International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.3, No.4, pp 2228-2244, Oct-Dec 2011

Biocolorants and its implications in Health and Food Industry - A Review

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Abstract: Color is the main feature of any food item as it enhances the appeal and acceptability of food. During processing, substantial amount of color is lost, and make any food commodity attractive to the consumers, synthetic or natural colours are added. Several types of dyes are available in the market as colouring agents to food commodities but biocolorants are now gaining popularity and considerable significance due to consumer awareness because synthetic dyes cause severe health problems. Biocolorants are prepared from renewable sources and majority are of plant origin. The main food biocolorants are carotenoids, flavanoids, anthocyanidins, chlorophyll, betalain and crocin, which are extracted from several horticultural plants. In addition to food coloring, biocolorants also act as antimicrobials, antioxygens and thereby prevent several diseases and disorders in human beings. Although, biocolorants have several potential benefits, yet tedious extraction procedures, low colour value, higher cost than synthetic dyes, instability during processing etc., hinder their popularity. Although, it is presumed that with the use of modern techniques of biotechnology, these problems in extraction procedures will be reduced, yet to meet the growing demand, more detailed studies on the production and stability of biocolorants are necessary while ensuring biosafety and proper legislation.
Keywords: Biocolorants, Anthocyanins, Betalains, Carotenoids, Flavonoids, Synthetic dyes, Biotechnology.

INTRODUCTION

Color is the main feature of food, which determines it appeal to the consumers. Biocolorants are those coloring agents, which are obtained from the biological sources**.** Biocolorants are mainly derived from pigments like anthocyanidin, carotenoids etc., However, there are biocolorants, which are not pigments in any state like structural color and light emitting luciferin[1]. Color is added to food for one or more of the following reasons: to replace color in the food, which is lost during processing, to enhance color of the food already present, to minimize batch-to-batch variations, to color otherwise uncolored food, and to supplement food with nutrients[2].

The chemistry of natural colours cannot fail to fascinate and intrigue and has become the most important part of any commodity[3]. In the past few years, the availability and use of natural colorants has greatly increased[4] as a consequence of perceived consumer preference as well as legislative action, which has continued the delisting of approved artificial dyes[5]. The current consumer preference for naturally derived colorants is mainly because these are healthy and have good quality. Moreover, synthetic colorants tend to impart undesirable taste and are harmful to human beings, as these are responsible for for allergenic and intolerance reactions[6]. As a resulting, there has been a worldwide interest in the development of food colorants from natural sources[7 & 8]. The use of food colorants as additives in the food industry is highly useful for both food manufacturers and consumers in determining the acceptability of processed food[9 & 10]. Currently, the European Union has authorized approximately 43 colorants as food additives, whereas approximately 30 colour additives are approved in the United States[11]. In both Europe and the US, most of the listed colour additives are derived from natural sources[12].

HISTORY OF BIO-COLORANTS

From time immemorial, color has been an important criterion for acceptability of products like textiles, cosmetics, food and other items[13]. In Europe, it was practiced during the Bronze Age. The earliest written record of the use of natural dyes was found in China dated 2600 BC[14]. According to Aberoumand[15] that in Indian subcontinent, dyeing was known even in the Indus Valley period (2500 BC) and has been substantiated by findings of colored garments of cloth and traces of madder dye in the ruins of Mohenjodaro and Harappa civilization (3500 BC). In Egypt, mummies have been found wrapped in colored cloth. Chemical tests of red fabrics found in the tomb of King Tutankhamen in Egypt showed the presence of alizarin, a pigment extracted from madder. The cochineal dye was used by the people of Aztec and Maya culture period of Central and North America. By the 4th century AD, dyes such as woad, madder, weld, Brazilwood, indigo and a dark reddish-purple were known[1]. Brazil was named after the woad found there[16]. Henna was used even before 2500 BC, while saffron is mentioned in the Bible[17]. Use of natural biocolarants in food is known from Japan in the shosoin text of the Nara period (8th century), which contains references regarding coloring soybean and adzuki-bean cakes. Thus, it appears that colored processed foods had been taken by the people of some sections during that period. According to Aberoumand[15] and Mortensen[2], the study of color was intensified in the late 19th century with the aim to understand:

- The phenomenon for survival of animals and plants,
- The relationship between color and evolution theories; and
- The role imparting in comparative physiology.

