanins content per serving (He & Giusti, 2010). From the available literatures it is envisaged that more than 90% anthocyanins contain glucose as a glycosylating sugar and Cy-3-glu is most widespread anthocyanin in nature (Andersen & Jordheim, 2008; Kong et al., 2003). Besides fruits and vegetables, many processed products like jam, jelly, wines, and juices are also rich source of anthocyanin which offers a sufficient amount of anthocyanin in human diet. With the advancement consumer is becoming more health conscious and synthetic dye has been replaced with natural anthocyanin rich color. Acylated anthocyanins use is increasing significantly in food industry as a natural colorant due to stability. Elder berry, capsicum and grape, flower petals and karonda like fruits have potential use in food industry due to rich in anthocyanin content. It has been reported that natural anthocyanin didn't harm human health on excess oral consumption (Brouillard, 1982). Its commercial application in food industry has been permitted by many countries like Japan, USA, and Europe including India. Natural colored vegetables like radish, sweet potato, black carrot, red cabbage etc. are also used as anthocyanin basket owing to their richness of acylated anthocyanins. Radish and red fleshed potatoes are using as alternative against Federal Food Drug and Cosmetic Red No. 40 (Allura red) (Shipp & Abdel-Aal, 2010). Recently, natural anthocyanins rich beverage has been prepared from fruits of Karonda (*Carissa carandus*) named as "Lalima" (Krishna et al., 2017). It is reported that colorless lemon based drink enriched with Lalima contains 469.2 μg additional anthocyanin (cyanidin-3-glucoside equivalent). The flavonol glucoside from the rose petals can be extracted and used as natural colorant. This phenolic compound is extracted through distillation from *Rosa damascena* Mill. petal which is enriched with sufficient amount of kaempferol and quercetin and its derivatives. Onion is another rich source of quercetin compound which imparts gold and yellow color. Is reported *Opuntia stricta* as a potential source of anthocyanins having red color. Another species of opuntia contains both betacyanins and betaxanthins whereas *O. stricta* conatins only betacyanins (Castellar, Obon, Alacid, & Fernandez-Lopez, 2003; Shamina et al., 2007a, 2007b).

However, differences in pH level affect its stability; thus, majority of natural anthocyanins are used in acidic food products. Krishna et al. (2017) extracted food colorant from Karonda with ethanol solvent. Acetone can be used as efficient solvent for extraction of anthocyanins from red fruits (Garcia-Viguera, Zafrilla, & Tomas-Barberan, 1998; Gil et al., 2000). Identification and characterization of type of anthocyanin is carried out by UV spectrophotometric analysis. Maximum absorbance of anthocyanins occurs at a wavelength of 520−540 nm in visible spectra of spectrophotometer. Some recent advances like use of NMR is also reliable to identify anthocyanins especially acylated anthocyanin (Castaneda-Ovando, Pacheco-Hernandez, Paez-Hernandez, Rodriguez, & Galan-Vidal, 2009; Kosir & Kidric, 2002). The successful application of anthocyanin in food industry can be only possible after suitable procedure and methods for application.

9.5 Betalains

Betalains are another most important group of plant pigment, which are abundantly present in many plants like beetroot, bougainvillea, portulaca, mirabilis, and opuntia. Betalains are heterocyclic and water soluble nitrogenous compound which can be further divided into two major classes according to their chemical structure: betacyanins (red violet color) such as betanin, prebetanin, isobetanin and neobetanin and betaxanthins, responsible for orange-yellow coloring, comprising vulgaxanthin I and II and indicaxanthin (Azeredo, 2009; Saponjac et al., 2016). Betalains are mainly produced by plants belonging to order Caryophyllales.

Betalins are vacuolar nitrogenous compounds having a core structure (protonated 1,2,4,7,7-pentasubstituted 1,7 diazaheptamethin system) known as betalamic acid (Khan & Giridhar, 2015). Betalamic acid condensed with cyclo-DOPA (L-3,4-dihydroxy-phenylalanine)/its glucosyl derivatives, and amino acids/its derivatives which results to formation of betalains viz. betacyanins (violet) and betaxanthins (yellow), respectively. Peterson and Joslyn (1958) mentioned that before 1957, Betalins were categorized as nitrogenous anthocyanin. Production of betanidin on its hydrolysis and isolation of indicaxanthin evidenced that this contains a separate pigment containing system of 1,7-diazaheptamethin which is responsible for their chroma (Mabry, Wyler, Parkih, & Dreiding, 1967; Piattelli, Minale, & Prota, 1964; Wyler and Dreiding,1957). Khan and Giridhar (2015) have reviewed systemically the various aspects such as production, biosynthesis and eco-physiological factors of betalins in a comprehensive mode. Betalins are hydrophilic in nature and due to this, extraction is carried out in aqueous methanol or methanol at pH 5 supplemented with ascorbic acid (Strack, Vogt, & Schliemann, 2003). This stability could be enhanced at 0.25% (w/v) (Khan & Giridhar, 2014). Piattelli et al. (1964) isolated a compound from cactus pear plant (*Opuntia ficusindica*) and identified as indicaxanthin. Indicaxanthin compound (λ_{max} 260, 305, 485 nm) on alkali fusion in the absence of oxygen yielded proline and 4-methylpyridine-2,6-dicarboxylic acid.

Betalains occur in almost all plant parts including flowers, fruits, bracts, inflorescence, and petioles. Since last few decades, a limited betalins sources have been used widely. The most common source like beet root; dragon fruit, opuntia, and amaranth have been used in most of investigation elucidating pigment extraction and/or purification for food uses, and their biological activities in crude and purified extract material (Gandía-Herrero, Escribano, & García-Carmona, 2014; Moreno, García-Viguera, Gil, & Gil-Izquierdo, 2008). Accumulation betacyanins in betalainoplast in vacuoles of tepal cells firstly in *Rebutia* spp. was confirmed by Iwashina, Ootani, and Hayashi (1988). Commercial production of such pigments is not fully exploited because of the lack of quantification methods, species which contain more tissue and extraction technologies. It is estimated that global production of betalins stands at 96.8 Gt out of 99.99% contribution is from beetroot alone. Remaining contribution is obtained from red pitaya, amaranth seeds and opuntia. It was also stated that low production and extraction of betalains might be due to less stability during processing resulting in significant loss. Apart from this, only betacyanins are considered as economically viable and stable as loss of betaxanthins is higher as compare to betacyanins during extraction (Delgado-Vargas, Jiménez, & Paredes-López, 2000; Herbach, Stintzing, & Carle, 2006). Betanin is only abundant betacyanin which has been approved for commercial use in food and pharmaceutical industries (Silva et al., 2016). Several studies have suggested that purified form of betanin can be stable at low temperature and alkaline pH during storage; thus, it may be useful as food colorant and antioxidant additive in meat industry. High antioxidant potential in betalains is very well known (Esatbeyoglu, Wagner, Schini-Kerth, & Rimbach, 2015). For coloring, beet root power and beet extracts are readily available and chiefly used worldwide. Studies suggested that betalains do not change its hue at different pH in food and beverages (JebaKezi & Judia Harriet Sumathy, 2014). Betalains also have good stability against pH and light (Chethana, Nayak, & Raghavarao, 2007; Gonçalves, Da Silva, DeRose, Ando, & Bastos, 2013). Besides color imparting phenomenon, it has enormous health benefit properties.

