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"Polyphenols for improving food
quality and nutraceuticals"

Review

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Polyphenols as Colorants

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ABSTRACT

The market for natural colors for food applications is ever increasing. Consumers are seeking simpler and cleaner labels for their foods and are looking for alternatives to synthetic ingredients. As a result, interest in natural products including natural colorants is obvious. This review explores the potential of polyphenols and polyphenol derived molecules as colorants. Polyphenols are classified into flavonoids or non-flavonoids and are known for their antioxidant and biological activity. Anthocyanins are well known colored flavonoids but other polyphenols including many aurones and chalcones derivatives are also colored. Curcumin, demethoxycurcumin, bisdemethoxycurcumin and cyclocurcumin are isolated from turmeric. Turmeric extract is used as a colorant in many food applications owing to its high heat stability. Anthraquinone based pigments occur in nature, one of which is tetrahydroxylated anthraquinone – carminic acid. Carminic acid and its lake are commonly used pigments and provide a red to pink hue depending on pH of a food or beverage. Usually colorless polyphenols can give rise to colored products by virtue of presence of enzymes or reaction with other substrates or both. Polyphenol oxidase (PPO) plays an important role in these reactions. Typically polyphenol content is lower after these reactions occur. Tea pigments are a good example where PPO oxidizes Epicatechin and epicatechin-3-O-gallate (EGCG) and other polyphenols. The reaction products being very unstable in nature interact chemically and generate pigments. During cocoa processing the nibs undergo fermentation, leading up to enzymatic reactions causing color development at the expense of polyphenols. Avocado seeds undergo similar reactions in presence of PPO and oxygen. Similarly in wine, new pigments called pyranoanthocyanins are generated from anthocyanins. During juice extraction in apples, colored products are formed from colorless chalcones as a result of PPO activity. PPO products have been thought to be polymeric in nature but in the reviewed studies that is not the case. Among the polyphenols discussed in the study, aurones, chalcones and Hydroxy-anthraquinones (HAQs) have not been exploited to full potential. More research on optimizing isolation of pigments and using in food systems is needed. Regulatory hurdles will need to be cleared to enable their use in food applications.

KEYWORDS: Natural colors; Polyphenols; Polyphenol oxidase; Anthocyanin; Anthraquinone; Carminic acid; Thearubigins; Theaflavin; Phloretin xyloglucoside oxidation product; Pyranoanthocyanin.

ABBREVIATIONS: PPO: Polyphenol Oxidase; EGCG: Epicatechin and epicatechin-3-O-gallate; FDA: Food and Drug Administration; EG: Epigallocatechin; EGC: Epigallocatechin-3-O-gallate; HAQ: Hydroxy-anthraquinone.

INTRODUCTION

Polyphenols can be most simply defined as molecules having more than one hydroxyl group in their structure. They are ubiquitous molecules which act as secondary metabolites in plants. They can have a simple structure like phenolic acids or very complex like tannins. They can be structurally classified as flavonoids or non-flavonoids. Non-flavonoids include phenolic acid derivatives, stilbenes, tannins and lignins. These compounds are mostly colorless or light yellow/brown and rarely contribute to the color of foods. This review will focus on polyphenols that are colored and also the polyphenols which produce color as a result of processing includ-

ing fermentation, enzyme activity or heat.

Colorants for foods can be categorized as natural or synthetic. However the definition of the term natural colorants is fairly limited per the FDA. For example using strawberry anthocyanins to color strawberry fruit preparation will be termed as natural color. All other colorants are deemed artificial by the U.S. based and other regulating agencies. However natural colors, *i.e.* colorants derived from natural sources, fall under color additives exempt from certification per the regulations devised by United States (US) Food and Drug Administration (FDA). Currently 37 pigments are exempt, which means every batch of these colorants does not need to be certified through FDA. There is another category, constituting synthetic colorants, of which every batch needs FDA certification. Currently 9 synthetic dyes are permitted; however there are limitations for some as to which applications they can go into. The seven which can be added to most food applications are Blue No 1, Blue No. 2, Green No 3, Red No. 3, Red No. 40, Yellow No. 5 and Yellow No. 6.¹

