CC 13, Unit 2 : FOOD PIGMENTS

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Introduction:

Food pigments are naturally occurring compounds present in plants and animal tissues. It plays a very important role in enhancing the aesthetic appeal of food. Until the discovery of the synthetic dyes, mankind has been relying on pigments extracted from plants, animals, and minerals as food colorants. The plant kingdom, with its multitude of colors, generates vast interest among many researchers and is most widely studied as a major source of food pigments. Flavonoids, carotenoids, and chlorophyll are the major contributors to the natural colors of most plants, with betalains and curcumin playing a minor yet significant role. To understand more about food pigments, their sources, structure and stability the topic is divided into 8 sessions

1) Sources of natural pigments
2) Classification of pigments
3) Flavonoids
4) Carotenoids
5) Chlorophyll
6) Betalains
7) Caramels
8) Heme pigments

1) Sources of natural pigments

Pigments are found widespread in nature from the simplest prokaryotic organisms like cyanobacteria, and throughout the kingdoms of fungi, plants and animals. Sources of natural pigments are flowering plants *Magnoliophyta* of the plant kingdom; scale insects, cochineal and lac; fungi, *Blakeslea trispora* and *Monascus* spp.; and cyanobacteria *Arthrospira* spp. Plant pigments are used widely and are sources of chlorophyll, flavonoids, carotenoids and betalains. Chlorophyll is found abundantly in the aerial part of plants such as leaves. Anthocyanins are a group of flavonoids that provide the red–purple shade of many fruits, in particular berries, for example, strawberries and black currants. Carotenoids are found in sweet potato, carrot, pumpkin, dark green leafy vegetables, *Dunaliella salina* which is an algae and *Blakeslea trispora* which is a fungus. Other important classes of pigments are the anthraquinones found in root of *Morinda angustifolia*, aloe latex, senna, rhubarb, fungi, lichens and cochineal and; betalains which are found abundantly in beetroot. Heme pigments are responsible for the color of meat in animals. Myoglobin is found in the muscle tissue whereas hemoglobin is the pigment responsible for the color of blood. Fresh meat and blood are the major sources of these pigments. When sugars are treated under anhydrous conditions with heat, or at high concentration with dilute acid, caramelization occurs, with the formation of amber to dark brown colored caramel pigments.

2) Classification of pigments: Based on their solubility they are classified as water soluble and water insoluble pigments. Chlorophyll and carotenoids are water insoluble in nature whereas as flavonoids and betalains are water soluble pigments. Based on the source they are classified as food pigments of plant origin, animal origin and synthetic pigments. Pigments of plant origin include chlorophyll-a, chlorophyll-b, carotene, lycopene, anthocyanins, flavonols, tannins, betanins and betaxanthins. Pigments of animal origin include heme compounds such as myoglobin, oxymyoglobin, metmyoglobin, nitrosomyoglobin, nitrosometmyoglobin, globin myohemochromogen which range between purplish red to brownish red in color; choleglobin, nitrihemin and verdohaem which are green in color and bile pigments which are yellow in color. The color of these pigments greatly depends on the state of iron i.e Fe^{2+} or Fe^{3+} state. Synthetic colorants include brilliant blue FCF, indigo, carmoisine, sunset yellow FCF and fast green FCF. Based on their chemical structure they are classified as tetrapyrroles, tetraterpenoids, *O*-heterocyclic compounds or quinones and *N*-heterocyclic compounds. Heme compounds and chlorophylls are grouped under tetrapyrroles, carotenoids under tetraterpenoids, flavonoids or phenolics under quinones and betalains under *N*-heterocyclic compounds.

3) Flavonoids:

Anthocyanin, chalcone, and flavones belong to a group of compounds collectively known as flavonoids. Anthocyanins are pigments found in a wide variety of edible plant materials, such as the skin of red apples, plums, and grapes in addition to strawberries, cranberries, raspberries, elderberries, blueberries and red cabbage. The major source of anthocyanins is the grape skin. Extraction of anthocyanins generally involves the use of an alcoholic solvent. One of the conventional methods of extracting anthocyanin from macerated plant materials involves the use of low boiling point alcohols such as methanol, ethanol, and *n*-butanol which have been acidified with mineral acids like HCl. Anthocyanins impart blue, purple, violet, magenta, red and orange colors in food. The word anthocyanin is derived from two greek words anthos which means flower and kyanos which means blue. The basic structure of flavonoid group has the characteristic $C_6C_3C_6$ carbon skeleton. The color of the sub groups depend on the presence and number of substituents attached to the molecule. Different sub groups of flavonoids are displayed on the screen (Figure 1). Application of anthocyanins in food is restricted due to their ability to participate in a number of reactions, resulting in its decolorization. These include reactions with ascorbic acids, oxygen, hydrogen peroxide, and sulfur dioxide to form colorless compounds; formation of complexes with metal ions and proteins; and hydrolysis of the sugar moieties to form unstable anthocyanidins. Anthocyanins are also sensitive to pH, being more stable at lower pH. In addition, the anthocyanin colors vary with change in the pH. At pH1 and below, the anthocyanin pigment gives an intense red but becomes colorless or purple when the pH is increased to between 4 and 6. Meanwhile, the pigment turns a deep blue when the pH is between 7 and 8. Further increase in pH sees the anthocyanin pigment turning from blue to green and then to yellow. Such variation in color has been attributed to structural transformation in response to changes in pH. The stability of anthocyanin in the lower pH range means that anthocyanins are best suited for use in food of low pH. The degree of unsaturation in anthocyanidin structure makes it susceptible to oxidation changing the color from purple to brown. Stability of anthocyanins is higher at lower water activity i.e., 0.63-0.79. They are also susceptible to photodegradation. Generally, increased hydroxylation decreases stability while increased methylation increases the stability of anthocyanins. Also, the unsaturated nature of the anthocyanidin structure makes it susceptible to oxidation. At higher concentrations of SO_2 i.e., 0.8-1.5%, bleaching of anthocyanin takes place, while some metal ions such as Ca, Fe and Al protect the pigments from discoloration by forming complexes. Enzymes glycosidases and polyphenol oxidases hydrolyze glycosidic linkages yielding sugars and aglycones characterized by discoloration. Anthocyanins are currently being used to provide a natural red or blue coloring for foodstuffs. Successful application of the anthocyanins includes the coloring of canned fruit, fruit syrups, yogurt, and soft drinks. Commercial anthocyanins have also been used to intensify the color of wine.

4) Carotenoids:

Carotenoids are water insoluble pigments found in all higher plants and some animals. Animals cannot synthesize carotenoids, so their presence is due to dietary intake. For example the pink salmon flesh owes its color to carotenoids. Carotenoid is derived from the word "carota" which means carrot (*Daucus carota*), and xanthophyll is derived from the Greek words, yellow and leaf. Together with anthocyanins, carotenoids are the most complex class of natural food pigments with around 750 different structures identified. Plants such as carrots, vegetable oils, apricots, dark green leafy vegetables, kale, spinach, brocolli, grass, alfalfa, oil plam and nettle are sources of carotene. The predominant carotenes are α and β-carotene, and other carotenes such as phytoene, phytofluene, γ-carotene, and lycopene which are all precursors in the biosynthesis of α and β-carotene. Carotenoids impart red, orange and yellow color to the plants. Carotenoids can be divided into carotenes, which are hydrocarbons containing only carbon and hydrogen, and xanthophylls are oxygenated derivatives which are made up of carbon, hydrogen, and oxygen. They are made up of eight isoprenoid units joined in such a manner that the arrangement of isoprenoid units is reversed at the centre of the molecule so that the two central methyl groups are in a 1,6-positional relationship and the remaining non-terminal methyl groups are in a 1,5-positional relationship. All carotenoids may be formally derived from the acyclic $C_{40}H_{56}$ structure, having a long central chain of conjugated double bonds, by hydrogenation, dehydrogenation, cyclization, oxidation or any combination of these processes as displayed on the screen (Figure 2). Carotenoids like other pigments are susceptible to oxidation mainly due to their conjugated polyene chain. Thermal treatment of carotenoids in the presence of oxygen results in the formation of volatile compounds, larger non-volatile compounds, *cis* isomers and oxidation products. Exposure of carotenoids to acids produces ion-pairs which later dissociate to form carotenoid carbocation. Oxidation of carotenoids by free radicals such as singlet oxygen leads to the formation of beta carotene 5,8-endoperoxide and beta carotene 5,6-epoxide.