9.6 Carotenoids

Carotenoids are most common lipophilic natural pigments responsible for red, orange, and yellow color in fruits, vegetables, and sea foods. Yabuzaki (2017) reported more than 1000 chemically distinctive carotenoids in plants. Most of carotenoids absorb light in a spectral region (400−550 nm), thus increases the light harvesting spectrum (Hashimoto, Uragami, & Cogdell, 2016). Carotenoids have good health promoting potential due to their inherent antioxidant properties. Investigation on beta carotene revealed that it has provitamin-A activity (Grune et al., 2010). Similarly, lycopene also a type of carotenoids which is capable to reduce the risk of prostate cancer and zeaxanthin and lutein reduces age related macular generation and also cognitive functions (Carpentier, Knaus, & Suh, 2009; Giovannucci, 2002; Johnson, 2012). Like other pigments, carotenoids also have stability problems and become instable under adverse conditions. The major factors are processing conditions, duration, adverse storage, packaging materials and exposure to light (Rodriguez-Amaya, 2015b). Carotenoids are commercialized in food industry as a colorant produced by chemical extraction methods. There are several fruits, vegetables, flowers and grasses which are rich sources of carotenoids. It is commercially extracted from Paprika, saffron, tomato, marigold, lycopene, annatto and many other plants (Rodriguez-Amaya, 2015b). Orange fleshed sweet potato is also rich source of α carotene and β-carotene which is used to overcome deficiency of vitamin A (Hermanns et al., 2020). Citrus fruits are also reported to be abundant source of carotenoids. On the basis of carotenoids, citrus cultivars are divided into three groups, as β-cryptoxanthin rich, violaxanthin rich, and low in β-cryptoxanthin and violaxanthin (Ikoma, Matsumoto, & Kato, 2016). It is well explained that there is direct correlation between carotenoids content and color of skin. In citrus peel, composition and ratios of the carotenoids affect the fruit color (Xu, Tao, Liu, & Deng, 2006).

Carotenoids are C40 tetraterpenes/tetraterpenoids formed from eight C5 isoprenoid units. Chemically, it is characterized by centrally conjugated double bond, which serves as the chromophore. This chromophore has light absorbing properties which gives attractive colors to carotenoids and also responsible for their typical functions and properties. In plants carotenoids biosynthesis take place in plastids (Li et al., 2016) including chromoplast in flower, fruits, roots and chloroplast in leafy vegetables. Carotenoids synthesized through methylerythritol phosphate (MEP) pathway by utilizing isoprenoid precursors. In this pathway, the enzyme 1-Deoxy-*D*-xylulose 5-phosphate synthase catalyzes the first reaction of *D*-glyceraldehyde 3-phosphate with pyruvate (Rodriguez-Amaya, 2015b). Further, biosynthesis of carotenoids can be divided into two sub categories: first carotenes (includes different carotenes) and another class is xanthophylls (includes Beta cryptoxanthin, zeaxanthin, antheraxanthin, violaxanthin, neoxanthin, zeinoxanthin, lutein etc.). Phytoene is first carotenoid compound of MEP pathway. Citrus accumulates wide array of carotenoids in both peel and pulp tissues depending upon tissue specific regulation. A number of pathways were reported for synthesis of carotenoids in citrus peel. Abscise of the β-cryptoxanthin and zeaxanthin yield red color β-citraurin in the peel of citrus (Hermanns et al., 2020). Recently, Zheng et al. (2019) discovered a natural variation in CCD4b promoter which was found to be most genetic determinant for natural variation in red coloration of citrus peel. Existence of red orange flesh color of melons (*Cucumis melo*) was reported due to predominant β carotene. CmOr was reported to be chief gene governing fruit orange-flesh color (Gur et al., 2017). Similarly, water melon produces a range of carotenoids which impart an attractive red, yellow and orange flesh. Lycopene was reported to be predominant in red fleshed water melon. It is reported that ClLCYB locates in a major flesh color QTL and strongly associated with red flesh color in most modern watermelon cultivars (Guo et al., 2019). Papaya is also having both red and yellow color flesh. Red flesh of papaya is associated with lycopene content whereas yellow fleshed papaya fruits rich in β-carotene and β-cryptoxanthin. In a discovery by Blas et al. (2010) chromoplast and chloroplast localized CpCYCB and CpLCYB were reported to be critical for lycopene accumulation in red-fleshed papaya. Besides, carotenoid accumulation in papaya was also regulated by ethylene and environment signals (Fabi & Do Prado, 2019). Lycopene is considered as ζ carotene, but it is predominant pigment in most of fruits and vegetables like tomato, watermelon, papaya, guava, grapefruit, and pitanga (Rodriguez-Amaya et al. 2008). An appreciable amount of α-carotene is present in carrot, red palm oil, and squashes and pumpkin, whereas γ-carotene are found in rose hips and pitanga abundantly. Rose hips also contain Rubixanthin, a derivative of γ-carotene, which is major pigment of hips (Hornero-Méndez & Mínguez-Mosquera, 2000). β-Cryptoxanthin, a xanthophyll is chiefly present in many orange-fleshed fruits, such as peach, nectarine, papaya, persimmon, tree tomato, and *Spondias lutea* (Rodríguez-Amaya et al., 2008; Rodriguez-Amaya, 2015a). Lutein is also chiefly present in yellow flowers, green leafy vegetables and some cucurbits. However, zeaxanthin is minor carotenoids and only present in limited form because of checking its biosynthesis at β carotene. Some of species specific carotenoids also detected in little amount. Capsanthin and capsorubin are major example which is abundantly present in red pepper (Rodriguez-Amaya, 2015a). Lactucaxanthin, a carotenoid with two ε-rings is rarely present in lettuce (Rodriguez-Amaya, 2015b).

Astaxanthin is pink color xanthophyll, which is derived from carotenoid. It has very high antioxidant potential and also used as a natural colorant. However, it is found in algae, yeast, fishes, and some crustacean byproducts. This is a chief source of color in marine industry. Astaxanthin is preferably more common as synthetic color and available under the commercial brand name CarophyllPink and canthaxanthin as CarophyllRed (Bowen et al., 2002; Gouveia et al., 2002; Storebakken & No, 1992).