The global natural food color market was estimated at US \$1,144.0 million in 2014, and is expected to reach \$1,697.6 million by 2020, reflecting a growth rate of 6.8% during forecast period. Synthetic colorants are not expected to grow at the same rate. The global natural food color market represented 54.9% of the total food color market in 2014 and is expected to account for nearly 60% of the overall market by 2020. The key applications of natural food colors are confectionary & bakery, beverages, packaged foods, dairy products, frozen foods, condiments, dressings, functional foods and pet foods.²

Some polyphenols possess color because they absorb light between 400-700 nm. Food coloring molecules absorb light due to presence of conjugated double bonds, creating a delocalized electron system. The color reflected depends on the number of conjugated bonds in the structure. Polyphenols are known for their antioxidant, biological and health promoting properties – properties which will not be dealt with in this review. The focus of this review is to explore polyphenolic colorants. Polyphenols may inherently display colors or may need to be processed before color is produced. Both of these classes are discussed below.

POLYPHENOLS THAT ARE COLORED

(A) Anthocyanins: Anthocyanins are well known and utilized pigments and vary in hue from orange, red, blue or purple in color. Purple sweet potato, purple carrot, cabbage & red radish are commonly used sources of these pigments. These pigments belong to flavonoids class of polyphenols. Anthocyanins show a lot of diversity but are based on select anthocyanidin structures. Such diversity is produced by chemical combination of C6-C3-C6 anthocyanidin structure with sugars and/or acyl groups. The basic structure is drawn in Figure 1 below. The structures differ in the number and position of hydroxyl and methyl ester groups.

The six most abundant structures are depicted below in Table 1 below. Generally, as the number of methyl esters increase in the structure, the molecule becomes bluer in color.

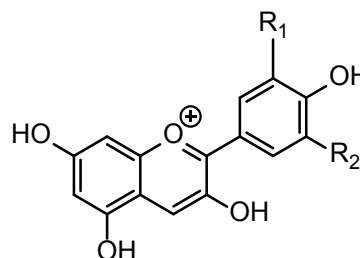


Figure 1: Basic structure of anthocyanin.

Anthocyanidin	R1	R2	λ_{max} (nm) color
Pelargonidin	H	H	494/orange
Cyanidin	OH	H	506/orange-red
Peonidin	OCH3	H	506/orange-red
Delphinidin	OH	OH	508/red
Petunidin	OCH3	OH	508/red
Malvidin	OCH3	OCH3	510/bluish-red

Table 1: Anthocyanidins commonly seen in nature, their B ring substitutes and colors.³

Stability of these pigments can be increased by intermolecular pigmentation by adding other polyphenolics, which interact with the molecule without forming a covalent bond. These groups prevent the nucleophilic attack by water molecule. Anthocyanins can also self-associate at C4 position or undergo intramolecular co-pigmentation owing to the presence of phenolics in their structure.

(B) Curcuminoids: Turmeric extract is approved as a color additive in the US by FDA in part 73, subpart A – 73.600 and 73.615 corresponding to turmeric and turmeric oleoresin respectively.¹ The extract contains several pigments, curcumin being the predominant one – existing in keto and enol form, and also three other curcumin derivatives namely demethoxycurcumin, bisdemethoxycurcumin and cyclocurcumin. After extraction with solvent, the pigments are crystallized, which can then be further processed for food applications. However their solubility is limited both in oil and water. The color of curcumin is yellow in neutral state ($1 < \text{pH} < 7$) and is orange in charged state ($\text{pH} > 7$). However these pigments will start to demonstrate change in hue at lower pH, beginning around $\text{pH} < 5$. These pigments are extremely stable under heat but not very stable under light.

