

Biosynthesis of Anthocyanins and their Applications as Food Additives

Irakoze Evangeline¹, Nduwayezu Barithazar² and Gakuba Emmanuel^{3*}

^{1,3}Chemistry Education, African Center of Excellence in Innovative Teaching and learning Mathematics and Science (ACE-ITLMS), College of Education, University of Rwanda, Rwanda; ²Department of Food Science and Technology, School of Agriculture and Food Science, College of Agriculture Animal Science and Veterinary Medicine, University of Rwanda, Rwanda
egakuba16@gmail.com* +250785240665

Received: February 08 2020/Accepted: 26 March 2020/Published: 07 September 2020

Abstract

An essential attribute of a food product is its visual appeal, where the color is the most evident. As most food products are acknowledged by consumers concerning color, food dyeing is a significant part of the food manufacturing process and is an imperative characteristic that governs the acceptability of a product by consumers. Food pigments are frequently chemical-based and because of the detrimental effects of chemicals used in the food industry, there is currently a growing tendency toward natural food coloring. Anthocyanins have been recommended as favorable food additives with a significant role in human health. Anthocyanins are the leading group of water-soluble colorants in the plants, which are liable for the red, purple and blue color apparent in fruits, vegetables, flowers and grains, and a microbial systems, such as, *Saccharomyces cerevisiae*, *Escherichia coli* were used for the synthesis of anthocyanins, where the synthesis of anthocyanin cyaniding 3-O-glucoside (C3G) reached a maximum titer of 350 mg/L. Anthocyanins are appraised to be largely eaten up by mankind worldwide because of being natural pigments found in fruits, red wine, and vegetables. Curiosity in anthocyanin-rich foods and extracts has increased lately due to their promising health benefits. Anthocyanins have been revealed to display antioxidants, anti-diabetic, anti-carcinogenic, and containing visual activities, and so on. Discoveries of their valuable health properties assist their importance as natural food pigments, functional foods, and dietary additives. The present review describes up to date benefits of anthocyanins and their biosynthesis.

Keywords: Food colorants, anthocyanins, colour stability, anthocyanidin, biosynthetic pathway.

Introduction

The consumers accept food products depending on different factors where the color is the most important (Giusti and Wrolstad, 2003; Pazmino-Duran *et al.*, 2001). The food color is the important property used first by consumers to decide the quality of a food product, and colorants have been used for centuries by the food industries to improve and reinstate the original look of food or to safeguard homogeneousness, as a sign of food quality. Color is an important component of diets since the visual appearance is the first features perceived by the senses and is used by customers for quick recognition and decisive approval of foods and the color affect the esthetics, safety, and sensory characteristics of food (Giusti and Wrolstad, 2003; Clydesdale, 2009). Consumers made their first impressions referred on the food color, such as the immaturity, ripeness, or over-ripeness of fruit; the burnt of the toast; the freshness of food; the food can be judged to be safe to eat or not and can be likely to taste good or not based on these first impressions.

The introduction of color to food is a tactic to satisfy the expectations because the color is closely connected with these expectations (Aberoumand, 2011). There are many reasons to add color to food that include, (a) to reinstate the novel food aspect as the occurrence alters throughout processing and storage, (b) to guarantee the color consistency to bypass dissimilarities in quality pigment by seasonal variations, (c) to strengthen colors which are usually found in diet and the customer will relate this enhanced pigment with food quality, (d) to safeguard the taste and light-sensitive vitamins, (e) to provide an attractive occurrence to food, while deprived of the colorant diet will not be an appetizing item, (f) to conserve the uniqueness or aspect through which food is accepted, (g) to assist in the visual tryst of the food quality, (h) to color otherwise uncolored food (Delgado-Vargas *et al.*, 2000a; Griffith, 2005; Aberoumand, 2011; Chaitanya Lakshmi, 2014).

Food colors are categorized depending on several factors; by their origin, food colors can be separated into four groups which are: a) natural colors, b) nature-identical colors, c) synthetic colors and d) inorganic colors. The pigment made by living organisms is classified in natural colors. Ordinarily, the alteration of constituents from living organisms, like vegetable carbon, caramel, and Cu-chlorophyllin are also thought-through as natural pigments however they are not found in nature, apart from carbon (Alan, 2006; Aberoumand, 2011).

Natural food pigments are very varied in structure and from a diversity of sources, although they can be gathered into a few groups of which the three utmost significant are the flavonoids, tetrapyrrols, and tetraterpenoids. Anthocyanins are a class of flavonoids that give the red-purple colour of various fruits, especially berries, such as strawberries, elderberries, and black currants. The most predominant member of tetrapyrrols found in all higher plants is chlorophyll. Whereas a tetraterpenoids which are prevalent as chlorophyll is carotenoids since they as well are member of the photosynthetic gear. Moreover, they offer the red-orange-yellow shade of numerous fruits (Aberoumand, 2011). Nature identical colors are colorants made by man that is found in nature too, for instance, riboflavin, canthaxanthin, and β -carotene. Synthetic colorants are pigments made by man that is not found in nature, these are regularly azo-dyes. Inorganic dyes can be made in nature or replicated by synthesis. The food pigments can be distributed into two groups in line with the chemical structure of their chromophore: a) Chromophores with conjugated systems, that is, anthocyanins, betalains, carotenoids, synthetic colors, caramel, and lakes. b) Metal-coordinated porphyrins, namely chlorophyll, myoglobin, and their byproducts (Aberoumand, 2011; Bartley *et al.*, 1991; Delgado-Vargas *et al.*, 2000a).

The safety of man-made pigments has been questioned for long time, leading to a decrease in the number of acceptable pigments. Attention in natural pigments has widely raised up as a result of both legislative action and shopper appreciation for the use of synthetic seasonings in their foods (Pazmino-Duran *et al.*, 2001; Griffith, 2005). Here in review, we focused on anthocyanins, a natural food colorant consist of various group of strongly colored colorants accountable for the attractive and frequently significant orange, red-purple and blue colors of various fruits, vegetables, flowers, leaves, roots, and other plant storage parts. They are water-soluble, which enables their assimilation into aqueous food systems, and have been eaten up for centuries without unfavorable effects (Pazmino-Duran *et al.*, 2001; Giusti and Wrolstad, 2003; Aberoumand, 2011).

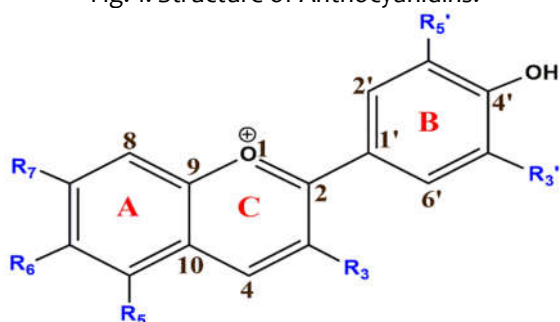
Anthocyanins

The two most important groups of colorants that are visible with naked eyes are chlorophyll and anthocyanins, respectively. Anthocyanins (from the Greek *Anthos*: flower, and *kyanos*: dark blue) are chemically belongs to the flavonoid (flavan like) cluster of phytochemicals, that is a group largely found in teas, honey, wines, fruits, vegetables, nuts, olive oil, cocoa and cereals, and chemically centered on a C₁₅ skeleton with a chromane ring holding an additional aromatic ring B in position 2 (C₆-C₃-C₆) and with one or more sugar molecules attached at diverse hydroxylated positions of the basic structure. Anthocyanins are substituted glycosides of salts of phenyl-2-benzopyrylium anthocyanidins (Delgado-Vargas *et al.*, 2000a; Roy *et al.*, 2009). In addition to the color qualities, curiosity in anthocyanins has raised on account of their promising health benefits. Health benefits linked with anthocyanin extracts consist of the visual sharpness, antioxidant ability, treatment of numerous blood circulation conditions arisen from capillary frailty, anti-angiogenic properties, vasoprotective and anti-inflammatory agents, platelet aggregation inhibitors, preservation of normal blood vessel permeability, managing diabetes, antitumor and chemoprotective agents, radioprotective agents and probably others owing to their various activities on numerous enzymes and metabolic processes. These potentials make anthocyanins agreeable other possibilities to synthetic dyes (Giusti and Wrolstad, 2003; Roy *et al.*, 2009).