Apocarotenoids are the form in which the carbon skeleton has been shortened by removal of fragments from one or both ends of the usual C40 structure. The most common examples of natural apocarotenoids are bixin mostly found in annatto, and crocetin, the chief constituent of saffron color (Rodriguez-Amaya, 2015b). As already mentioned, carotenoids are lipophilic in nature, majority of carotenes are water insoluble pigments but soluble in organic solvents such as acetone, petroleum ether, hexane, alcohol, ethyl ether, chloroform etc. Xanthophylls are better dissolved in methanol and ethanol (Rodriguez-Amaya, 2015b). β-Carotene and the xanthophyll lutein can be soluble in tetrahydrofuran (Craft & Soares, 1992). All the carotenoids have numerous health benefits and it is recommended to take more carotene rich diet for healthy body.

9.7 Porphyrin pigments (chlorophylls)

Natural porphyrin pigments are chlorophylls and chlorophyllins. The green chlorophylls are found in chloroplast where they furnish the process of photosynthesis and facilitate the assimilation of carbon dioxide. Green chlorophylls consist of 4-pyron core with a centrally located atom of Mg. In general chlorophylls are olive green to dark green in color obtained from extraction of green leaves. It is very less stable and sensitive to high heat and temperature. Chlorophylls are used in different beverages, jam, jellies, syrups, pickles and also as medicinal tonic. They have very good health potential and help in curing many diseases, improve metabolism and enhance amount of hemoglobin in blood.

Green natural colorants are constituent of chlorophyll and its derivatives. European current legislation (Regulation EC No. 1333/2008 and its amendment) has allowed two major natural green colorants E140 and E141 which is related with chlorophylls and its derivative extractions respectively. Green colorants can be extracted from different chlorophyll rich fruits, vegetables and other grasses. Herbs and vegetables especially leafy vegetables are abundant source of chlorophyll. Chlorophyll a and b are most dominant chlorophylls used as natural colorant. Some of researchers explained the natural green color named as chlorophyll or E140i which is directly obtained through solvent extraction from natural green plants and mainly composed of chlorophyll a and b and their pheophytin derivatives. Another colorant named as E140ii or chlorophyllin. However, there is no clear cut definition and chemical structure of chlorophyllin but Willstätter (1915) reported that it is chlorophyll derivatives which produced after saponification of chlorophyll without changing in color. Earlier, it was assumed that chlorophyllin composed of that intact Mg molecule but now this theory has rejected and this term is not so common because colorant green pigment include the chemical structure without magnesium molecule. For fixing of green color copper is used. It is reported that water soluble pigment E141ii is most common in food industry and requires saponification and copper treatment for more transformation into green from chlorophylls (Viera et al., 2019). Chlorophylls are stable in their natural existence but prone to change their structure during processing and handling. Most common factors are pH, temperature and nature of component. Major reaction such as substitution of central Mg ion by H ion leads to drastic changes in color of pigment and possibly this is major lacuna in loss of green color during processing of food materials. It is well explained that Mg derivatives are green in color but Mg free mainly pheophytins and pheorphorbides are brown in color. To fix this green color, several attempts such as can coatings, use of alkali agents etc. have been made by researchers but this was found to be least effective with some other negative effects like off flavor (Schwartz et al., 2017). Subsequently, a successful approach has been developed and patented. In a new approach the H ion has been substituted by addition of zinc and copper to form more stable green color metallochlorophylls which consequently "re-green" the food product. A long term investigation was done to fix this process in vegetables during processing (Jones, White, Gibbs, Butler, & Nelson, 1977; LaBorde & von Elbe, 1990; Schanderl, Marsh, & Chichester, 1965). In the continuation, an another patent was registered by Continental Can Company termed as "Veri-Green" in which vegetables were blanched in a Zn^{+2} or Cu^{+2} enriched brine solution to form Zn and Cu pheophytins to make canned beans more greener (Segner, Ragusa, Nank, & Hoyle, 1984; Von Elbe, Huang, Attoe, & Nank, 1986). However, a large amount of Zn is required for this against the permissible limit of 75 ppm by FDA. Therefore, encapsulation is necessary to improve this procedure more efficient. Some of the vegetables are spray dried and their encapsulated green color is using in food industry. It is very difficult to produce natural green hue. In country like Indonesia, natural chlorophyll is being extracted from Suji (*Dracaena angustifolia Medik*) *Roxb.* plant and using in food industry (Aryanti, Nafiunisa, & Willis, 2016; Prangdimurti, Muchtadi, Astawan, & Zakaria, 2006). However, chlorophylls from this plant are highly prone to enzymatic and non enzymatic degradation.

9.8 Regulatory mechanism for food colors

Food colors and natural pigments are not classified separately by Codex, but they are kept in the category of food additives. Certain limits have been standardized for the handling of synthetic colors but they should have same safety standards as other natural food colors. Licensing and certification process is mandatory for synthetic food colors (Shamina et al., 2007a, 2007b). Legal advisory and regulated use of food colors is governed jointly by WHO and FAO. The CAC along with Codex Committee on Food Additives and Contaminants-CCFAC and Joint Expert Committee on Food Additives—JECFA prepare documents of regulatory practices for safe production of the food which is accepted by majority of countries in all around the world (Luckey, 1968). Moreover, these regulations vary country to country. European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) are well known agencies in this line (Martins et al., 2016). The FDA is primarily responsible for safe handling and use of food additives, thus a food manufacturer must first get approval from FDA to use new colorant into food.

FDA issues a certificate to manufacture, sale, and use for synthetic colors, but natural colors are exempt from certification process. In USA certified color additives and color additives color additives exempt from certification terms applied. Shamina et al. (2007a, 2007b) mentioned in a comprehensive review that only 43 colorants are authorized as food additives by Council of the European Union, and an E number has been issued. Out of, 16 colorants are based on plant origin and some extracts from juices, fruits and vegetables are also used as colorants. Lehto et al. (2016) collected regulatory information of food colors and compared these regulations in European Union and United States. Only approved colors can be used in both EU and US. Food colors are used as food additive agents and regulated under the comprehensive set of regulations such as regulation

(EC) No. 1331/2008 (European Commission, 2008a) categories the common authorization processes, regulation (EC) No. 1333/2008 (European Commission, 2008b) deals with food additives and its amendment and regulation (EC) No 1129/2011 (European Commission, 2011) presents the rules for food colors. The use of permitted food colors, maximum limits and instruction for uses are also described under regulation (EC) No. 1333/2008. There is also a provision for new color which was not earlier in the market (before 1997) and to be commercialized needs to be evaluated under Regulation (EC) No 258/97 on novel foods (European Commission, 1997) and under the new Regulation (EU) No 2015/2283 (European Commission, 2015) from 2017 onwards, before commencement in the market.