Thus, studies on biocolorant were greatly impulsed by their multiple functions[18 & 19]. The art of coloring spread widely with the advancement of civilization[16 & 20]. Primitive dyeing techniques included sticking plants to fabric or rubbing crushed pigments into cloth. The methods became more sophisticated with time and techniques using natural dyes from crushed fruits, berries and other plants, which were used to boil with the fabric to give light and water fastness (resistance)[21]. Today, dyeing is a complex and specialized science.

THE NEED FOR BIOCOLORANTS

Colors derived from minerals (lead chromate, copper sulphate) may cause serious health problems[22] and environment hazardous effects[23]. Thus, in the last few decades, synthetic additives have been severely criticized, and consumers show reluctance towards these products, consequently they prefer to use the natural colorants [23 & 24]. In the 1960s in the US, the environmental activists made several demonstrating against the use of synthetic colorants and this attitude was spread out widely. Activists campaigned for the natural colorants highlighting their nutritional characteristics as a sales tool. As a result, the number of permitted artificial colors has reduced considerably, and the interest of the consumers in natural colorants has increased significantly [25 & 26]. Because of health and hygiene, nutrition, pharmaceutical activities, fashion and environmental consciousness, indicate relative dependency on natural products besides, of good market value fetched by the natural colored products[1], and as of now, natural colorants have become the major alternatives to synthetic colorants[13]. Possible reasons for use of colorants in food substances are enumerated[27] as under:

- To maintain the original food appearance even after processing and during storage;
- To assure the color uniformity for avoiding seasonal variations in color tone;
- To intensify normal color of food and thus to maintain its quality;
- To protect the flavor and light susceptible vitamins making a light-screen support; and
- To increase acceptability of food as an appetizing item.

MARKET VALUE OF THE BIO-COLORANTS

Natural colors lost their appeal with the synthetic colors arrived on the scene, as they provide less consistency, heat stability and color range than their chemical alternatives. The market for natural carotenes has declined since the introduction of synthetic colour. Moreover, natural colors are more expensive and unstable in nature. The leading markets for natural colours in the EU are the UK, Germany, France, Italy and Spain[28]. Recently, there is also a growing market in emerging economy countries like China, India and South Korea[1].The demand for natural colours is increasing day-by-day because of the following reasons:

- Health-promoting properties of biocolorant food;
- Natural colours has been the consumer priority;
- Low-fat content' is the objective for many new or improved food formulations, replacing fats with thickeners or other food additives;
- Increased consumer preferences for organic food;
- Variety and internationalisation of food colour and flavours.

The market for natural food colours is estimated to increase by approximately 10% annually [28]. Many of the raw materials for colours and flavours require growing conditions which are more favourable in countries outside the EU. This makes EU a large importer of colours and flavours. Total imports of natural colours, flavours and thickeners by the EU amounted to Euro 2,055 million or 475 thousand tonnes in 2008[28]. Developing countries like India and china may play a major role in supplying natural colours either in processed forms or as raw materials to the EU markets, due to their favourable climatic and production conditions coupled with the rise in their middle income family. In essence, the message to consumers that "Natural is Better" is gaining popularity day-by-day. Although, natural colors are on the rise but they are unlikely to be a total replacement for synthetic dyes because the area of land required for production of natural colorants yielding plants increasing[29] due to inadequate strategies and

- Increasing demand for natural food in comparison to synthetic one;

horticultural practices on this crops. It was estimated that to provide sufficient vegetable dyes to dye cotton alone, about 462 million ha would be needed, ie, 31% of the world's current agricultural land[30], which appear unlikely. Thus, natural dyes is likely to occupy a small niche market, unless technology of horticultural practices and pigments extraction is redefined and standardisation on modern scientific lines.