Classification of anthocyanins

The anthocyanin structure that is based on C₆-C₃-C₆ is the basis of the ubiquity of colors formed by its chemical grouping with glycosides and/or acyl groups and by its interplay with other molecules and/or media conditions. It exists in more than 17 anthocyanidins, with dissimilarities in the number and location of hydroxyl groups and/or methyl ether groups, however, the most common anthocyanidin constituents of anthocyanins are 6. To obtain anthocyanin compounds from these 17 structures, the mixtures must consist of at least one sugar (Paliwal *et al.*, 2016). Therefore, anthocyanins have also been categorized in reference with the number of sugar molecules that form their compounds; namely, monosides, biosides, triosides; and, fascinatingly, the number of possible compounds is significantly raised by considering the sugar variety and all the probable structural positions of glycosylation, even though the order of sugar existence in natural anthocyanins is glucose, rhamnose, xylose, galactose, arabinose, and fructose. Moreover, several anthocyanins have revealed in their structures ester bonds between sugars and organic acids, in other words, acylated anthocyanins, and in nature, the utmost frequent acyl groups are coumaric, caffeic, ferulic, p-hydroxybenzoic, synapic, malonic, acetic, succinic, oxalic, and malic.

Fig. 1. Structure of Anthocyanidins.



Furthermore, the interchange of hydroxyl and methoxyl groups impacts the color of anthocyanins. Increases in the number of hydroxyl groups enhance the color to a more bluish tint (Delgado-Vargas *et al.*, 2000b). Contrarily, increases in the number of methoxyl groups rise redness (Fig. 1). Thus, it makes sense and simple to figure out the collection of colors perceived in nature that is manufactured from a single structure (Harborne and Sherratt, 1961; Francis and Markakis Pericles, 1989; Delgado-Vargas *et al.*, 2000a).

Table 1. Natural Anthocyanidins.

Anthocyanidin	Abbreviation	Substitution position	Produced colors
Substitution with a methyl ether group			
Capensinidin	Cp	5, 3', 5'	Bluish red
Europenidin	Eu	5, 3'	Bluish red
Hirsutidin	Hs	7, 3', 5'	Bluish red
Malvidin	Mv	3, 5'	Purple
5-Methyl-cyanidin	5MeCy	5	Orange red
Peonidin	Pn	3'	Magenta
Petunidin	Pt	3'	Purple
Pulchellidin	Pl	5	Bluish red
Rosinidin	Rs	7, 3'	Red
Substitution with an hydroxyl group			
Apigeninidin	Ap	5, 7, 4'	Orange
Aurantininidin	Au	3, 5, 6, 7, 4'	Orange
Cyanidin	Cy	3, 5, 7, 3', 4'	Magenta and Crimson
Delphinidin	Dp	3, 5, 7, 3', 4', 5'	Purple, mauve, and blue
6-Hydroxy-cyanidin	6-OHCy	3, 5, 6, 7, 3', 4'	Red
Luteolinidin	Lt	5, 7, 3', 4'	Orange
Pelargonidin	Pg	3, 5, 7, 4'	Salmon, Orange
Triacetidin	Tr	5, 7, 3', 4', 5'	Red

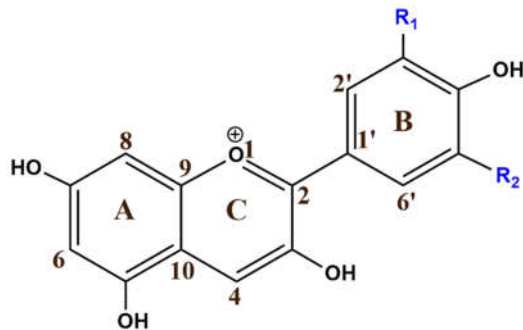
Red colored names denote the most common anthocyanidins; Source, (Francis and Markakis Pericles, 1989; Delgado-Vargas *et al.*, 2000a).

Biochemistry of Anthocyanins

Anthocyanins are a class of more than 500 molecules that offer the red, purple and blue colors of countless vegetables and fruits. Anthocyanins are members to a broad group of chemicals generally referred to as flavonoids that is a subclass of a bigger group of chemicals recognized as polyphenolics. Chemically, anthocyanins are glycosylated, polyhydroxy or polymethoxy derivatives of 2-phenylbenzopyrylium and hold two benzoyl rings (A and B) isolated by a heterocyclic (C) ring (Aam *et al.*, 2018). The distinctions of anthocyanins in structure are caused of variations in the number of hydroxyl groups in the compound, the amount of methylation of these hydroxyl groups, the nature and number of the sugar component linked to the phenolic (aglycone) molecule and the site of the bond, in addition to the nature and number of aliphatic or aromatic acids bound to the sugars. Depending on the number and position of the hydroxyl and methoxyl groups as substituents, diverse anthocyanins have been characterized, and six of them are usually found in fruits and vegetables, namely, cyanidin, pelargonidin, peonidin, delphinidin, petunidin, and malvidin (Aza-González *et al.*, 2012; Lila, 2004a; McGhie Tony and Walton Michaela, 2007; Shipp and Abdel-Aal, 2010). These anthocyanidins are frequently linked to a saccharide residue like glucose, galactose, rhamnose, or arabinose as 3-glycosides or 3,5-diglycosides (Jang *et al.*, 2005; McGhie Tony and Walton Michaela, 2007; Shipp and Abdel-Aal, 2010).