Similarly, there are regulatory provisions in US for federal colors additive which were enforced under US Federal Food, Drug and Cosmetic Act. There are many amendments such as The Food Additive and the Color Additive Amendment, the Food Safety and Modernization Act-2012, the Fair Packaging and Labeling Act-1966 and the Public Health Security and Bioterrorism Preparedness and Response Act (Bioterrorism Act) which came into existence in 2002 also part of this series (Barrows, Lipman, & Bailey, 2003; Northcutt & Parisi, 2013). The main regulatory body for food colors is US Food and Drug Administration which regulates these set of rules under the Title 21 of the Code of Federal Regulations (CFR, 2016). The definition of these doesn't cover the separate classification of colors in the form of natural or synthetic and also their source of origin. The broad classification of approved colors in US is done into two categories that is, certified colors which include all artificial colors and need to be certified by FDA and colors exempt from certification that contain natural pigments (Lehto et al., 2016). Another important point with regard to natural pigments is that all certification exempted color must have purity, specifications and used limitations prescribed under regulatory standards and these must verify by the users not from the FDA. The substances or food materials which impart their own color (like chocolate in milk) when added to the food cannot be considered as a color (21 CFR Section 70.3). In present time there are total 39 colors are authorized and approved for uses in EU whereas in USA only 27 natural colors

Table 9.3 Common available plant extract-based colorants in India.

S. No.	Name of color group	Color name
1.	Carotenoids	Beta carotene
		Beta-apo 8′-carotenal
		Methylester of Beta-apo 1′ carotenoic acid
		Ethylester of Beta-apo 8′ carotenoic acid
		Canthaxanthin
2.	Chlorophyll	Chlorophyll
3.	Riboflavin (Lactoflavin)	Riboflavin (Lactoflavin)
4.	Caramel	Caramel
5.	Annatto	Annatto
6.	Saffron	Saffron
7.	Curumin or turmeric	Turmeric

Adopted from Gazette of India, Food Safety Standard Authority of India (FSSAI), GoI, India released on August 1, 2011. https://fssai.gov.in/upload/uploadfiles/files/FSSAI-regulations.pdf (retrieved 08.06.2021).

are approved and 9 synthetic colors approved. In spite of different regulatory procedures the overall aim is ensure to make food safer under all circumstances.

In India, as per regulation of Food Safety Standard Authority of India (FSSAI) Regulation 4.2.1 Food additives, unauthorized coloring material except a specifically permitted by these rules is prohibited. Additional coloring material other than mentioned on label attached with article is also prohibited. However, some of the natural colors either produced naturally or extracted synthetically may be used. Common permitted natural colors and synthetic colors are mentioned in Tables 9.2 and 9.3, respectively.

The maximum limit of permitted synthetic food colors has been decided by the authority. For natural colors it is 200 ppm in candy, crystallized and glazed fruits. Beyond that quantity no one can use synthetic colors. As per regulation of FSSAI "The maximum limit of permitted synthetic food colors or mixture thereof which may be added to any food article enumerated 85 in Regulation (vii) 4.2.1 (1) of these Regulations shall not exceed 100 parts per million of the final food or beverage for consumption, except in case of food articles mentioned in clause (c) Regulation 3.1.7 of these Regulations where the maximum limit of permitted synthetic food colors

Table 9.4 International numbering system for different natural food color under the category food additives governed by Codex Alimentarius.

S. No.	International numbering system	Food color name
1.	100	Curcumins
2.	100 (i)	Turmeric
3.	160a (ii)	Natural extracts
4.	160b	Annatto extracts
5.	160c	Paprika Oleoresins
6.	160d	Lycopene
7.	160e	Beta-apo-carotental
8.	160f	Beta-apo-′-carotenic acid, methyl or ethyl ester
9.	161a	Flavoxanthin
10.	161b	Lutein
11.	161c	Krytoxanthin
12.	161d	Rubixanthin
13.	161e	Violoxanthin
14.	161f	Rhodoxanthin
15.	161g	Canthaxanthin
16.	162	Beet red
17.	163	Anthocyanins
18.	163 (ii)	Grape skin extracts
19.	163 (iii)	Black currant extracts
20.	164	Gardenia yellow

Class names and international numbering system for food additives CAC/GL36−1989 (last amendment 2011). Accessed from http://www.fao.org/fao-who-codexalimentarius/shproxy/en/?lnk = 1&url = https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B36%-1989%252FCXG_036e.pdf (retrieved 08.06.2021).

Table 9.5 List of color additives exempted from certification (natural colors) by Food and Drug Administration.

21 CFR section[a]	Straight color	EEC[b]	Year approved	Uses and restrictions
Section73.30	Annatto extract	E160b	1963	Foods generally.
Section73.40	Dehydrated beets (beet powder)	E162	1967	Foods generally.
Section73.75	Canthaxanthin	E161g	1969	Foods generally, NTE 30 mg/lb of solid or semisolid food or per pint of liquid food; May also be used in broiler chicken feed.
Section73.85	Caramel	E150a-d	1963	Foods generally
Section73.90	β-Apo-8′-carotenal	E160e	1963	Foods generally, NTE15 mg/lb solid, 15 mg/pt liquid.
Section73.95	β-Carotene	E160a	1964	Foods generally
Section73.100	Cochineal extract	E120	1969	Foods generally
			2009	Food label must use common or usual name “cochineal extract”; effective January 5, 2011
	Carmine	E120	1967	Foods generally
			2009	Food label must use common or usual name “carmine”; effective January 5, 2011
Section73.125	Sodium copper chlorophyllin	E141	2002	Citrus-based dry beverage mixes NTE 0.2 percent in dry mix; extracted from alfalfa
Section73.140	Toasted partially defatted cooked cottonseed flour	——	1964	Foods generally
Section73.160	Ferrous gluconate	——	1967	Ripe olives
Section73.165	Ferrous lactate	——	1996	Ripe olives
Section73.169	Grape color extract	E163	1981	Nonbeverage food
Section73.170	Grape skin extract (enocianina)	E163	1966	Still & carbonated drinks & ades; beverage bases; alcoholic beverages (restrict. 27 CFR Parts 4 & 5).
Section73.200	Synthetic iron oxide	E172	1994	Sausage casings NTE 0.1% (by wt.).
			2015	Hard and soft candy, mints and chewing gum
			2015	For allowed human food uses, reduce lead from ≤ 20 to ≤ 5 ppm
Section73.250	Fruit juice		1966	Foods generally
			1995	Dried color additive
Section73.260	Vegetable juice		1966	Foods generally
			1995	Dried color additive, water infusion
Section73.300	Carrot oil	——	1967	Foods generally
Section73.340	Paprika	E160c	1966	Foods generally
Section73.345	Paprika oleoresin	E160c	1966	Foods generally

(*Continued*)

Table 9.5 List of color additives exempted from certification (natural colors) by Food and Drug Administration. ***Continued***

