Frequently asked questions:

1) What are pigments? Mention the natural sources from which they can be derived.

Ans: Pigments are natural colors present in plant and animal tissue. Pigments are found widespread in nature from the simplest prokaryotic organisms like cyanobacteria, and throughout the kingdoms of fungi, plants and animals. Plant pigments are used widely and are sources of chlorophyll, flavonoids, carotenoids and betalains. Cochineal and lac are insects that produce pigments. Fungi, *Blakeslea trispora* and *Monascus* spp and cyanobacteria *Arthrospira* spp are few prokaryotic organisms capable of producing pigments. Of the animal source, heme pigments are responsible for the color of meat.

2) How are pigments classified? Give examples for different classifications.

Ans: Pigments are classified based on the following:

a) *Solubility:* Based on solubility pigments are classified as water soluble and water insoluble pigments. Examples for water soluble pigments are betalains and flavonoids whereas water insoluble pigments are chlorophyll and carotenoids.

b) *Source:* Based on the source pigments are classified as that 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.

c) *Chemical structure:* 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) Write a note on application of anthocyanins in food industry.

Ans: 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) What is the influence of pH on the stability of anthocyanins?

Ans: Anthocyanins are sensitive to pH, being more stable at lower pH. The anthocyanin colors vary with change in the pH. At pH 1 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.

5) What are carotenoids? Write a note on their sources and structure.

Ans: Carotenoids are water insoluble pigments found in all higher plants which impart yellow to orange red color. Plants such as carrots, vegetable oils, apricots, dark green leafy vegetables, kale, spinach, brocolli, grass, alfalfa, oil plam and nettle are sources of carotene. Carotenoids can be divided into carotenes, which are hydrocarbons containing only carbon and hydrogen, and xanthophylls which 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.

6) Write a note on stability of carotenoids.

Ans: 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.

7) Write a note on annatto.

Ans: 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. Annatto is slightly more reddish in application than β-carotene. 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. 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.

8) What is the basic structure of chlorophyll? Write a note on different forms of chlorophyll.

Ans: The basic structure of 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. 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. 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.

9) Write a note on pigments derived from beetroot.

Ans: The two types of pigments derived from beetroot are betacyanins and betaxanthins. Betacyanins are red in color and betaxanthins are yellow in color. Betaxanthins are present as indicaxanthin, vulgaxanthin I and II. Hydrolysis of betacyanin leads to either betanidin or isobetanidin or a mixture of the two isomeric aglycones. Beetroot and amaranth leaves are good sources of betalains. 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.

10) Write the importance of caramelization of sugar with respect to food pigments.

Ans: Heating of sugar under anhydrous condition leads to the production of caramel colors ranging between 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.

11) Write a note on pigments derived from animal source.

Ans: Heme pigments are primarily derived from animal source. 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. The chromophore component responsible for light absorption and color is a porphyrin known as heme. 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.

12) Write a note on oxygenated carotenoids.

Ans: 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 which is typically less than 10%. Containing only 10 conjugated double bonds, lutein is more yellowish-green than oil palm carotenes.

13) Write a note on lycopene.

Ans: The best-known sources of lycopene are tomatoes, watermelon, guava, and pink grapefruit. 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. 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.

14) Write a note on stability of chlorophyll.

Ans: The coordinated magnesium in the chlorophyll structure is easily lost during extraction and processing, especially under the action of acid. It is 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. They behave 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 enzyme 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.

15) What is the effect of change in pH and temperature on betalain pigments?

Ans: 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.