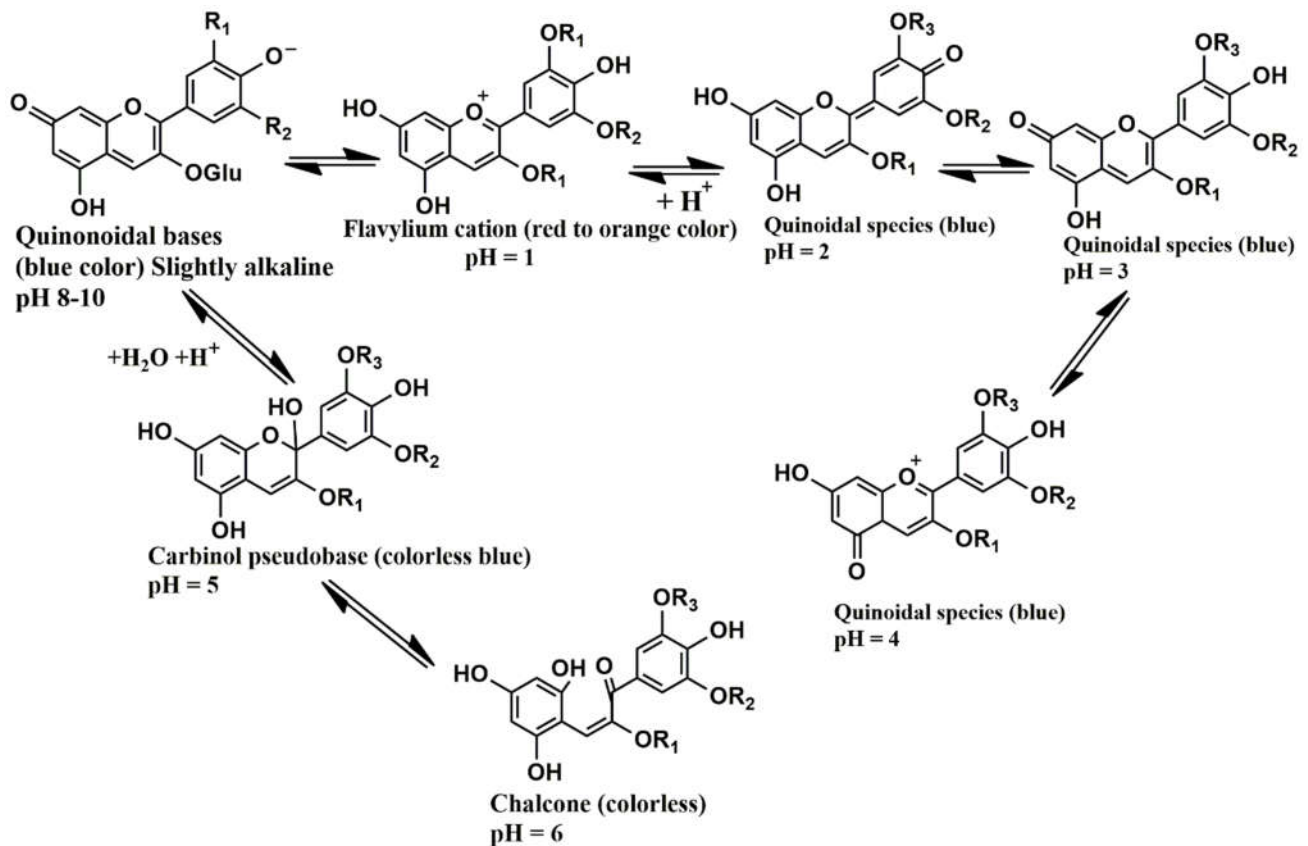
Color dissimilarities among anthocyanins are mostly decided by the exchange configuration of the B-ring of the anthocyanidin, the arrangement of glycosylation, and the level and nature of esterification of the sugars with aliphatic or aromatic acids and by the pH, temperature, kind of solvent and the appearance of co-pigments. Along with the pH of the medium, anthocyanins may alter from extremely red or orange in acidic environments ($\text{pH} < 2$) as a result of the existence of eight conjugated double bonds carrying a positive charge. At pH values between 2 and 4, the quinoidal blue species predominated. At pH values between 5 and 6 only two colorless varieties can be found (carbinol pseudobase and chalcone, respectively). At pH values greater than 7, the anthocyanins are deteriorated based on their substituent groups. Color stability then decreases to a neutral stance however some anthocyanins displayed a stability rise ending at limited highest around 8-9. For instance, the 3-glucosides of malvidin, peonidin, and pelargonidin that presented their best bluish colours at this range of pH values. The existence of methoxyl groups and the lack of ortho-dihydroxylation on the B-ring appears to favor this blue color in the alkaline region (Giusti and Wrolstad, 2003; Mazza, 2007; Shipp and Abdel-Aal, 2010; Miguel, 2011) (Fig. 2).

Fig. 2. More commonly found anthocyanidins in nature.



Anthocyanidin	R ₁	R ₂	Color ; λ _{max} (nm) visible
Cyaniding	OH	H	Orange-red (Magenta); 506
Pelargonidin	H	H	Orange; 494
Peonidin	OMe	H	Orange-red (Magenta); 506
Delphinidin	OH	OH	Red; 508
Petunidin	OMe	OH	Red; 508
Malvidin	OMe	OMe	Purple (bluish-red); 510

Fig. 3. Structural transformations of anthocyanins upon pH changes
(Source: Stintzing and Carle, 2004; Miguel, 2011).



Other factors like oxygen, ascorbic acid, and/or metal ions, light, and numerous enzymes can degrade color, for instance, a quick red shade is observed on a strawberry side that is facing the sun than the opposite side. This is particularly important when colors added in into foods and calculating the shelf-life of the product. For retaining the color and functionality of anthocyanins, it is of vital importance to increase stability for the reason that they are extremely reactive compounds that gladly degrade or react with other ingredients in mixtures to yield colorless or brown compounds. Glycosylation principally at the C-3 results in decreased maximum wavelength absorption however intensifies stability and solubility. Acylation of sugar residues with cinnamic namely p-coumaric, caffeic, ferulic, or aliphatic like acetic, malonic, succinic acids further enhances the anthocyanin stability. Normally, the 3-glucosylated structures are more stable than 5-glycosides, aliphatic acyl-anthocyanins, and aromatic acyl derivatives, respectively (Alkema and Seager, 1982; Stintzing and Carle, 2004; McGhie Tony and Walton Michaela, 2007; Shipp and Abdel-Aal, 2010) (Fig. 3).

Biological activities of anthocyanins

Apart from the nutritional values of food, they have several other valuable effects on human health. Anthocyanins found in some food and beverages has revealed to exhibit a vital role in the avoidance of numerous diseases like cancer; cardiovascular diseases requiring mechanisms of antioxidant activity, detoxification activity, anti-proliferation, induction of apoptosis; anti-inflammatory activity; inhibition of digestive enzymes (α -glycosidase, α -amylase, protease, and lipase), which is a clinical therapeutic target for regulatory type II diabetes and obesity; development of the immune system; enhancement of night vision along within the retard of the aging process, reducing, for instance, the risk of degenerative disorders, like Alzheimer's disease (Pandey and Rizvi, 2009). Moreover, anthocyanins can also intervene in: (1) reduction of the permeability of the blood-brain barrier to toxic materials; acylated anthocyanins from red cabbage were revealed to avoid toxins paraquat-induced oxidative stress; (2) *in vitro* inhibition of platelet aggregation, that is cardioprotective agents; (3) antitumor activity, inhibition of angiogenesis, anthocyanins of fruit extracts, such as, blueberry, bilberry, cranberry, strawberry, and raspberry, were revealed to prevent both the beginning of carcinogenesis and the development of tumors (esophageal, colon, liver); specifically cyanidin and delphinidin repressed the progress of certain tumor cells by prevention of the epidermal growth-factor receptor, whereas the equivalent glycosides were inactive, and malvidin from grapes less active; structural components was found liable for the extraordinary effect of berry anthocyanins on the vascular endothelial growth-factor