21 CFR section[a]	Straight color	EEC[b]	Year approved	Uses and restrictions
Section73.350	Mica-based pearlescent pigments		2006	Cereals, confections, and frostings, gelatin desserts, hard and soft candies (including lozenges), nutritional supplement tablets and gelatin capsules, and chewing gum.
			2013	Distilled spirits containing not less than 18% and not more than 23% alcohol by volume but not including distilled spirits mixtures containing more than 5% wine on a proof gallon basis.
			2015	Cordials, liqueurs, flavored alcoholic malt beverages, wine coolers, cocktails, nonalcoholic cocktail mixers and mixes and in egg decorating kits.
Section73.450	Riboflavin	E101	1967	Foods generally
Section73.500	Saffron	E164	1966	Foods generally
Section73.530	Spirulina extract		2013	Candy and chewing gum
			2014	Coloring confections (including candy and chewing gum), frostings, ice cream and frozen desserts, dessert coatings and toppings, beverage mixes and powders, yogurts, custards, puddings, cottage cheese, gelatin, breadcrumbs, and ready-to-eat cereals (excluding extruded cereals)
Section73.575	Titanium dioxide	E171	1966	Foods generally; NTE 1% (by wt.)
Section73.585	Tomato lycopene extract; tomato lycopene concentrate	E160	2006	Foods generally
Section73.600	Turmeric	E100	1966	Foods generally
Section73.615	Turmeric oleoresin	E100	1966	Foods generally

[a] *Title 21, Code of Federal Regulations (CFR) by Federal Food Drug and Cosmetic Act, 21 CFR Section73.*
[b] *International Numbering Sys. (European Commission Regulation), European Union.*
Note: Accessed from: https://www.fda.gov/industry/color-additive-inventories/summary-color-additives-use-united-states-foods-drugs-cosmetics-and-medical-devices#ftnote1 (accessed 08.06.21).

shall not exceed 200 parts per million of the final food or beverage for consumption." In addition to that, purity of color should be maintained and according to rule, the colors specified in Regulation (v) of 4.2.1 (1) of these Regulations, when used in the preparation of any article of food shall be pure and free from any harmful impurities. Besides, there are some setups of rules (Part 6.16) for selling of synthetic and natural colors. They says that no person can manufacture and sale the synthetic food colors and mixture directly without having any license or label on container mentioning "Food Colors" or chemical/common name of that mixture.

At international level, a specifically international numbering system for food additives adopted by Codex Committee on Food Additives and Contaminants for the identifying food additive in ingredient lists by a numerical approach (Table 9.4). These numerical series also include several synthetic and natural colors and extracts (Table 9.5).

9.9 Challenges with natural colors

Natural colorants are primarily derived from plants, animals, and microbes. However, colorants extracted from the plants changed the perception of consumers. They become more interesting and preferable because of safety and healthy characteristics. Despite certain advantages, plant-based colors have several production and stability challenges. It is reported that plant colorants have higher production cost in the initial, and they are unstable (Prince, 2017; Sen, Barrow, & Deshmukh, 2019). Other challenges with natural colors are heat and light sensitivity and interaction with other ingredients (Prince, 2017). In addition to that colors behavior is highly dependent on the application and that is a tough task to understand by manufacturer for making formulations. Anthocyanin based plant colorants are reported to be highly unstable and highly susceptible to degradation (Jiménez-Aguilar et al., 2011; Sagdic et al., 2013; Zhu et al., 2015). It is also evident that due to external factors, packaging materials, and interference in chemical structure, natural color loses its attractiveness (Jiménez-Aguilar et al., 2011; Lemos, Aliyu, & Hungerford, 2012; Zhu et al., 2015). The degree of susceptibility also varies from source to source from which colorants extracted. It has reported that anthocyanin extracted from red tulip is less sensitive as compare to violet though the plant belongs to same family (Sagdic et al., 2013). Besides, there is no definite legislation separately for natural colors. They are merged with food additives. Therefore, no certain regulatory standards and norms for uses have been assigned to natural colorants. Now a day, consumer satisfaction not only limited to sensory attributes but also with their health impacts, safety and quality (Giusti &Wrolstad, 2003; Kammerer, Kammerer, Valet, & Carle, 2014; Xi et al., 2007). Thus, food industry must satisfy the costumer with its claim for safe, healthy, and reliable in nature and therefore they need strong collaboration with research institutes and clinical agencies.

9.10 Conclusion

Natural food colors have increased demand because of their safety, biological, and health potential over synthetic colors. Uses of natural colors like curcumin, betalains, anthocyanin, paprika, annatto extracts etc. find wide application in the food industry. Their regulatory mechanism has been determined by FDA and different regulatory bodies countrywide to use them in a safe manner. As compared to synthetic dyes, these natural colors are exempted from certification and perceived safer by the human beings. Despite many advantages, extraction process, stability, and longevity are still challenging for natural colorants and need to be addressed by technological interventions. However, producers are bound to keep all regulatory mechanisms, methods of use, and criteria in controlled and regulatory manners.

References

Amchova, P., Kotolova, H., & Ruda-Kucerova, J. (2015). Health safety issues of synthetic food colorants. *Regulatory Toxicology and Pharmacology*, *73*(3), 914–922.

Andersen, O. M., & Jordheim, M. (2008). Anthocyanins—Food applications. In: *Presented at proceeding of 5th international congress pigments foods: For quality and health*, 14–16 August, Helsinki, Finland.

Arnold, D., LeBizec, B., & Ellis, R. (2009). Malachite green in *Residue evaluation of certainveterinary drugs. FAO JECFA Monographs*, *6*.

Aryanti, N., Nafiunisa, A., & Willis, F. M. (2016). Ekstraksi dan Karakterisasi Klorofildari Daun Suji (*Pleomele Angustifolia*) sebagai Pewarna Pangan Alami. *Jurnal Aplikasi Teknologi Pangan*, *5*(4), 129–135. Available from https://doi.org/10.17728/jatp.183129.

Azeredo, M. C. (2009). Betalains: Properties, sources, applications, and stability—A review. *International Journal of Food Science & Technology*, *44*, 2365–2376.

Barrows, J. N., Lipman, A. L., & Bailey, C. J. (2003). *Color additives: FDA's regulatory process and historical perspectives*. Magaz: Food Saf.

Bateman, B., Warner, J. O., Hutchinson, E., Dean, T., Rowlandson, P., Gant, C., . . . Stevenson, J. (2004). The effects of a double blind, placebo controlled, artificial food colorings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. *Archives of Disease in Childhood*, *89*, 506–511.

Batu, A., & Molla, E. (2008). Lokum Üretiminde Kullanılan. Katkı Maddeleri. *Gıda Teknolojileri Elektronik Dergisi* (1), 33–36.

Blas, A. L., Ming, R., Liu, Z., Veatch, O. J., Paull, R. E., Moore, P. H., & Yu, Q. (2010). Cloning of the papaya chromoplast-specific lycopene β-cyclase, CpCYC-b, controlling fruit flesh color reveals conserved microsynteny and a recombination hot spot. *Plant Physiology*, *152*, 2013–2022.

Borcaklı, M. (1999). The use of antimicrobial substances in food production and providing microbiological security, TMMOB publications, Ankara, March p. 16–21.