CHOICE OF COLOUR

Commonly single natural coloring agents may not give the desired effect, the background color and of neighbouring colored substances make a large impact in the color look[31]. Product concepts, requiring blue or green, limit the choice from certified colors only. Bluish purple can be achieved with carmine, but it does not create a true blue[32]. Annatto or turmeric tends to represent a cheese color or have an eggy tone compared with the bright color produced with the FD&C-yellow. Now-a-days, fluorescent colors are also getting importance in food industry as consumers favor foods to glow under conditions[31]. Turmeric is highly fluorescent, thus it is commonly used in food. Normally, the physical and chemical properties of food product limit the choice of a colorant. A list of available shades for food grade biocolorant is **given in Table 1.**

Table 1. Different shades available from a wide range of food grade biocolorants

Color	**Wavelength interval (nm)**	**Biocolorants**	**Colorant status**	**Colorant amount**	Shade
Red	~630 – 700	Red Beet Juice (Betanine) Paprika	Powder	0.3% - 0.45	
			Emulsion (oil soluble)	40,000 IU	
			Emulsion (water soluble)	40,000 IU.	
Orange	~590 – 630	Kesar Orange / Mango Annatto (Bixin) Annatto (Norbixin)	Powder	1.5%	
			Emulsion (oil soluble)	1.5%	
			Emulsion (water soluble)	1.5%	
Yellow	~560 - 590	Turmeric	Powder	5%	
Green	~490 – 560	Chlorophyll	Emulsion (oil soluble)	-	
			Emulsion (water soluble)		
Blue-violet	~400 - 490	Anthocyanin	Powder	-	

Source: Chattopadhyay et al. [1]

MAIN CHROMOPHORES AND THEIR POTENTIAL SOURCES

The colorants that occur naturally in food plants have been the source of the traditional colorants of raw as well as the processed food[33]. However, they can also be obtained from microorganisms and animals, but few of them are available in sufficient quantities for commercial use as food colorant[15]. Although, biocolorants are structurally much diversified and from a variety of sources, the three most important are: tetrapyrrols, tetraterpenoids, and flavonoids[2]. The main pigments and their potential natural sources are discussed below.

Carotenoids, these are one of the most important groups of natural pigments[34]. These are lipid-soluble, yellow–orange–red pigments found in all higher plants and some animals[35]. Animals cannot synthesize carotenoids, so their presence is due to dietary intake. The most important carotenoids are carotenes which including (alpha carotene, beta-carotene, beta-cryptoxanthin, lutein, and lycopene) and xanthophyll including violaxanthin, neoxanthin, zeaxanthin and canthxanthin[1 & 34].

ß-Carotene is orange-yellow in color, oil soluble but can be made into a water dispersible emulsion. Carrot (*Daucus carota*) is a good source of ß-carotene[36]. But most ß-carotene for commercial use is now derived from algae. Oil palm[2 & 34], orange, apricot, mango, and peach and pepper[38] contributed significantly in increasing ß-cryptoxanthin and ß-carotene concentrations of foods. Besides being used as colorants, carotenes are also used for nutritional purposes as provitamin A agents as in margarine where they also provide color or as dietary supplements[2].

Lycopene, being a precursor in the biosynthesis of carotene, it is found in plants containing carotene, usually at a very low (sometimes undetectable) concentration. Lycopene is an expensive pigment and is very prone to oxidative degradation which is much more so than carotene[2], but highly stable under a wide range of temperature and pH, hence used as common food colorant. It is available in liquid form or as cold-water dispersible powder. Though lycopene is found in abundance in tomatoes in large proportion, but was also identified in about 70 plant species including red pepper, Kapia pepper, onion, , *Rosa rubiginosa* (rose hip), *Taxus baccata* (yew), *Calendula officinalis* (marigold) and *Citrullus lanatus* (watermelon)[4] also contain lycopene at low concentration. Further, red cabbage juice[1] and carrot has long been a component of tomato blends[40], indicating that both contains appreciable quantities of lycopene.