(C) Aurones and Chalcones: Aurones are another class of flavonoids that express colors, though they are not very abundant. Aurones are the pigments responsible for coloring flowers of some popular ornamental plants, such as snapdragon and cosmos. They also exhibit a strong and broad range of biological activities.⁴ They can also be formed by oxidation of chalcones during extraction and purification. Chalcones act as an intermediate

for many color producing pigments in biosynthetic process.³

Safflower pigments are based on C-glycosyl quino-chalcone structures and are red (carthamin) or yellow in color (safflower yellow A and safflower yellow B). These pigments are not however approved by FDA as color additive. Limited research has been done on these pigments with regards to their performance in food systems.

(D) Hydroxy-anthraquinones (HAQs): HAQ based pigments exist in nature and structures containing more than one phenoxy substitute can be technically classified as polyphenolics. The color for pigments varies from yellow to red. These pigments are mainly found in plants like *Rubiaceae*, *Polygonaceae*, *Rhamnaceae*, *Fabaceae*, *Liliaceae*, *Bignoniaceae* and *Pedaliaceae*, in lichens and in the animal kingdom.

HAQs can be classified different ways. When classified according to their structure, they can be called emodin type pigments or alizarin type pigments. Emodin type pigments occur in plants of family *Fabaceae*, *Rhamnaceae* and *Polygonaceae*, some lichens and in fungi. These pigments show substitution on both aromatic rings of anthraquinones and at least two hydroxyl groups in both R1 and R8 positions (Figure 2A). The other class is alizarin type pigments –which have substitution on only one ring and at least one hydroxyl group in position R1 (Figure 2B below). These pigments also exist in animal kingdom and an example is carminic acid. The basic structure is presented in Figure 3 below.⁵ Carminic acid is a red dye extracted from several insect taxa including *Porphyrophora hemelii* (Armenian cochineal) and *Dactylopius coccus* (American cochineal). They are native to tropical South America and Mexico, where their host, *Opuntia* cacti grow. The pigments are classified as emodin type anthraquinones, and are polyphenolic in nature. They

are extracted from dried gravid insects using boiling water. Lac also has HAQ based pigments like laccaic acid A, laccaic acid B and laccaic acid C which are all tetra HAQ. Lac pigments are extracted from *Laccifer lacca*. The FDA has approved carminic acid as a colorant to be added to foods in the USA. However the pigments from lac are not approved as colorant for foods.⁶ The main coloring compounds from *Rubiaceae* family are alizarin, pseudopurpurin, purpurin etc. The roots of European madder (*Rubiatinctorum*) are used as hair dye and for other cosmetic uses.⁵

HAQs can also be classified on the basis of origination. They can be derived from plants – an example being the roots of European madder (*Rubiatinctorum*). Structurally, these belong to alizarin type pigments. These pigments are di- and tri- hydroxyl HAQ glycosides but aglycones exist as well. However, there are some families which have emodin type pigments. For example family *Fabacea e*(cassia *spp.*), *Liliacea e*(Aloes *spp.*) etc. have pigments, including emodin, aloe-emodin and rhein, chrysophanol. HAQs can also be derived from insects (carminic acid, already discussed above), lichens and fungi. Lichens produce mostly emodin type of structures, which are yellow to red in colors. HAQ pigments are widespread in nature and have been also found abundantly in microorganisms, particularly in filamentous fungi belonging to *Penicillium spp.* and *Aspergillus spp.* with different hues. An example is pigment emodin from strains of *Penicillium citrinum* and *P. islandicum*. Some strains of *Aspergillus* produce yellow and red hydroxyquinones including emodin, physcion, questin and rubrocristin. However, by using *Aspergillus* and *Penicillium* strains, several mycotoxins can be co-produced in the medium, e.g., secalonic acid D, citrinine, oxaline or aspergiolide. Many of these toxins are pigmented naphthoquinones. Apart from mycotoxigenic fungi, there are other filamentous fungi which have the ability to produce these