Bowen, J., Soutar, C., Serwata, R., Lagocki, S., White, D. A., Davies, S. J., & Young, A. J. (2002). Utilization of (3S, 3_S) astaxanthin acyl esters in pigmentation of rainbow trout (Oncorhynchus mykiss). *Aquaculture Nutrition*, *8*, 59–68.

Brouillard, R. (1982). Chemical structure of anthocyanins. *In P. Markakis (ed.), Anthocyanins as food colors* (pp. 1–40). New York: Academic Press.

Brouillard, R., & Delaporte, B. (1977). Chemistry of anthocyanin pigments. 2. Kinetic and thermodynamic study of proton transfer, hydration, and tautomeric reactions of malvidin 3-glucoside. *Journal of the American Chemical Society*, *99*, 8461–8468.

Burrows, J. D. (2009). Palette of our palates: A brief history of food coloring and its regulation. *Comprehensive Reviews in Food Science and Food Safety*, *8*(2), 394–402.

Carpentier, S., Knaus, M., & Suh, M. (2009). Associations between lutein, zeaxanthin, and age-related macular degeneration. *Critical Reviews in Food Science and Nutrition*, *49*, 313–326.

Castaneda-Ovando, A., Pacheco-Hernandez, L., Paez-Hernandez, E., Rodriguez, J. A., & Galan-Vidal, C. A. (2009). Chemical studies of anthocyanins: A review. *Food Chemistry*, *113*, 859–871.

Castellar, R., Obon, J. ,M., Alacid, M., & Fernandez-Lopez, J. A. (2003). Color properties and stability of betacyanins from Opuntia fruits. *Journal of Agricultural and Food Chemistry.*, *51*(9), 2772–2776.

Chethana, S., Nayak, C. A., & Raghavarao, K. S. M. S. (2007). Aqueous two phase extraction for purification and concentration of betalains. *Journal of Food Engineering*, *81*(4), 679–687.

Craft, N. E., & Soares, J. H. (1992). Relative solubility, stability, and absorptivity of lutein and beta-carotene in organic solvents. *Journal of Agricultural and Food Chemistry*, *40*(3), 431–434.

Delgado-Vargas, F., Jiménez, A. R., & Paredes-López, O. (2000). Natural pigments: Carotenoids, anthocyanins, and betalains–characteristics, biosynthesis, processing, and stability. *Critical Reviews in Food Science and Nutrition*, *40*, 173–289.

Delgado-Vargas, F., & Paredes-López, O. (2003). Anthocyanins and betalains. In *Natural Colorants for Food and Nutraceutical Uses* (pp. 167−219). Boca Raton: CRC Press.

Esatbeyoglu, T., Wagner, A. E., Schini-Kerth, V. B., & Rimbach, G. (2015). Betanin—A food colorant with biological activity. *Molecular Nutrition & Food Research*, *59*, 36−47.

European Commission. (1997). Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. *Official Journal of the European Union*, *L43*, 1−6.

European Commission. (2008a). Regulation (EC) No 1331/2008 of the European Parliament and of the Council of December 16, 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavorings. *Official Journal of the European Union*, *L354*, 1−6.

European Commission. (2008b). Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. *Official Journal of the European Union*, *L354*, 16−33.

European Commission. (2011). Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. *Official Journal of the European Union*, *L295*, 1−177.

European Commission. (2015). Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. *Official Journal of the European Union*, *L327*, 1−22.

Fabi, J. P., & Do Prado, S. B. R. (2019). Fast and furious: Ethylene-triggered changes in the metabolism of papaya fruit during ripening. *Frontiers in Plant Science*, *10*, 535.

Feingold, B. F. (1975). Hiperkinesis and learning disabilities linked to artificial food flavorsand colors. *American Journal of Nursing*, *75*, 797−803.

Frick, D., & Meggos, H. (1988). Federal food, drug and cosmetic colors. *Food Technology*, *7*, 49−56.

Gandía-Herrero, F., Escribano, J., & García-Carmona, F. (2014). Biological activities of plant pigments betalains. *Critical Reviews in Food Science and Nutrition*. Available from https://doi.org/10.1080/10408398.2012.740103.

Garcia-Viguera, C., Zafrilla, P., & Tomas-Barberan, F. A. (1998). The use of acetone as an extraction solvent for anthocyanins from strawberry fruit. *Phytochemical Analysis: PCA*, *9*, 274−277.

Gil, M. I. G., Tomas-Barberan, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry*, *48*, 4581−4589.

Giovannucci, E. (2002). A review of epidemiologic studies of tomatoes, lycopene and prostate cancer. *Experimental Biology and Medicine*, *227*, 852−859.

Giusti, M. M., & Wrolstad, R. E. (2003). Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal*, *14*, 217−225. Available from https://doi.org/10.1016/S1369-703X(02)00221-8.

Gonçalves, L. C. P., Da Silva, S. M., DeRose, P. C., Ando, R. A., & Bastos, E. L. (2013). Beetroot-pigment-derived colorimetric sensor for detection of calcium dipicolinate in bacterial spores. *PLoS One*, *8*, 737−740.

Gouveia, L., Choubert, G., Pereira, N., Santinha, J., Empis, J., & Gomes, E. (2002). Pigmentation of gilthead seabream, *Sparus aurata* (L. 1875) using *Chlorella vulgaris*(Chlorophyta, Volvocales) microalga. *Aquaculture Research*, *33*, 987−993.

Grune, T., Lietz, G., Palou, A., Ross, A. C., Stahl, W., Tang, G., . . . Biesalski, H. K. (2010). β-Carotene is an important vitamin A source for humans. *The Journal of Nutrition*, *140*, 2268S−2285S.

Gulrajani, M. L. (2001). Present status of natural dyes. *Indian Journal of Fibre & Textile Research*, *26*, 191−201.

Guo, S., Zhao, S., Sun, H., Wang, X., Wu, S., Lin, T., . . . Xu, Y. (2019). Resequencing of 414 cultivated and wild watermelon accessions identifies selection for fruit quality traits. *Nature Genetics*, *51*, 1616–1623.

Gur, A., Tzuri, G., Meir, A., Sa'ar, U., Portnoy, V., Katzir, N., . . . Tadmor, Y. (2017). Genome-wide linkage-disequilib- rium mapping to the candidate gene level in melon (*Cucumis melo*). *Scientific Reports*, *7*, 9770.

Hari, R. K., Patel, T. R., & Martin, A. M. (1994). An overview of pigment production in biological systems: Functions, biosynthesis, and applications in food industry. *Food Reviews International*, *10*(1), 49–70.

Hashimoto, H., Uragami, C., & Cogdell, R. J. (2016). Carotenoids and photosynthesis. *SubcellBiochem*, *79*, 111–139.

Hastaoğlu, E., Can, Ö. P., & Vural, H. (2018). The effects of colorants used in hotel kitchens in terms of child health. *Avrupa Bilim ve Teknoloji Dergisi* (14), 10–16.