Lycopene

The structure of lycopene is similar to carotenoids with molecular formula $C_{40}H_{56}$. Being a precursor in the biosynthesis of β-carotene, lycopene can be expected to be found in plants containing β-carotene usually at a very low concentration. The best-known sources of lycopene are tomatoes, watermelon, guava, and pink grapefruit. In solution, lycopene is orange and not bright red as in the tomato. Lycopene is hardly used as a colorant because it is a rather expensive pigment and is very prone to oxidative degradation.

Lutein and zeaxanthin are oxygenated carotenoids with the molecular formula $C_{40}H_{56}O_2$. The name Lutein is derived from the Latin word for yellow. Commercially, the most interesting source is Aztec marigold (*Tagetes erecta*) in which lutein is primarily found esterified with saturated fatty acids; lauric, myristic, palmitic, and stearic acid. Lutein made from Aztec marigold also contains some zeaxanthin (typically less than 10%). Containing only 10 conjugated double bonds, lutein is more yellowish-green than oil palm carotenes.

Annatto

Annatto is both the name of the colorant and the tree providing the colorant. Other names of the tree (*Bixa orellana*) are lipstick tree and achiote. The colorant is extracted from the seeds, which are covered by a red, resinous coating containing the pigments. The main pigment is bixin which is a *cis*-isomer. Smaller amounts of *trans*-bixin, norbixin which is a demethylated bixin, and *trans*-norbixin are also present. Annatto may be extracted using either of three methods: extraction with hot vegetable oil, extraction with organic solvents, or extraction with alkali. Extraction with vegetable oil or organic solvents gives a colorant containing bixin as the main coloring principle, whereas extraction with alkali leads to saponification of bixin yielding the water-soluble norbixin, which may be precipitated by acid to give a powder. Norbixin is used to color cheese (e.g., cheddar) because it binds to the proteins. It may also be used to color beverages with neutral pH, e.g., flavored milk drinks, but not with low pH because of precipitation. Annatto is slightly more reddish in application than β-carotene.

Paprika

Paprika is a well-known spice made from the fruit pods of *Capsicum annuum*. Paprika contains the orange–red pigments capsanthin and capsorubin and a number of yellow pigments, most notably violaxanthin, zeaxanthin, β -cryptoxanthin, and β -carotene.

5) Chlorophyll

Chlorophyll is the green pigment utilized by all higher plants for photosynthesis. It is derived from the Greek words for green and leaf. The most important member of the tetrapyrroles is chlorophyll, which are green pigments found in all higher plants, algae and photosynthetic bacteria. Chlorophyll is derived from porphin and has a four cyclic tetrapyrrole bridged by single carbons with coordinated magnesium in the center. Following these are pyrrole carbon atoms on the periphery of the porphin which are also bridged by carbon atoms. Substituted porphins are called porphyrins as displayed on the screen (Figure 3). In plants, there are two forms of chlorophyll; chlorophyll *a* and *b* which only differ in the substitution at C-3 position of the tetrapyrrole ring. Chlorophyll *a* has a methyl group whereas chlorophyll *b* has a formyl group. It is water insoluble in nature and can be extracted with the use of organic solvents. Chlorophyll can be made water-soluble by saponification, in which case it is called chlorophyllin. The coordinated magnesium is easily lost during extraction and processing, especially under the action of acid and displaced by hydrogen atoms, yielding a yellow–brown pigment pheophytin. Copper forms a complex with pheophytin. These complexes are often referred to as copper chlorophylls or copper complexes of chlorophyll or copper pheophytins. Cu-chlorophyll behaves very much like chlorophyll except that it is more of brighter green and much more stable since copper is not easily displaced. Chlorophyll can be degraded by chlorophyllase to form chlorophyllides and pheophorbides. However the enzyme is active between 60-80°C. Structurally chlorophyll *b* is more stable than chlorophyll *a* due to the electron withdrawing effect of C-3 formyl group. pH plays a vital role on the stability of chlorophyll, where the stability is higher in alkaline pH than acidic pH. Chlorophylls oxidize when dissolved in alcohol or other solvents and exposed to air. This process is called allomerization. Chlorophyll can act as a sensitizer and generate singlet oxygen while carotenoids quench these free radicals and protect the plant from photodegradation. When the protection is lost, chlorophyll under light and oxygen are irreversibly bleached. The process results in opening of the tetrapyrrole ring and fragmentation into low molecular weight compounds such as methyl ethyl maleimide.