Xanthophylls are oxygenated carotenes, derived from the Greek words for yellow and leaf[2]. Orange to yellow colour of yew tree, *Taxus baccata* is due to rhodoxanthin of xanthophylls[4] and Rubixanthin produces yellow colour in dog rose, *Rosa canina* [21].

Lutein is also a very common carotenoid. The name is derived from the Latin word for yellow (compare xanthophyll, vide supra). Marigold, (*Tagetes erecta*) flowers are by far the most abundant natural source for commercial lutein[41&42]. Lutein is primarily found esterified with saturated fatty acids like lauric, myristic, palmitic, and stearic acid[43]. Lutein is more yellowish-green than oil palm carotenes. Lutein is not allowed as a food colorant in the USA except for chicken feed[2]. Lutein, is also found in Zucchini (*Cucurbita pepo L. var. giromontia*), green vegetables like cabbage, parsley, spinach, etc. and some fruits[33].

Annatto, a yellow to orange color has been used for over two centuries mainly for colouring dairy products especially cheese and is derived from the outer layer of seeds of the tropical tree *Bixa orellana*[44]. The chief coloring principle is the carotenoid, bixin and norbixin[45].The pH and solubility affect the color hue; the greater the solubility in oil, the brighter is the color. Water soluble, oil soluble, and oil/water dispersible forms of annatto are available. 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 and it is slightly more reddish in application than β- carotene[2]. Since it precipitates at low pH, it is also available as emulsion, an acid proof state[46]. The food colorants obtained from paprika (*Capsicum annuum*) including red colour due to red carotenoids which are dominated by canthaxanthin and capsorubin and yellow colour imparted by xanthophylls includes ß-cryptoxanthin, zeaxanthin, antheraxanthin and ß-carotene[47]. Their combination also produces a bright orange to red-orange colour in food products. The oleoresin is oil soluble, when emulsified becomes water dispersible.

The saffron coloring matter is crocin which is extracted from the dried stigmas and styles of the saffron plant**,** *Crocus sativa*[49]. It is water soluble and considered as the most expensive colorant as well as spice[50&51]. The flower is light purple with thread-like red stigma, is the valued material. The odour of saffron is sometimes described as sea air to express its color shade and fragrance. The color appears as a powerful yellow in applications such as saffron rice[52]. Crocin also found in Cape jasmine or gardenia, *Gardenia angusta* fruits[2].

Flavonoids, the flavonoids are a diverse group of polyphenolic compounds[53] contribute to the yellow color of horticultural products. They are widely distributed in the plant kingdom and over 4000 structurally unique flavonoids have been identified in plant sources[53]**.** These are divided into six different major classes (flavonols, flavanones, flavones, isoflavones, flavonols and anthocyanidins) based on differences in molecular backbone structure[54]. Flavonols may to fade in strong light but flavones remain more permanent but paler in colour. The leading representatives of flavone pigments are *apigenin, kaempferol, quercetin, myricetin, luteolin, tricin, izoramnetin*[38]. The minor flavonoids are chalones - coreopsidoside and mareoside found in daisy family, aurones- sulphuroside in fustic, daisy family, genistein in pea family, osajin and pomiferin in Osage-orange, *Maclura pomifera*[29]. Some of the major flavanoids are:

Quercetin is one of the most important flavonoids. The richest sources of quercetin are: apples, onions, plants of Cruciferae family, *Sambucus nigra*[55], in fact the main source of quercetin is from quercitron, isolated from the inner bark of an oak, *Quercus tinctoria*[56] but it is also present in horse chestnuts, onion skins, tea and sumac, and *Citrullus colocynthis*[57].

Luteolin is one of the principle compounds of yellow dye, which produces the most vibrant and lightfast. The dye and weld or dyer‘s rocket (*Reseda luteola*) was cultivated for extraction of luteolin in northern Europe[58, 59 & 4]. Its major use was the dyeing of gold braid. The perennial plant saw-wort*, Serratula tinctoria* L. was also a yellow dye yielding plant due to the present of ecdysteroid[60, 61 & 62], luteolin and luteolin-7-O glucoside[63] content in the leaves[64 & 65].