He, J., & Giusti, M. M. (2010). Anthocyanins: natural colorants with health-promoting properties. *Annual Review of Food Science and Technology*, *1*, 163–187.

Herbach, K. M., Stintzing, F. C., & Carle, R. (2006). Betalain stability and degradation—Structural and chromatic aspects. *Journal of Food Science*, *71*, R41–R50.

Hermanns, A. S., Zhou, X., Xu, Q., Tadmor, Y., & Li, L. (2020). Carotenoid pigment accumulation in horticultural plants. *Horticultural Plant Journal*, *6*(6), 343–360.

Hornero-Méndez, D., & Mínguez-Mosquera, M. I. (2000). Carotenoid pigments in Rosa mosqueta hips, an alternative carotenoid source for foods. *Journal of Agricultural and Food Chemistry*, *48*(3), 825–828.

Ikoma, Y., Matsumoto, H., & Kato, M. (2016). Diversity in the carotenoid profiles and the expression of genes related to carotenoid accumulation among citrus genotypes. *Breed Science*, *66*, 139–147.

Iwashina, T., Ootani, S., & Hayashi, K. (1988). On the pigmented spherical bodies and crystals in tepals of cactaceous species in reference to the nature of betalains or flavonols. *Botanical Magazine Shokubutsu-Gaku-Zasshi*, *101*, 175–184.

Jackman, R. L., Yada, R. Y., Tung, M. A., & Speers, R. A. (1987). Anthocyanins as food colorants—a review. *Journal of Food Biochemistry*, *11*(3), 201–247.

JebaKezi, J., & Judia Harriet Sumathy, V. (2014). Betalain—A boon to the food industry. *Discovery.*, *20*(63), 51–58.

Jiménez-Aguilar, D. M., Ortega-Regules, A. E., Lozada-Ramírez, J. D., Pérez-Pérez, M. C. I., Vernon-Carter, E. J., & Welti-Chanes, J. (2011). Color and chemical stability of spray-dried blueberry extract using mesquite gum as wall material. *Journal of Food Composition and Analysis.*, *24*(6), 889–894. Available from https://doi.org/10.1016/j.jfca.2011.04.012.

Johnson, E. J. (2012). A possible role for lutein and zeaxanthin in cognitive function in the elderly. *The American Journal of Clinical Nutrition*, *96*(5), 1161S–1165S.

Jones, I., White, R., Gibbs, E., Butler, L., & Nelson, L. (1977). Experimental formation of zinc and copper complexes of chlorophyll derivatives in vegetable tissue by thermal processing. *Journal of Agricultural and Food Chemistry*, *25*, 149–153.

Kammerer, D. R., Kammerer, J., Valet, R., & Carle, R. (2014). Recovery of polyphenols from the by-products of plant food processing and application as valuable food ingredients. *Food Research International*, *65*, 2–12. Available from https://doi.org/10.1016/j.foodres.2014.06.012.

Khan, M. I., & Giridhar, P. (2014). Enhanced chemical stability, chromatic properties and regeneration of betalains in *Rivina humilis* L. berry juice. *LWT – Food Science Technology*, *58*, 649–657.

Khan, M. I., & Giridhar, P. (2015). Plant betalains: Chemistry and biochemistry. *Phytochemistry*, *117*, 267–295.

Kong, J. M., Chia, L. S., Goh, N. K., Chia, T. F., & Brouillard, R. (2003). Analysis and biological activities of anthocyanins. *Phytochemistry*, *64*(5), 923–933.

Kosir, I. J., & Kidric, J. (2002). Use of modern nuclear magnetic resonance spectroscopy in wine analysis: Determination of minor compounds. *Analytica Chimica Acta*, *458*, 77–84.

Krishna, H., Chauhan, N., & Sharma, B. D. (2017). Evaluation of karonda (Carissa carandus L.) derived natural colourant cum nutraceuticals-supplement. *RESEARCH ARTICLE PAGE*, 28.

LaBorde, F. F., & von Elbe, J. H. (1990). Zinc complex formation in heated vegetables purees. *Journal of Agricultural and Food Chemistry*, *38*, 484−487.

Lehto, S., Buchweitz, M., Klimm, A., Strassburger, R., Bechtold, C., & Ulberth, F. (2016). Comparison of food color regulations in the EU and the US—Review of current provisions. *Food Additives & Contaminants: Part A*. Available from https://doi.org/10.1080/19440049.2016.1274431.

Lemos, M. A., Aliyu, M. M., & Hungerford, G. (2012). Observation of the location and form of anthocyanin in purple potato using time-resolved fluorescence. *Innovative Food Science and Emerging Technologies*, *16*, 61−68. Available from https://doi.org/10.1016/j.ifset.2012.04.008.

Li, L., Yuan, H., Zeng, Y., & Xu, Q. (2016). Plastids and carotenoid accumulation. *Carotenoids in Nature*, 273−293.

Luckey, T. D. (1968). Introduction to food additives. In T. E. Furia (Ed.), *Handbook of food additives* (2nd ed., pp. 1−27). United States: CRC Press LLC.

Mabry, T. J., Wyler, H., Parkih, I., & Dreiding, A. S. (1967). The conversion of betanidin and betanin to neobetanidin derivatives. *Tetrahedron*, *23*, 3111−3127.

Martins, N., Roriz, C. L., Morales, P., Barros, L., & Ferreiral, I. C. F. R. (2016). Food colorants: Challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. *Trends in Food Science*, 1−72.

McCann, D., Barrett, A., Cooper, A., Crumpler, D., Dalen, L., Grimshaw, K., . . . Prince, E. (2007). Food additives and hyperactive behavior in 3-year-old and 8/9-year-old children in the community: A randomised, double-blinded, placebo controlled trial. *Lancet*, *370*, 1560−1567.

Mittal, R., Sharma, A., & Singh, G. (2007). Food colors from plants: Patenting scenario. *Indian Food Industry*, *26*(3), 52−58.

Moreno, D. A., García-Viguera, C., Gil, J. I., & Gil-Izquierdo, A. (2008). Betalains in the era of global agri-food science, technology and nutritional health. *Phytochemistry Reviews.*, *7*, 261−280.

Mortensen, A. (2006). Carotenoids and other pigments as natural colorants. *Pure and Applied Chemistry*, *78* (8), 1477−1491.

Msagati, T. A. M. (2013). *Chemistry of food additives and preservatives* (7th ed., p. 148)New York: John Wiley and Sons publishers.

Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A*, *1054*(1−2), 95−111.

Northcutt, J. K., & Parisi, M. A. (2013). Major food laws and regulations. In P. Curtis (Ed.), *Guide to US food laws and regulations* (pp. 73−96). Chishester: Wiley Blackwell.

Omaye, S. (2004). *Food and nutritional toxicology*. New York. New York: CNC Press.

Özcan, G., Artık, N. ve, & Üner, Y. (1997). Gıda katkı maddelerinin tü-ketici bilinci ve insan sağlığı açısından irdelenmesi. *TMMOB, Eylül*, 1−31.