6) Betalains: Betalains and anthocyanins have similar color. They are of two types, betacyanins and betaxanthins. Betacyanins are red in color and betaxanthins are yellow in color. Beetroot and amaranth leaves are good sources of betalains. When compared to anthocyanins, betalains are stable to a wider pH range. They are present in the vacuoles and are water soluble in nature. The general formula of betalains represents condensation of primary or secondary amine with betalamic acid. Betacyanins are optically active and have chiral carbons at C-2 and C-15 position. Hydrolysis of betacyanin leads to either betanidin or isobetanidin or a mixture of the two isomeric aglycones. Betaxanthins are present as indicaxanthin, vulgaxanthin I and II. Betaxanthins differ from betacyanins where the indole nucleus is replaced with an amino acid where it is proline in case of indicaxanthin and glutamic acid in case of vulgaxanthin. Under mild alkaline conditions, betanin degrades to betalamic acid and cyclodopa-5-*O*-glucoside. These two degradation products also form during heating of acidic betanin solutions or during thermal processing of products containing beetroot, but more slowly. Decrease in water activity will decrease the degradation rate of betanin. As in the case of other pigments betalains are also sensitive to oxidation which accelerates in the presence of light.

7) Caramels:

Caramels are water-soluble food colors. It is made by heat treatment of carbohydrates, in general in the presence of acids, alkalis, or salts, in a process called caramelization. It is produced from commercially available nutritive sweeteners consisting of fructose, dextrose (glucose), invert sugar, sucrose, malt syrup, molasses, starch hydrolysates and fractions. It is more fully oxidized than caramel candy, and has an odor of burnt sugar and a bitter taste. Its color ranges from pale yellow to amber to dark brown. Caramel color is one of the oldest and most widely used food colorings, and is found in many commercially produced foods and beverages, including batters, beer, brown bread, buns, chocolate, cookies, cough drops, spirits and liquor. They are widely used in chocolate-flavored confectionery and coatings, custards, decorations, fillings and toppings. Soft drinks especially colas have caramel as coloring and flavoring agent. Caramel color is widely approved for use in food globally but application and use level restrictions vary by country since caramels at higher levels are harmful.

8) Heme: Heme pigments are responsible for the color of meat. Myoglobin and haemoglobin are the primary pigments present in meat. The colors imparted by these pigments and their secondary compounds are purplish red, bright red, brown, reddish brown, brown and green. The color of the pigments depends on the state of iron, state of globin, state of oxidation and mode of formation. Myoglobin is a globular protein consisting of a single polypeptide chain and comprised of 150 amino acids. The molecular mass is 16.8kD. The protein portion of the molecule is known as globin. The chromophore component responsible for light absorption and color is a porphyrin known as heme. The porphyrin ring is formed by four pyrrole rings joined together and linked to a central iron atom. Thus, myoglobin is a complex of globin and heme as displayed on screen (figure 4). Haemoglobin consists of four myoglobins linked together as a tetramer. When molecular oxygen binds with myoglobin, oxymyoglobin is formed which has a bright red color whereas deoxymyoglobin is purplish red in color. When oxidation of myoglobin occurs the iron atom is converted from ferrous to ferric state forming metmyoglobin which is brown in color. In the presence of hydrogen peroxide ferrous or ferric site of heme forms choleglobin, a green colored pigment. Lipid oxidation accelerates pigment oxidation which can be prevented by addition of antioxidants such as ascorbic acid and vitamin E. Irradiation of meat changes the color of the meat since iron in myoglobin is susceptible to change in chemical environment.

Conclusion:

Food pigments have an important role as colorants in food industries in enhancing the aesthetic appeal. Pigments are found in minute micro-organisms as well in the animal and plant kingdom. The structure and stability of pigments determine the retention or loss of color. Various factors influence the stability of pigments which include temperature, pH, oxidation and photodegradation. Apart from imparting color, few of these pigments also serve as antioxidants. Therefore, pigments are an important part of food.