expression and release, in other words, antiangiogenetic effect; (4) antimutagenic activity; (5) stimulation of insulin in pancreatic cells; (6) protection against liver impairment; (7) favorable effects on neurodegenerative developments in Parkinson's or Alzheimer's disease; this is possibly because of the capacity of anthocyanins to decrease inflammation and oxidative stress in brain (Liu *et al.*, 2005; Thenmozhi *et al.*, 2016). It was revealed that berries ameliorate dopamine release in the brain associated with improved communication between brain cells. High consumption of anthocyanins via nourishments rich in fruits and vegetables could hinder or inverse age-linked changes in brain and behavior; (8) antiulcer properties; (9) protection against UV-B radiation (290-320 nm); especially cyanidin acts as a skin photoprotective agent (Giusti, 2003; Bagchi, 2004; Lila, 2004b; Gochenaour, 2006; McGhie, 2007; Dilip and Konishi, 2007; Susanne, 2008; Aramwit, 2010; Jaime, 2010; Miguel, 2011; Simona, 2011; Tsuda, 2012). The use of anthocyanins for therapeutic purposes lasts for numerous years due to their pharmacological properties. Therefore, crude extracts of *Vaccinium myrtillus* administered orally, intravenously, or intramuscularly are capable to lessen the capillary permeability and fragility. OptiBerry is a health improvement molecule in the form of a synergistic mixture of six selected extracts from wild blueberry and bilberry (*Vaccinium myrtillus* and *Vaccinium corymbosum*), cranberries, elderberries, raspberries, and strawberries. This compound has displayed superb antioxidant and antiangiogenetic activities, and also antiatherosclerosis, anticarcinogenic and antibacterial properties (effective against *Helicobacter pylori* pathogen liable for the manifestation of gastrointestinal disorders including gastric ulcer and cancer). The results show that OptiBerry has low cytotoxicity, thus, safe food and dietary additive. By means of powders, anthocyanins (grapes, tomatoes, red cabbage) are used as natural food supplements in confectionery and soft drinks. Anthocyanins from red cabbage are stable over a wider range of pH, thus are used as natural substitutions to synthetic blue pigments for foods with neutral pH. Anthocyanins of hibiscus are used both in soft drinks and medical herbs (Simona, 2011).

Biosynthesis of anthocyanins in plants

Different factors determine the number of flavonoids in plants, such as, genetic variations between dissimilar plant species, the action of environmental factors like light, the temperature on the plant during development, methods of cultivation that is agronomic factors, and additional processing of plant materials. Anthocyanins are universal flavonoid colorants present in most plant parts that play a vital role in attracting pollinators and distributing seeds, and safeguard the plant against pathogens, herbivores, and environmental stresses such as UV-B light (Das, 2012; Simona, 2011).

The anthocyanins' biosynthetic pathway starts with the pentosephosphates pathway, continuous with shikimate pathway, and follows the pathway of flavonoids. Flavonoids are manufactured in the cytosol. Flavonoid production in plants begins with the amino acid phenylalanine followed by the removal of the amino group to make cinnamic acid, which will be hydroxylated to the p-coumaric acid, active in the form of Coenzyme A thioester (Ferreyra *et al.*, 2012). The biosynthetic enzymes make a super-molecular complex referred to as metabolon through protein-protein interaction and are attached in the endoplasmic reticulum membrane. The biosynthetic enzymes are members of several enzyme groups, for instance, 2-oxoglutarate-dependent dioxygenases (OGD), cytochromes P450 (P450), and glucosyltransferases (GT), and it is proposed that plants drafted these enzymes from a previously existed metabolic pathways. Phylogenetic examination shows that genes encoding enzymes with the equivalent or analogous activity had differed before the speciation of seed plants. Chalcone synthase (CHS), a polyketide synthase, is the first devoted enzyme in the pathway, and it catalyzes the synthesis of THC (tetrahydrochalcone) from one molecule of 4-coumaroyl CoA and three molecules of malonyl CoA. THC is quickly and stereospecifically isomerized to the colorless (2S)-naringenin by chalcone isomerase (CHI). (2S)-Naringenin is hydroxylated at the 3-position by flavanone 3-hydroxylase (F3H) to produce (2R,3R)-dihydrokaempferol, a hydroflavonol. F3H is a member of the OGD family. F3H also catalyzes the hydroxylation of eriodictyol and pentahydroxyl flavanones to dihydroquercetin and dihydromyricetin, respectively. Flavonoid 3'-hydroxylase (F3'H) and flavonoid 3',5'-hydroxylase (F3'5'H), that are P450 enzymes, facilitate the hydroxylation of dihydrokaempferol(DHK) to make (2R,3R)-dihydroquercetin and dihydromyricetin, respectively. F3'H and F3'5'H regulate the hydroxylation configuration of the B-ring of flavonoids and anthocyanins, and are compulsory for cyanidin and delphinidin synthesis, respectively. They are the main enzymes that decide the structures of anthocyanins and therefore their color. F3'H and F3'5'H catalyze the hydroxylation of flavanones, flavonols, and flavones (Han *et al.*, 2010; Schwinn *et al.*, 2014; Deng *et al.*, 2018).

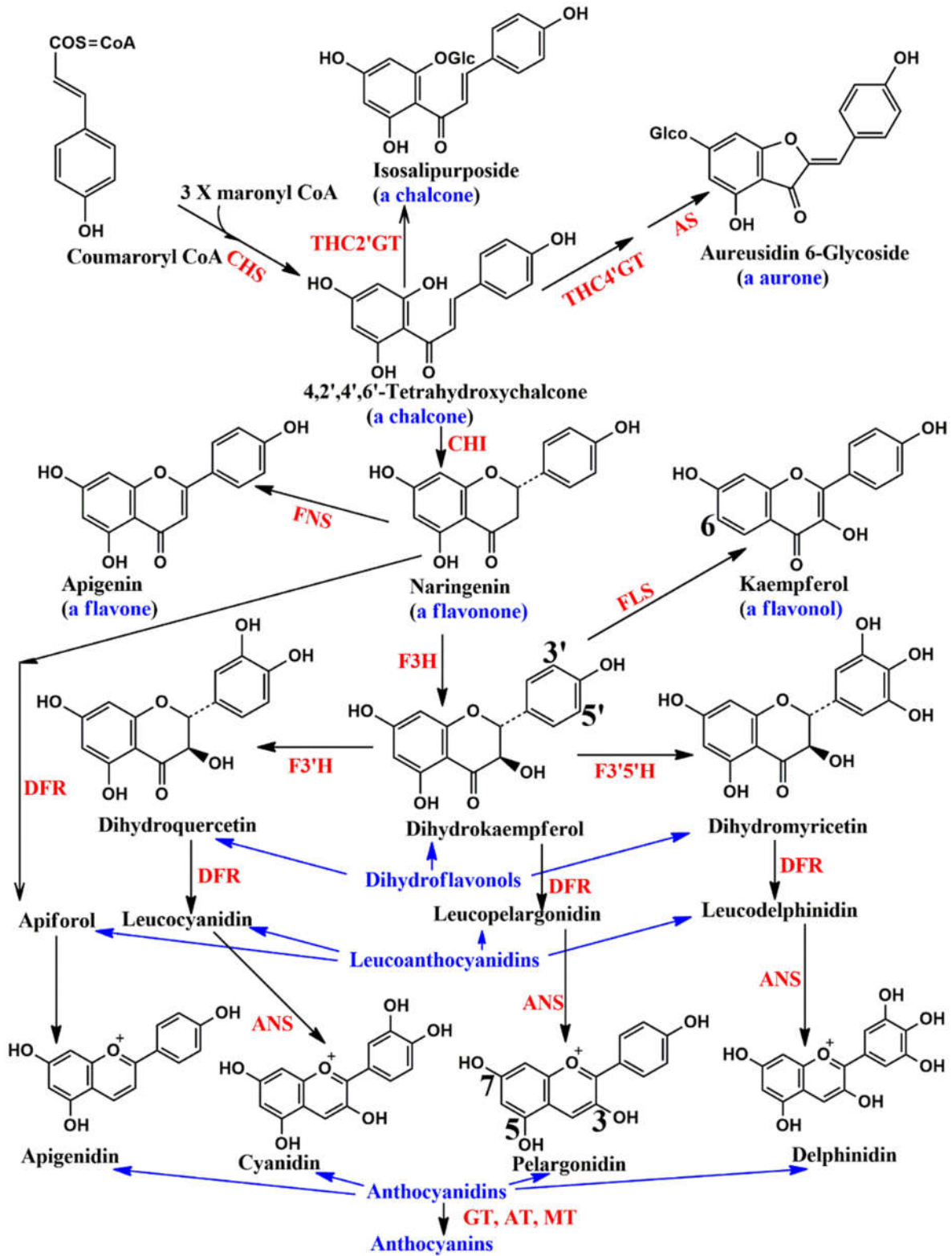
Many essential horticultural crops, for example, roses (*Rosa hybrida*), chrysanthemums (*Chrysanthemum morifolium*), and carnations, do not yield delphinidin and consequently lack violet and/or blue color diversities. This is accredited to the reason that they do not own the F3'5'H gene, possibly because they lost it throughout their evolution. Fascinatingly, some species of Asteraceae have regained the F3'5'H activity from their F3'H gene by convergent evolution. Transgenic blue/violet carnations and roses have been evolved by expressing a heterologous F3'5'H gene.