Pandey, R. M., & Upadhyay, S. K. (2012). *Food additive* (5, pp. 1−31). İndia: InTech.

Parkinson, T. M., & Brown, J. P. (1981). Metabolic fate of food colorants. *Annual Review of Nutrition*, *1*, 175−205.

Peterson, R. G., & Joslyn, M. A. (1958). Nature of betanin, the pigment of red beet. *Nature*, *182*, 45−46.

Piattelli, M., Minale, L., & Prota, G. (1964). Isolation, structure and absolute configuration of indicaxanthin. *Tetrahedron*, *20*, 2325−2329.

Pollock, I., & Warner, J. O. (1990). Effect of artificial food colors on childhood behavior archives of disease in childhood. *Archives of Disease in Childhood*, 65−74.

Prangdimurti, E., Muchtadi, D., Astawan, M., & Zakaria, F. R. (2006). Aktivitasantioksidandaun suji.pdf. *Jurnal Teknologi dan Industri Pangan*, *17*(2), 79−86.

Prince, J. (2017). Natural shows its true color. *Nutritional Outlook*, *20*(9), 66.

Rodriguez-Amaya, D. B. (2015a). Carotenes and xanthophylls as antioxidants. In *Handbook of antioxidants for food preservation* (pp. 17−50). Woodhead Publishing.

Rodriguez-Amaya, D. B. (2015b). *Food carotenoids: Chemistry, biology and technology*. Oxford: IFT Press-Wiley.

Rodríguez-Amaya, D. B., Kimura, M., Godoy, H. T., & Amaya-Farfan, J. (2008). Updated Brazilian database on food carotenoids: Factors affecting carotenoid composition. *Journal of Food Composition and Analysis*, *21*(6), 445−463.

Sabnis, R. W., Pfizer, I., & Madison, N. J. (2010). *Biolojical dyes and stains, synthesis and industrial applications* (pp. 1−521). Canada: Wiley Publication.

Sagdic, O., Ekici, L., Ozturk, I., Tekinay, T., Polat, B., Tastemur, B., ... Senturk, B. (2013). Cytotoxic and bioactive properties of different color tulip flowers and degradation kinetic of tulip flower anthocyanins. *Food Chemical Toxicol*, *58*, 432−439.

Saponjac, V. T., Canadanovic-Brunet, J., Cetkovic, G., Jakisic, M., Djilas, S., Vulic, J., & Stajcic, S. (2016). Encapisulation of beetroot pomace extract: Rsm optimization, storage and gastrointestinal stability. *Molecules (Basel, Switzerland)*, *21*, 584.

Schanderl, S. H., Marsh, G., & Chichester, C. (1965). Colour reversion in processed I. Studies on regreened vegetables pea purges. *Journal of Food Science*, *30*, 312−316.

Schwartz, S. J., Cooperstone, J. L., Cichon, M. J., Von Elbe, J. H., & Giusti, M. M. (2017). *Colorants: In Fennema's food chemistry* (2017, pp. 681−752). Boca Raton, FL: CRC Press.

Segner,W. P., Ragusa, T. J., Nank,W. K., & Hoyle, W. C. (1984, September 25). Process for the preservation of green color in canned vegetables. U.S. Patent No. 4473591.

Sen, T., Barrow, C. J., & Deshmukh, S. K. (2019). Microbial pigments in the food industry—Challenges and the way forward. *Frontiers in Nutrition*, *6*, 7. Available from https://doi.org/10.3389/fnut.2019.00007.

Shamina, A., Shiva, K. N., & Parthasarathy, V. A. (2007a). Food colors of plant origin. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, *2*(087). Available from https://doi.org/10.1079/PAVSNNR20072087.

Shamina, A., Shiva, K. N., & Parthasarathy, V. A. (2007b). Food colors of plant origin. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, *12*, 24.

Shipp, J., & Abdel-Aal, E. S. M. (2010). Food applications and physiological effects of anthocyanins as functional food ingredients. *The Open Food Science Journal*, *4*(1).

Silva, D. V., Silva, F. O., Perrone, D., Pierucci, A. P. T. R., Conte-Junior, C. A., Alvares, T. S., ... Paschoalin, V. M. F. (2016). Physicochemical, nutritional, and sensory analyses of a nitrate-enriched beetroot gel and its effects on plasmatic nitric oxide and blood pressure. *Food & Nutrition Research.*, *60*, 29909.

Singh, S. (2006). Impact of color on marketing. *Management Decision*, *44*, 783−789.

Storebakken, T., & No, H. K. (1992). Pigmentation of rainbow trout. *Aquaculture (Amsterdam, Netherlands)*, *100*, 209−229.

Strack, D., Vogt, T., & Schliemann, W. (2003). Recent advances in betalain research. *Phytochemistry*, *62*, 247−269.

Viera, I., Pérez-Gálvez, A., & Roca, M. (2019). Green natural colorants. *Molecules*, *24*(1), 154.

Von Elbe, J. H., Huang, A. S., Attoe, E. L., & Nank, W. K. (1986). Pigment composition and color of conventional and Veri-Green canned beans. *Journal of Agricultural and Food Chemistry*, *34*, 52−54.

Willstätter, R. (1915). Chlorophyll. *Journal of the American Chemical Society*, *37*, 323−345.

Wyler, H., & Dreiding, A. S. (1957). Kristallisiertes betanin. *Vorlaufige Mitteilung Helvetica Chimica Acta*, *40*, 191−192.

Xi, L., Qian, Z., Xu, G., Zheng, S., Sun, S., Wen, N., . . . Zhang, Y. (2007). Beneficial impact of crocetin, a carotenoid from saffron, on insulin sensitivity in fructosefed rats. *The Journal of Nutritional Biochemistry.*, *18*(1), 64−72. Available from https://doi.org/10.1016/j.jnutbio.2006.03.010.

Xu, J., Tao, N., Liu, Q., & Deng, X. (2006). Presence of diverse ratios of ly- copene/ ß-carotene in five pink or red-fleshed citrus cultivars. *Science Horticulture*, *108*, 181−184.

Yabuzaki, J. (2017). Carotenoids database: Structures, chemical finger-prints and distribution among organisms. *Database*. Available from https://doi.org/10.1093/database/bax004.

Zheng, X., Zhu, K., Sun, Q., Zhang, W., Wang, X., Cao, H., . . . Deng, X. (2019). Natural variation in CCD4 promoter underpins species-specific evolution of red coloration in citrus peel. *Molecular Plant*, *12*, 1294−1307.

Zhu, Z., Wu, N., Kuang, M., Lamikanra, O., Liu, G., Li, S., & He, J. (2015). Preparation and toxicological evaluation of methyl pyranoanthocyanin. *Food Chemical Toxicology*, *83*, 125−132. Available from https://doi.org/10.1016/j.fct.2015.05.004.