Anthocyanidins are the highly coloured flavonoids[29]. Anthocyanins are the glycosides of anthocyanidins and are found more in plants than the parent anthocyanidins[29]. Anthocyanins are a class of compounds belonging to phenolic substances widely distributed in vegetables, giving rise to the blue–purple– red–orange color of flowers and fruits. The name has been derived from two Greek words antho and cyaniding meaning flower and dark blue respectively. Until now, more than 540 anthocyanin pigments have been identified in nature[66], with most of the structural variation coming from glycosidic substitution at the 3 and 5 positions and possible acylation of sugar residues with organic acids. The most common anthocyanidins are cyanidin (redpurple), delphinidin (blue-purple), malvidin (deep purple), peonidin (red), petunidin (purple) and pelargonidin (orange-red)[7], and the distribution of this pigment in the horticultural plants is not even[55]. Some fruits contain a single type of anthocyanin (e.g. cyanidin in apple, cherry, fig, etc), some contain two major types (cyanidin and peonidin as cherry and canberry); or some with several anthocyanins[67 & 45] giving a variety of colors like red, purple, yellow and blue as in grape or raspberry or strawberry. Anthocyanin are used to color a number of non beverage foods, including gelatin desserts, fruit fillings and certain confectionaries[1]. Grape peel extract (enocianina) also imparts a reddish purple color to beverages[68]. Garden Huckleberry (*Solanum scabrum*) contains petunidin[69] and pelargonidin is found in Red radish (*Raphanus sativus* L.) roots[70] and onion (*Allium cepa*) solid wastes[71] and cyanidin 3-glucoside and cyanidin 3-rutinoside is found in mulberry, *Morus* spp[72] and coffee husk[73]. Other potential sources of red food colorants are banana bract and *Oxalis triangularis*[74], black carrot cultivars[75], elderberries (*Sambucus nigra*), red cabbage (*Brassica oleracea*), roselle (*Hibiscus sabdariffa*), blood orange (*Citrus sinensis*), black chokeberry (*Aronia melanocarpa*), sweet potato (*Ipomoea batatas*)[77, 7 & 2], , *Rubia tinctorum*[78& 79]. The plants that impart red to purple colour spectrum are Garden Huckleberry, *Solanum scabrum* Mill.[80&69], Roselle, *Hibiscus sabddariffa* L.[81], Radish, *Raphanus sativus* L.[70] and red and purple radishes[82].

Chlorophyll is the green pigment utilized by all higher plants for photosynthesis. The name derives from the Greek words for green and leaf (compare to xanthophyll). Chlorophyll is a cyclic tetrapyrrole with coordinated magnesium in the center. In plants, there are two forms of chlorophyll (*a* and *b*) which only differ in the substitution of the tetrapyrrole ring. The oleoresin thus obtained typically contains 10–20 % chlorophyll and some carotenoids (mainly lutein and ß-carotene), which are co-extracted by the organic solvents, yielding a yellow–brown pigment (pheophytin). It is used in jam, jelly, candy, ice cream and in several other products, but chlorophyll finds limited use as a colorant because of the lability of the coordinated magnesium and the associated color change, chlorophyll (in)[2]. Chlorophyll is extracted from edible plants, nettle, grass, or alfalfa, silkworm droppings and mulberry leaves[2].

Anthracenes are of two major groups, the anthracenes contain several well-known dyes. Anthraquinones are the largest group of quinones, best known for their use as mordant dyes. However, their importance, as with most other natural dyes, diminished with the development of the synthetic dye industry. They occur in many different plants and are generally present as the glycosides in young plants[56]. The biosynthetic pathway for anthraquinones is ambiguous. They may be derived from shikimic acid, mevalonic acid or polyketides[83]. Different anthraquinones are alizarin, mungistin, purpurin from Madder family; emodin from Persian berries, kermes and lac and Napthoquinones, e.g. juglone (walnut) and alkanin; hypericin (St. John's wort). Anthraquinone dyes require mordants (metal ions complexed to the fabric to be dyed), which makes the dyeing process more complicated[56]. Other plant yielding anthraquinone red dyes are madder including Indian madder (*R. Cordifolia* Linn) and Naga madder (*R. sikkimensis*)[84]. Galium species, particularly *Galium tinctorium, Galium mullugo* (great ladies bedstraw or Wild madder), *Galium verum* (yellow ladies bedstraw) and *Galium aperine* (goosegrass or cleavers) are inferior dyes to the alizarin obtained from madder. Several other species, which although, are not considered viable contributors to the natural dye market are *Anchusa tinctoria*, *Lithispermum* spp. *Carthamus tinctoria*[56]. Indian mulberry (*Morinda citrifolia*) commonly known as Noni, also a potential yielding anthraquinones[85] and flavonoids[86 & 87].