Dihydroflavonols are reduced to resulting in 3,4-cis leucoanthocyanidins throughout the use of dihydroflavonol 4-reductase (DFR). In different plant species, like petunia (*Petunia hybrida*) and cymbidium (*Cymbidium hybrida*), DFR is more specific to a substrate and unable to employ dihydrokaempferol. Subsequently, these species lack anthocyanins based on pelargonidin and consequently lack flowers of an orange/brick red shade. Furthermore, anthocyanidin synthase (ANS, also referred to leucoanthocyanidin dioxygenase), that belongs to the OGD family, catalyzes the synthesis of analogous dyed anthocyanidins. The genes encoding the enzymes listed above have been isolated and characterized from flowers of numerous plants, along-with petunia, snapdragon (*Antirrhinum majus*), gentian (*Gentiana triflora*), torenia (*Torenia hybrida*), morning glories, and other tissues of maize, Perilla and Arabidopsis (Delgado-Vargas, 2000; Tanaka, 2008; Gou, 2011; Sharma, 2011; Simona, 2011; Das, 2012) (Fig. 4).

Production of anthocyanins through fermentation

The best-characterized and understood pathways in plant secondary metabolism are the anthocyanin pathway. This pathway is characterized by both genetically and enzymatically (Levisson *et al.*, 2018; Petroni and Tonelli, 2011). It offshoots from the flavonoid pathway after the formation of flavanones, for instance, naringenin and eriodictyol. The accessibility of genes for the complete pathway unlocks the opportunity for the expression of the full plant biosynthetic pathway in a microbial host and therefore generates a workable suitable platform for the biotechnological assembly of specific anthocyanin derivatives. Naringenin is synthesized through the phenylpropanoid pathway, beginning from the aromatic amino acids phenylalanine or tyrosine. Yeast strains engineered using the pathway towards naringenin has been in engineered *Saccharomyces cerevisiae* (Koopman *et al.*, 2012; Lyu *et al.*, 2017; Levisson *et al.*, 2018). Beginning with naringenin, anthocyanidins are synthesized by flavanone 3-hydroxylase (F3H; syn. FHT), dihydroflavonol 4-reductase (DFR), and anthocyanidin synthase (ANS; syn. leucoanthocyanidin dioxygenase). ANS has been identified as a multifunctional protein catalyzing several reactions with distinct flavonoid substrate intermediates (Wellmann *et al.*, 2006; Martens *et al.*, 2010; Levisson *et al.*, 2018). The last step to a basic anthocyanin is catalyzed by an anthocyanin 3-O-glucosyltransferase (3GT). Additional variations of the anthocyanidin skeleton can be integrated at different sites in this pathway, for example via the action of flavonoid hydroxylases, methyltransferases, other glycosyltransferases, and acyltransferases (Levisson *et al.*, 2018; Zha and Koffas, 2018) (Fig. 5).

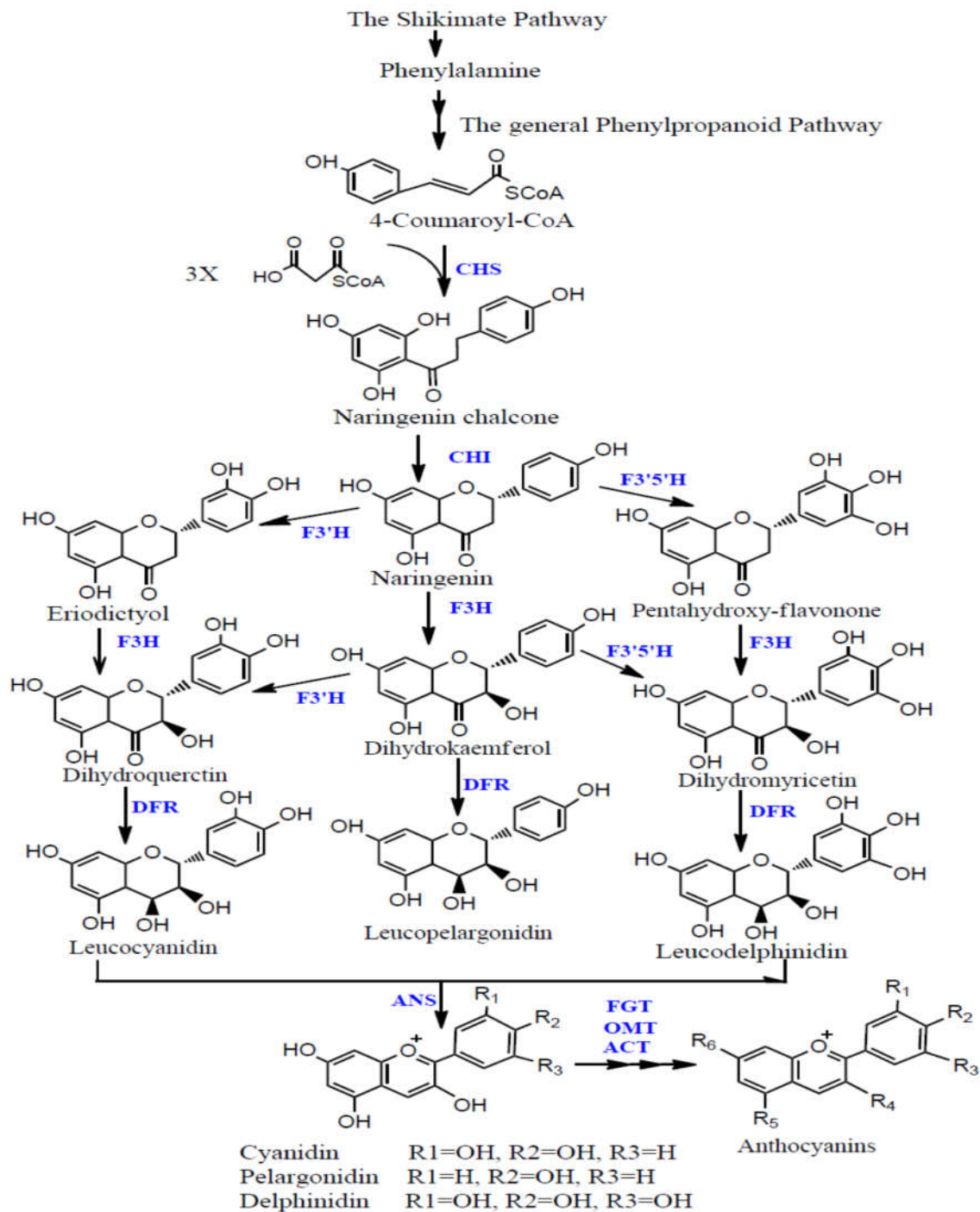
Fig. 4. The anthocyanins biosynthetic pathway (Tanaka, 2008).