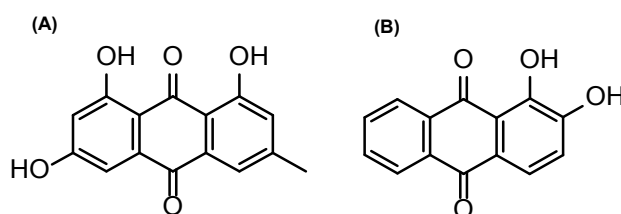


Figure 2: Structure of two types of hydroxyquinones. (A) Emodin type and (B) Alizarin type.

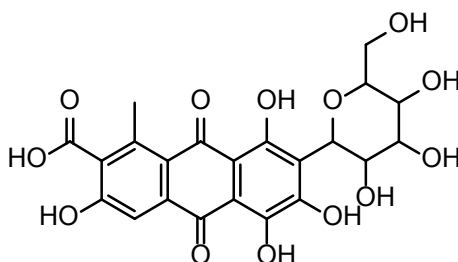


Figure 3: Structure of carminic acid.

pigments without coproduction of mycotoxins.⁵

Water solubility is a useful property for these colorants, as most of the food applications are water based. HAQs are also relatively heat and light stable in addition to being water soluble generally. However, except for carminic acid, these are not used in food on a large scale. More research needs to be carried out on these pigments to enable their use in foods.

Pigments Formed as a Result of Processing

This group consists of polyphenols which are either faintly colored or colorless but develop color as they are processed – the processing can consist of heat treatment, fermentation, exposure to oxygen or just breaking the intact cells. Polyphenols, being reactive compounds can be degraded and modified through both enzymatic and non-enzymatic reactions during processing. Some of the common enzymes that can oxidize polyphenols are PPO, peroxidases, glycosidase, esterase etc. Oxidation reactions caused by PPO and peroxidase enzymes tend to produce brown or black pigments which result from polymerization of products formed due to enzyme activity. These reactions have negative effects on fresh fruits and vegetables during transport and fruit juice and wines before fermentation. The substrate and enzyme typically interact when cells are broken down – during maceration, juice extraction, or simply processing for example fermenting green tea. Even if these reactions are unwanted in most conditions, they play an important role in the color of some products, like black tea. As will be seen below, different colors are formed as a result of enzyme and/or subsequent non-enzymatic reactions – yellow (apple juice), red (pyranoanthocyanins), orange (avocado seed) to brown (cocoa) and black (tea).

Interestingly some polyphenols inhibit the activity of PPO, like auronnes.

Discussed below are some interesting and potentially beneficial examples demonstrating color development from polyphenols.

(A) Tea pigments: Unfermented green tea is rich in flavonols, which make up about 30% of the dry weight. Enzymatic oxidation reactions during fermentation are essential for the production of semi-fermented oolong tea and fermented black tea. Black tea is an important polyphenol rich beverage with higher polyphenol content than coffee or cocoa. During its processing, four major tea catechins originally contained in fresh leaves are enzymatically converted into various oxidation products. These compounds are Epigallocatechin (EG), Epigallocatechin-3-O-gallate (EGC), epicatechin and Epicatechin-3-O-gallate (EGCG). Black tea pigments can be classified into two groups – theaflavins and thearubigins. A series of theaflavins which are yellow-orange pigments, results from oxidative coupling of B-rings of galloocatechins.⁷ Theaflavin and its galloyl esters are well characterized catechin dimers with a characteristic benzotropolone unit produced by oxidative coupling between pyrogallol

type catechin and catechol type catechins. Theaflavin content in the black tea leaves is usually 0.8-2.8% depending on conditions of fermentation. The structure of theaflavin is shown in the Figure 4 below. While the arubigins constitute up to 60% of solids in dark tea infusions and are very important to not only color, but to flavor, these pigments are still being characterized.⁸ Flavanols EGC and EGCG can be also transformed by PPO into theasinensins and oolongtheanin could be obtained from EGCG and theasinensins. It has also been proposed that structures of thearubigins are similar to those of oolongtheanin.⁹