Betacyanins (betalains) are obtained from the red beet (*Beta vulgaris*) extract that are mainly used as a food colouring agents. These red dyes and the related group, betaxanthins (yellows) were initially thought to be flavonoids but now it is estimated they differ from flavonoids as they contain nitrogen and do not change colour reversibly as the anthocyanins do to pH[29]. Betanin is the major component (95%) of the pigments in the extract and have a good flavor. The beet root extract contains red, yellow and also a bluish-red color pigments depending on their content produced by a compound known as betanin which is stable at higher pH range than red cabbage extract[88]. It has wide application in different food commodity from beverages to candy and from dairy to cattle products[89]. Apparently, they are only present in a few plants of the Chenopodiacae besides red beet (*Beta vulgaris*). Bixin can also be obtained from seeds of Sinduri (*Bixa orella* Linn.), which imparts orange-yellow colour to the products[84]. Some plants of portulaca and goosefoot families also yield significant amount of betacyanins[29]. There is no limit in its upper usage level in the food products. Turmeric is bright yellow colorant can also be obtained from the ground powder of the rhizomes of turmeric (*Curcuma longa* Linn.) plant. Turmeric contains 3–5% volatile oils and 2.5–6% yellow pigments, the curcuminoids, of which curcumin predominates[45]. Solubility of turmeric compound depends on the processing medium. Turmeric oleoresin is water soluble; but oil extract can be added to fat based foods and at high pH, the extract turns orange. There is no usage restriction as long as the level conforms to Good Manufacturing Practices[1]. Indigo blue is obtained the best from dried leaves indogo, *Indigofera* spp[90], which contains glucoside indican or isatan B or Indigotin[21]. It is soluble in water and hydrolysed to indoxyl in the dyeing process. Oxidation, usually by exposure to air, turns the indoxyl to indigotin (or indigo blue), which is insoluble in water, ether and alcohol. Indigo blue is also known to be present in a small number of plants like woad (*Isatis tinctoria)*, a Japanese knotweed (*Polygonum tinctorium)*, common knotweed (*P. aviculare*), *Nerium tinctorium*, and *Lonchocarpus cyanescens*[29]. The structure and formulae of food grade biocolorants have been given in Table 2.