*Corresponding author

Fig. 5. The biosynthetic pathway of anthocyanins in plants.

The overall precursor phenylalanine, got from the shikimate pathway, go into the phenylpropanoid pathway to supply the intermediate coumaroyl-CoA for the synthesis of flavonoids. Coumaroyl-CoA go through condensation with malonyl-CoA to make naringenin chalcone, which goes through several alterations to produce various anthocyanin compounds. R₁-R₅ are functional groups involved in the variation of diverse carbons in anthocyanin molecules, for example glycosyl, acyl, methyl, and hydroxyl groups. Abbreviations of enzymes: CHS chalcone synthase, CHI chalcone isomerase, F₃H flavanone 3-hydroxylase, F₃'H flavonoid 3'-hydroxylase, F₃'5'H flavonoid 3', 5'-hydroxylase, DFR dihydroflavonol reductase, ANS anthocyanidin synthase, FGT flavonoid-glucosyltransferase, OMT O-methyltransferase, ACT acyltransferase (Source: Zha and Koffas, 2018).



Anthocyanin biosynthesis has been accomplished in *Escherichia coli*, through introducing parts of the anthocyanin biosynthetic pathway and via enriching the growth medium with precursors like coumaric acid, naringenin, catechin, or eriodyctiol (Yan *et al.*, 2005; 2008; Leonard *et al.*, 2008; Levisson *et al.*, 2018; Lim *et al.*, 2015; Zha and Koffas, 2017). Several approaches have been evolved to enhance further the anthocyanin yield in this system, as well as higher accessibility of UDP-glucose, switching the carbon metabolism to malonyl-CoA and optimizing cultivation parameters. These conducted to the synthesis of the anthocyanin cyaniding 3-O-glucoside (C3G) at a titer of 350 mg/L (Levisson *et al.*, 2018; Lim *et al.*, 2015). Lately, a polyculture of four *E. coli* strains was utilized to get de novo synthesis of pelargonidin 3-O-glucoside at a titer of 9.5 mg/L (Jones *et al.*, 2017; Levisson *et al.*, 2018). Nonetheless, despite advancement at the genetic and cultivation stages, the building of a single microbial strain able of making de novo anthocyanins and employing a simple carbon source is required.

Stability of anthocyanins pigments

Many factors are used to determine the stability of anthocyanin pigments, for example, structure and concentration of the colorant, pH, temperature, light intensity and quality, the existence of copigments, metal ions, enzymes, oxygen, ascorbic acid, sugars, and their degeneration products and sulfur dioxide, and so on. The equilibrium of four anthocyanin structures exists in acidic media, which are, flavylium cation, quinonoidal base, carbinol pseudobase and chalcone. The comparative quantities of these structures at equilibrium differ with pH and anthocyanin structure (Cevallos-Casals and Cisneros-Zevallos, 2004; Bakowska-Barczak, 2005). The stability of some anthocyanins is higher than that of others regarding their molecular structure. Normally, raised hydroxylation reduces stability, however, raised methylation raises it. The color of foods comprising anthocyanins that are rich in pelargonidin, cyanidin, or delphinidin aglycones is less stable than that of food having petunidin or malvidin aglycones. Anthocyanins holding galactose are more stable than those comprising arabinose. Moreover, acylation with hydroxylated aromatic organic acids grants higher stability, with few exceptions. The stability of anthocyanins can also upsurge with intermolecular copigmentation. Aqueous fruit, vegetable, and grain extracts, with high anthocyanin content, have mixtures of various chemicals that may function as copigments for intermolecular connection with anthocyanins. Nonetheless, not all chemicals improve copigmentation; for instance, sugars and their deterioration products serve to intensify the deterioration of anthocyanins. The frequency of anthocyanin deterioration is linked with the speed at which the sugar is deteriorated to furfural-type compounds resulted from the Maillard

reaction (Chandra *et al.*, 2001; Cevallos-Casals and Cisneros-Zevallos, 2004; Bakowska-Barczak, 2005; Wang *et al.*, 2010; Ma *et al.*, 2012).

Conclusion

Food pigments are frequently chemical-based and because of the detrimental effects of chemicals used in the food industry; customers are avoiding foods holding synthetic pigments that guide food industries to substitute them by natural pigments which include anthocyanins. Anthocyanins have been recommended as favorable nutritional products with a significant role in mankind welfare. They are the prevalent group of water-soluble colorants in the plants that are liable for the red, purple, and blue color in fruits, vegetables, flowers, and grains. Anthocyanins are appraised to be largely eaten up by human worldwide because of being natural pigments found in fruits, red wine, and vegetables. Attention in anthocyanin-rich foods and extracts has augmented lately as a result of their potential health benefits, such as antioxidants, anti-diabetic, anti-carcinogenic properties, and holding visual activities and so on. These aptitudes support the role of anthocyanins as natural food pigments, functional foods, and dietary additives. However, the color and stability of anthocyanin colorants are reliant on numerous factors, such as structure and concentration of the colorant, pH, temperature, light intensity and quality, presence of co-pigments, metallic ions, enzymes, oxygen, ascorbic acid, sugars, and their degradation products and sulfur dioxide. This can be solved by using more stable anthocyanin compounds, like methylated compounds or acylated compounds for long time storage food.

Acknowledgements

Authors sincerely acknowledge the valuable support provided by Fabien Nsanzabera for drawing different figures.

References

1. Aam, E., E S Mirghani, M., Kh, M., Na, K. and Mz, A. 2018. Challenges of Extraction Techniques of Natural Antioxidants and Their Potential Application Opportunities as Anti-Cancer Agents. *Health Sci. J.* 12: 12-19.
2. Aberoumand, A. 2011. A Review Article on Edible Pigments Properties and Sources as Natural Biocolorants in Foodstuff and Food Industry. *World J. Dairy Food Sci.* 6: 71-78.
3. Alan, M. 2006. Carotenoids and other pigments as natural colorants. *Pure Appl. Chem.* 78.
4. Alkema, J. and Seager, S.L. 1982. The Chemical Pigments of Plants. *J. Chem. Educ.* 59: 183-186.
5. Aramwit, P.B. and Nipaporn Srichana, Teerapol. 2010. The properties and stability of anthocyanins in mulberry fruits. *Food Res. Int.* 43: 1093-1097.