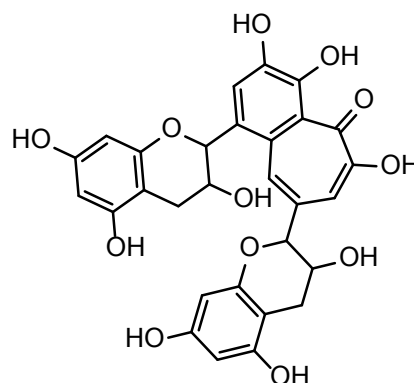


Figure 4: Structure of Theaflavin.

(B) Colored avocado seed: Avocado seed can be used as a potential source of colorant. An orange water soluble pigment is formed when avocado seed is crushed with water in presence of air. The pigment is generated from PPO activity with the color proportionally increasing as the dose of enzyme is increased. Typically PPO first forms quinones and thereafter other black or brown polymerized pigments result from these quinones. However, in this case an orange pigment is the end product. Also, this pigment was not formed by other common enzymes, like peroxidase – this was demonstrated by inactivating the enzymes in crushed avocado seed and then adding peroxidase and H₂O₂ externally. The phenolic content (mg/g Gallic Acid Equivalents) in the colored extract was only 77% of the uncolored extract, which also hinted at utilization of phenolics for formation of colored pigments.¹⁰

(C) Pigments in cocoa: Polyphenol oxidase mediated reactions occur in cocoa processing and are responsible to an extent, for brown color of cocoa. Although the activity of the enzyme is reduced rapidly during fermentation (50% activity on day 1 only 6% activity on day 2), the reactions still occur leading up to non-enzymatic oxidation and subsequent condensation into high molecular weight pigments which often, are colored. Reduction of polyphenols also occurs during this step.¹¹ This phenomenon is similar to the one occurring in avocado seed – increase in color at the expense of polyphenols.

(D) Pigments formed in apples: Apple skin is colored due to anthocyanins. However apple pulp is uncolored and apple juice color develops early during first stages of fruit processing. On maceration, plastidial PPO catalyze oxidation of vacuolar phe-

nolic compounds in the presence of oxygen generating colored O-quinones. These species being very unstable rapidly condense non-enzymatically to form colored compounds. Dihydrochalcones, namely phloridzin and phloretin etc., play a major role by being the substrates for PPO. Phloretin glycosides may also have synergistic effects on color development when in the presence of chlorogenic acid or catechins and could play a significant role in complex color of real apple juice.

Apple pulp was used to extract juice with or without presence of oxygen. Flavanol monomers, hydroxycinnamic acids, dihydrochalcones were the main phenolic classes acting as color precursor as their concentrations were markedly lower in juice made in the presence of oxygen. This means that these reactions use flavonoids and occur in the presence of oxygen and may be catalyzed by enzymes including PPO. As indicated by molecular weight determinations of the products, the brown color could not be attributed to polymerized high molecular weight pigments. Usually brown color is attributed to these high weight complex pigments. Yellow pigment Phloretin xyloglucoside oxidation product (PXGOPj) was identified as xyloglucose analogue of previously identified phloridzin colored product (POPj) – another colored pigments formed as a result of PPO activity. The only difference in these two molecules is that of the sugar – xylose and glucose respectively.¹²

(E) Pigments in wine: Pyranoanthocyanins are a class of red wine pigments formed as early as during alcoholic fermentation and also during aging of wine. General pathway involves an anthocyanin and a compound having polarizable double bond, reacting to give rise to a new pyrano ring (D ring in Figure 5) fused to anthocyanin molecule. The general structure of pyranoanthocyanins can be classified into (A) vitisin-like structures – which contain non-phenolic groups on carbon 10 or (B) hydroxyphenyl pyranoanthocyanins (Figure 5A and 5B) which are derived as a result of condensation with hydroxycinnamic acids.¹³ The two type of structures are shown in Figure 5 below.