Implications of biocolorants in food and pharmaceutical industry

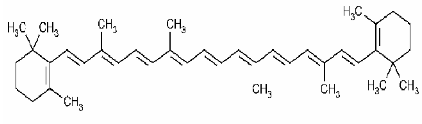
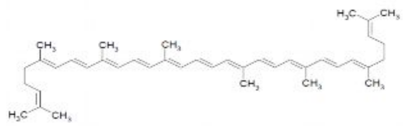
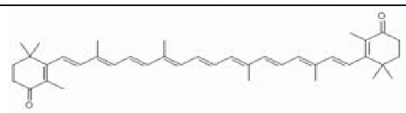
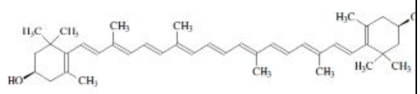
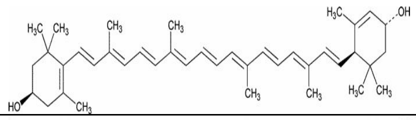
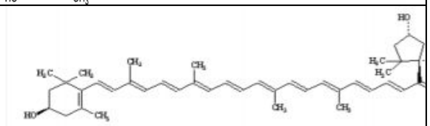
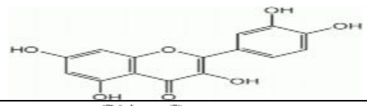
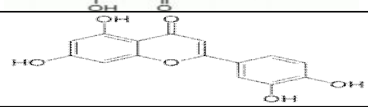
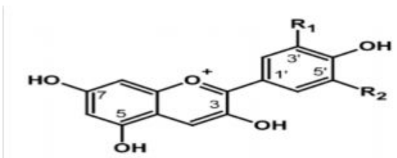
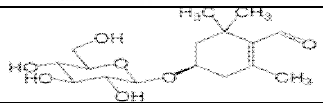
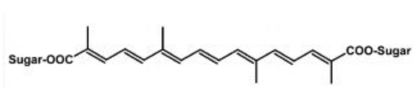
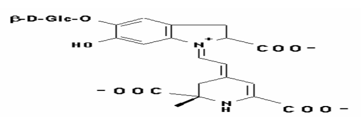
1. Food preservatives

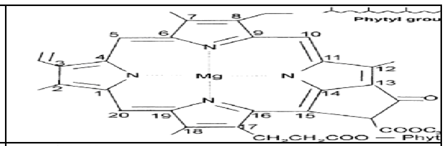
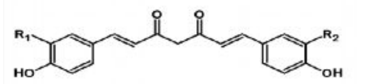
Most of the natural biocolorants possess antagonistic activity to certain bacteria, viruses and fungi for protecting the food from microbial spoilage[77]. Some are also active against protozoa (*Leishmania brasiliensis*), and insects (*Calliphora erythrrocephala*)[1]. Carotenoids are also to known to act as sun screen for maintaining the quality of food by protecting them from intense light. Norton[91] reported that corn carotenoids inhibit the synthesis of aflatoxin by *Aspergillus flavus* (90%) and by most of the *A. parasiticus* (30%) strains.

2. Quality control markers

Generally for maintenance of good manufacturing practices, level of anthocyanin is used as an indicator to evaluate the quality of colored food[92]. Anthocyanin profiles have been used to determine the quality of fruit jams. From anthocyanin profile, it can be easily detected that labeled black cherry jam which is prepared from common red cherries is real or not[1]. Besides, adulteration of blackberry jam with strawberries can also be detected efficiently by the analysis of pelargonidin and cyanidin-3-glucoside content[18].

Table 2. Structural and molecular formula of food grade biocolorants

Food grade biocolorants	Example		Molecular formula	Structural formula
Carotenoid	Carotene	Beta Carotene	$C_{40}H_{56}$	
		Lycopene	$C_{40}H_{56}$	
	Xanthophyll	Canthxanthin	$C_{40}H_{52}O_2$	
		Zeaxanthin	$C_{40}H_{56}O_2$	
		Lutein	$C_{40}H_{56}O_2$	
	Capsanthin		$C_{40}H_{56}O_3$	
Flavonoids	Quercetine		$C_{15}H_{10}O_7$	
	Luteolin		$C_{15}H_{10}O_6$	
Anthocyanidins	Pelargonidin (R1, R2=H)		$C_{15}H_{11}O_5^+$	
	Cyanidin(R1= OH, R2=H)		$C_{15}H_{11}O_6^+$	
	Delphinidin(R1, R2=OH)		$C_{15}H_{11}O_7$	
Irodoid	Picrocrocin		$C_6H_{26}O_7$	
	Crocin		$C_{44}H_{64}O_{24}$	
Betalain	Betaine		--	

Chlorophyll a	$C_{55}H_{72}O_5N_4Mg$	
Curcumin (R1, R2 = OCH_3)	$C_{21}H_{20}O_6$	

Sources: Anonymous[141]; Chattopachyay et al.[1]; Shafagha & Salimi[142]; Giaccio[143]