6. Aza-González, C., Núñez-Paleniús, H.G. and Ochoa-Alejo, N. 2012. Molecular Biology of Chili Pepper Anthocyanin Biosynthesis. *J. Mex. Chem. Soc.* 56: 93-98.
7. Bakowska-Barczak, A. 2005. Acylated Anthocyanins as Stable, Natural Food Colorants – A Review. *Pol. J. Food Nutr. Sci.* 14(55): 107–116.
8. Bartley, G.E., Viitanen, P.V., Pecker, I., Chamovitz, D., Hirschberg, J. and Scolnik, P.A. 1991. Molecular cloning and expression in photosynthetic bacteria of a soybean cDNA coding for phytoene desaturase, an enzyme of the carotenoid biosynthesis pathway. *Proc. Nat. Acad. Sci. USA* 88: 6532-6536.
9. Cevallos-Casals, B.A. and Cisneros-Zevallos, L. 2004. Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. *Food Chem.* 86: 69–77.
10. Chaitanya Lakshmi, G. 2014. Food Coloring: The Natural Way. *Res.J.Chem.Sci.* 4: 87-96.
11. Chandra, A., Rana, J. and Li, Y. 2001. Separation, Identification, Quantification, and Method Validation of Anthocyanins in Botanical Supplement Raw Materials by HPLC and HPLC-MS. *J. Agric. Food Chem.* 49: 3515–3521.
12. Clydesdale, F.M. 2009. Color as a factor in food choice. *Crit. Rev. Food Sci. Nutr.* 33: 83-101.
13. D. Bagchi, C.K.S., M. Bagchi, and Atalay, M.. 2004. Anti-angiogenic, Antioxidant, and Anti-carcinogenic Properties of a Novel Anthocyanin-Rich Berry Extract Formula. *Biochemistry (Moscow)*. 69(1): 2004, pp. 95
14. Das, P.K.S., Choi, D. H. and S. B. Park, Y. I. 2012. Sugar-hormone cross-talk in anthocyanin biosynthesis. *Mol. Cells.* 34: 501-507.
15. De Pascual-Teresa, S., Moreno, D.A. and Garcia-Viguera, C. 2010. Flavanols and anthocyanins in cardiovascular health: a review of current evidence. *Int. J. Mol. Sci.* 11: 1679-1703.
16. Delgado-Vargas, F., Jiménez, A.R. and Paredes-López, O. 2000a. Natural Pigments: Carotenoids, Anthocyanins, and Betalains — Characteristics, Biosynthesis, Processing, and Stability. *Crit. Rev. Food Sci. Nutr.* 40: 173–289.
17. Delgado-Vargas, F., Jiménez, A.R. and Paredes-López, O. 2000b. Natural Pigments: Carotenoids, Anthocyanins, and Betalains— Characteristics, Biosynthesis, Processing, and Stability. *Crit. Rev. Food Sci. Nutr.* 40: 173-289.
18. Deng, Y., Li, C., Li, H. and Lu, S. 2018. Identification and Characterization of Flavonoid Biosynthetic Enzyme Genes in *Salvia miltiorrhiza* (Lamiaceae). *Mol.* 23: 25-29.
19. Dilip Ghosh, P. and Tetsuya Konishi, P. 2007. Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. *Asia Pac. J. Clin. Nutr.* 16: 200-208.
20. F. Delgado-Vargas, A.R.J., and Paredes-López, O. 2000. Natural Pigments: Carotenoids, Anthocyanins, and Betalains— Characteristics, Biosynthesis, Processing, and Stability. *Crit. Rev. Food Sci. Nutr.* 40: 173–289.
21. Ferreyra, M.L.F., Rius, S.P. and Casati, P. 2012. Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Front. Plant Sci.* 3: 25-29.
22. Francis, F.J. and Markakis Pericles, C. 1989. Food colorants: anthocyanins. *Crit. Rev. Food Sci. Nutr.* 28: 273-314.
23. Giusti, M.M. and Wrolstad, R.E. 2003. Acylated anthocyanins from edible sources and their applications in food systems. *Biochem. Eng. J.* 14: 217-225.
24. Giusti, M.M.W. and Ronald E. 2003. Acylated anthocyanins from edible sources and their applications in food systems. *Biochem. Eng. J.* 14: 217-225.
25. Gochenaur, D.R.B.a.K. 2006. Direct vasoactive and vasoprotective properties of anthocyanin-rich extracts. *J. Appl. Physiol.* 100: 1164–1170.
26. Gou, J.Y.F., Liu, F. F., Weigel, C. J. and D. Wang, J. W. 2011. Negative regulation of anthocyanin biosynthesis in Arabidopsis by a miR156-targeted SPL transcription factor. *Pl. Cell.* 23: 1512-1522.
27. Griffith, J.C. 2005. Coloring Foods & Beverages: Natural and synthetic colors play several roles in foods and beverages. Here's how they are regulated in the United States. *Food Technol.* 59: 38-45.
28. Han, Y., Vimolmangkang, S., Soria-Guerra, R.E., Rosales-Mendoza, S., Zheng, D., Lygin, A.V. and Korban, S.S. 2010. Ectopic expression of apple F3'H genes contributes to anthocyanin accumulation in the Arabidopsis tt7 mutant grown under nitrogen stress. *Pl. Physiol.* 153: 806-820.
29. Harborne, J.B. and Sherratt, H.S.A. 1961. Plant Polyphenols: Flavonoids in Genotypes of *Primula Sinensis*. *Biochem. J.* 78: 298-306.
30. Jaime Guerrero C., L.C.P., Andrea Castilla C., Fernando Medel S., Heidi Schalchli S., Emilio Hormazabal U., Emma Bensch T. and Miren Alberdi, L. 2010. Antioxidant Capacity, Anthocyanins, and Total Phenols of Wild and Cultivated Berries in Chile. *Chil. J. Agric. Res.* 70: 537-544.
31. Jang, Y.P., Zhou, J., Nakanishi, K. and Sparrow, J.R. 2005. Anthocyanins Protect Against A2E Photooxidation and Membrane Permeabilization in Retinal Pigment Epithelial Cells. *Photochem Photobiol.* 81: 529–536.
32. Jones, J.A., Shirke, A.N., Vernacchio, V.R., Xiu, Y., McCutcheon, C.C., Collins, S.M., Englaender, J.A., Linhardt, R.J., Cress, B.F., Gross, R.A. and Koffas, M.A.G. 2017. Complete Biosynthesis of Anthocyanins Using *E. coli* Polycultures. *mBio.* 8: e00621-00617.
33. Koopman, F., Beekwilder, J., Crimi, B., Houwelingen, A.v., Hall, R.D., Bosch, D., Maris, A.J.v., Pronk, J.T. and Daran, J.-M. 2012. De novo production of the flavonoid naringenin in engineered *Saccharomyces cerevisiae*. *Microb. Cell Fact.* 11: 52-59.
34. Leonard, E., Yan, Y., Fowler, Z.L., Li, Z., Lim, C.-G., Lim, K.-H. and Koffas, M.A.G. 2008. Strain Improvement of Recombinant *Escherichia coli* for Efficient Production of Plant Flavonoids. *Mol. Pharm.* 5: 257–265.
35. Levisson, M., Patinios, C., Hein, S., Groot, P.A.d., Daran, J.-M., Hall, R.D., Martens, S. and Beekwilder, J. 2018. Engineering de novo anthocyanin production in *Saccharomyces cerevisiae*. *Microb. Cell Fact.* 17: 21-29.
36. Lila, M.A. 2004a. Anthocyanins and Human Health: An In Vitro Investigative Approach. *J Biomed Biotechnol.* 5: 306–313.
37. Lila, M.A. 2004b. Anthocyanins and Human Health: An In Vitro Investigative Approach. *J Biomed Biotechnol.* 2004: 306–313.
38. Lim, C.G., Wong, L., Bhan, N., Dvora, H., Xu, P., Venkiteswaran, S. and Koffas, M.A. 2015. Development of a Recombinant *Escherichia coli* Strain for Overproduction of the Plant Pigment Anthocyanin. *Appl Environ Microbiol.* 81: 6276-6284.
39. Liu, Z., Schwimer, J., Liu, D., Greenway, F.L., Anthony, C.T. and Woltering, E.A. 2005. Black Raspberry Extract and Fractions Contain Angiogenesis Inhibitors. *J. Agric. Food Chem.* 53: 3909–3915.