Interestingly pigments resembling the hydroxyphenyl

pyranoanthocyanins were recently isolated from staghorn sumac. These are two 7-O-methyl-pyranoanthocyanin-vinylcatechol aglycones, Sumadin A and Sumadin B, and their derivatives. These pigments however occur naturally in the fruits of this plant.¹⁴

CONCLUSION

Knowledge of new sources of natural colors is crucial since natural colors are becoming more important than synthetic. Isolating different pigments with distinct properties will help application scientists meet the needs of processors. Most polyphenols don't absorb in the visible region of the wavelength and hence do not express colors. Anthocyanins are well known for their coloring properties and different fruit and vegetable based sources are used abundantly to color foods. Turmeric extract is another well used example giving a yellow color to applications while providing good heat stability. There are other polyphenols which are not as well-known like auronones – more research is yet needed to understand them in food applications. HAQ based pigments are also not well exploited except carminic acid which is commercially used in many food applications. Carminic acid is one of the few water soluble colors with relatively high stability. Colorless polyphenols can participate in reactions leading up to colored products. Usually such reactions are thought to be bad for keeping quality of fruit and vegetables as they lead to formation of polymeric compounds which impact the color and nutrition of food products. However, enzyme reactions discussed in this review lead to the production of attractive yellow, orange and brown and black colors. PPO is the enzyme mostly responsible for these reactions. Tea pigments are a well known example of where the colored products also contribute to flavor. Cocoa is another well researched example; however the pigments in both these products are still being discovered. In avocado seed, pigments are formed as a result of reaction between PPO and polyphenols, and the research is still in the nascent phase. In wine, pyranoanthocyanins are condensation products formed due to anthocyanins and other fermentation products or hydroxycinnamic acids. Similarly, chalcones in apples undergo

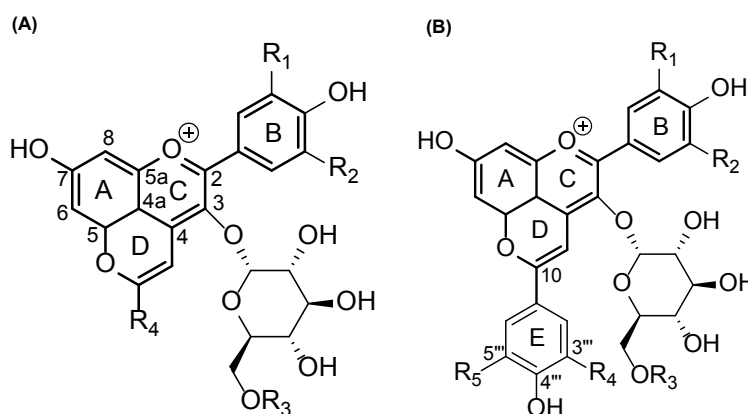


Figure 5: Two types of pyranoanthocyanins (A) Vitisin-like structures and (B) Hydroxyphenyl based.

enzymatic reactions to produce yellow colored pigments. In these substrates, multiple pigments are produced, all of which have not yet been identified.

To date, reaction products have been studied as a part of the matrix in which they are present. Minimal effort has been taken to isolate the pigments and apply them to different food systems. Natural colors can replace synthetic colors in most applications, but there are still some applications for which a suitable fit is absent. It is important to isolate these pigments and study their applications and stability in food matrices. Given the increasing demand of natural colorants, this is certainly possible in the coming years. In order for these colors to be commercially available, regulatory approvals will be required. Polyphenols are known for their health promoting properties, thus contribution to color can be seen as an additional benefit. This property also distinguishes them from synthetic colors which may not have any health promoting properties.

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