40. Lyu, X., Ng, K.R., Lee, J.L., Mark, R. and Chen, W.N. 2017. Enhancement of Naringenin Biosynthesis from Tyrosine by Metabolic Engineering of *Saccharomyces cerevisiae*. *J. Agric. Food Chem.* 65: 6638-6646.
41. Ma, C., Yang, L., Yang, F., Wang, W., Zhao, C. and Zu, Y. 2012. Content and Color Stability of Anthocyanins Isolated from *Schisandra chinensis* Fruit. *Int. J. Mol. Sci.* 13: 14294-14310.
42. Martens, S., Preuss, A. and Matern, U. 2010. Multifunctional flavonoid dioxygenases: flavonol and anthocyanin biosynthesis in *Arabidopsis thaliana* L. *Phytochem.* 71: 1040-1049.
43. Mazza, G.J. 2007. Anthocyanins and heart health. *Ann Ist Super Sanità.* 43: 369-374.
44. McGhie, T.K.W. and M. C. 2007. The bioavailability and absorption of anthocyanins: towards a better understanding. *Mol. Nutr. Food Res.* 51: 702-713.
45. McGhie Tony, K. and Walton Michaela, C. 2007. The bioavailability and absorption of anthocyanins: towards a better understanding. *Mol. Nutr. Food Res.* 51: 702-713.
46. Miguel, M.G. 2011. Anthocyanins: Antioxidant and/or anti-inflammatory activities. *J. Appl. Pharm. Sci.* 01: 07-15.
47. Miguel, M.G. 2011. Anthocyanins: Antioxidant and/or anti-inflammatory activities. *J. Appl. Pharm. Sci.* 01: 07-15.
48. Paliwal, H., Goyal, S., Singla, S. and Daksh, S. 2016. Pigments from natural sources: An overview. *Int. J. Pharm. Pharm. Sci.* 1: 01-12.
49. Pandey, K.B. and Rizvi, S.I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longev.* 2: 270-278.
50. Pazmino-Duran, E.A., Giusti, M.M., Wrolstad, R.E. and Gloria, M.B.A. 2001. Anthocyanins from *Oxalis triangularis* as potential food colorants. *Food Chem.* 75: 211-216.
51. Petroni, K. and Tonelli, C. 2011. Recent advances on the regulation of anthocyanin synthesis in reproductive organs. *Plant Sci.* 181: 219-229.
52. Roy, H.J., Lundy, S., Eriksen, C. and Kalicki, B. 2009. Anthocyanins. Pennington Nutrition Series No 1, Division of Education, Pennington Biomedical Research Center.
53. Schwinn, K., Miosic, S., Davies, K., Thill, J., Gotame, T.P., Stich, K. and Halbwirth, H. 2014. The B-ring hydroxylation pattern of anthocyanins can be determined through activity of the flavonoid 3 α -hydroxylase on leucoanthocyanidins. *Planta.* 240: 1003-1010.
54. Sharma, M.C.-C., Ahern, M., McMullen, K. R., Brutnell, M. and T. P. Chopra, S. 2011. Identification of the pr1 gene product completes the anthocyanin biosynthesis pathway of maize. *Genetics.* 188: 69-79.
55. Shipp, J. and Abdel-Aal, E.-S.M. 2010. Food Applications and Physiological Effects of Anthocyanins as Functional Food Ingredients. *Open Food Sci. J.* 4: 7-22.
56. Simona Oancea, L.O. 2011. Anthocyanins, from biosynthesis in plants to human health benefits. *Food Technol.* XV: 3-16.
57. Stintzing, F.C. and Carle, R. 2004. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends Food Sci. Technol.* 15: 19-38.
58. Susanne U. Mertens-Talcott, J.R., Jilma-Stohlawetz, P., Pacheco-Palencia, L. A., Meibohm, B., Talcott, S. T., and Derendorf, H. 2008. Pharmacokinetics of Anthocyanins and Antioxidant Effects after the Consumption of Anthocyanin-Rich *Açai* Juice and Pulp (*Euterpe oleracea* Mart.) in Human Healthy Volunteers. *J. Agric. Food Chem.* 30: 63-69.
59. Tanaka, Y.S. and Ohmiya, N. A. 2008. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *Pl. J.* 54: 733-749.
60. Thenmozhi, A.J., Manivasagam, T., Essa, M.M. 2016. Role of Plant Polyphenols in Alzheimer's Disease, in: Essa, M.M., Akbar, M., Guillemin, G. (Eds.), *The Benefits of Natural Products for Neurodegenerative Diseases*. Advances in Neurobiology, Springer International Publishing Switzerland, pp. 153-171.
61. Tsuda, T. 2012. Dietary anthocyanin-rich plants: biochemical basis and recent progress in health benefits studies. *Mol. Nutr. Food Res.* 56: 159-170.
62. Wang, B.C., He, R. and Li, Z.M. 2010. The Stability and Antioxidant Activity of Anthocyanins from Blueberry. *Food Technol. Biotechnol.* 48: 42-49.
63. Wellmann, F., Griesser, M., Schwab, W., Martens, S., Eisenreich, W., Matern, U. and Lukačič, R. 2006. Anthocyanidin synthase from *Gerbera hybrid* catalyzes the conversion of (+)-catechin to cyanidin and a novel procyanidin. *FEBS Lett.* 580: 1642-1648.
64. Yan, Y., Chemler, J., Huang, L., Martens, S. and Koffas, M.A. 2005. Metabolic engineering of anthocyanin biosynthesis in *Escherichia coli*. *Appl. Environ. Microbiol.* 71: 3617-3623.
65. Yan, Y., Li, Z. and Koffas, M.A.G. 2008. High-yield anthocyanin biosynthesis in engineered *Escherichia coli*. *Biotechnol. Bioeng.* 100: 126-140.
66. Zha, J. and Koffas, M.A.G. 2017. Production of anthocyanins in metabolically engineered microorganisms: Current status and perspectives. *Synth. Syst. Biotechnol.* 2: 259-266.
67. Zha, J. and Koffas, M.A.G. 2018. Anthocyanin Production in Engineered Microorganisms. In: Schwab W., Lange B., Wüst M. (eds) *Biotechnology of Natural Products*. Springer, Cham, pp. 81-97.

Cite this Article as:

Irakoze, E., Nduwayezu, B. and Gakuba, E. 2020. Biosynthesis of anthocyanins and their applications as food additives. *J. Acad. Indus. Res.* 9(1): 10-20.