3. Nutritional supplements

Biocolorants possess chemical compounds produced by plant cells, which are known as the *vegetal active principles.* These are sources for obtaining drug substances (biologically active) and many other natural compounds used in various industries such as food, pharmaceuticals, cosmetics, with important commercial value[55]**.** Carotenoids are also used as vitamin supplements[93], since β-carotene is the precursor of vitamin A. In under developed countries, the diet is primarily of rice, there is every possibility of inadequate supply of vitamin A, which leads to night blindness and in extreme cases to xerophthalmia. Riboflavin is another example of natural food grade biocolorant which is an essential vitamin source and available in milk and in several leafy vegetables, meat, and fish[89 & 94]. Yellow β-xanthins, in addition to their potential role as food colorant, may be used as a means of introducing essential dietary amino acids into foodstuffs[1].

4. Therapeutic properties

Biocolorants may also play an important role in human health as they contain some biologically active compounds, which possess a number of pharmacological properties like strong antioxidant, antimutagenic, anti-inflammatory and antiartheritic effect[95, 96 & 97]. Carotenoids also act as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen and also as a good source of anti-tumor agent[34]. Lycopene, is particularly effective at quenching the destructive potential of singlet oxygen[98]. Lutein, zeaxanthin and xanthophylls are believed to function as protective antioxidants in the macular region of the human retina[9, 99 & 100]. These compounds also act against aging, mascular degeneration, and senile cataracts[101]. Betacyanin also contain antioxidant and radical scavenging properties. Since betanin exerts a good bioavailability, red beet products may provide protection against certain stress related disorders[1]. It has been established that flavonoids present in different plant products show good antioxidant activity, sometimes better than the commercially available antioxidants[102]. Allomelanins (free of proteins) from plants are found to suppress growth of tumorigenic cells of mammals. Grape seed extract is the primary commercial source of a group of powerful antioxidants known as oligomeric proanthocyanidins (OPCs), also generically called pycnogenol, a class of flavonoids[1]. Canthaxanthin also shows antioxidant property[44]. Astaxanthin is another naturally occurring xanthophyll with potent antioxidant properties[98]. Other health benefits of biocolorants include enhancement of immune system function[103], protection from sunburn[104], and inhibition of the development of certain types of cancers[105]. Lycopene prevents oxidation of low-density lipoprotein (LDL) cholesterol and reduces the risk of developing atherosclerosis and coronary heart disease[106]. Epidemiological studies indicated that there is a correlation between the consumption of chlorophylls and decreased risk of colon cancer[107].

USE OF BIOTECHNOLOGY FOR THE DEVELOPMENT OF BIOCOLORANTS

Although there is a large number of biocolorants existing in nature, yet quite a meagre number of them are available from natural extracts[1]. Therefore, biotechnology could be a solution for providing more number of coloring compounds which are difficult to synthesize by chemical means or traditional methods of extraction. Over the past few years, there has been a good afford being made in the studies of biocolorants production through biotechnology. For biotechnological production of such colorants, plants and microorganisms are more suitable due to understanding of proper cultural techniques and processing[15].

1. Microorganism's cell culture for biocolorants production

A bacteria, *Bradyrhizobium* sp. strain are known to produce canthaxanthin (4,4'-diketo-β-carotene)[108] and the carotenoid gene cluster was fully sequenced[109]. This keto-carotenoid was also found in another microorganism, belonging to the genus *Halobacterium*[110]. Culture of *Flavobacterium* sp.[111] in a nutrient medium containing glucose or sucrose, sulphur-containing amino acids such as methionine, cystine or cysteine, pyridoxine and bivalent metal ions was able to produce zeaxanthin @ 190 mg/L, with a cell concentration of 16 mg/g dried cellular mass. The alga *Haematococcus lacustris* commercially used for production of astaxanthin using bioreactor[112]. Besides, echineone and canthaxanthin are also identified in *Haematococcus* cultures. *In vivo* [113] and *in vitro*[114] studies have shown that high astaxanthin production required high level of oxygen (aerobic conditions) and high C/N ratio but cell growth requires low C/N ratio. Also, it is suggested that the addition of ethanol during the second stage enhanced the production of astaxanthin 2.2 times whereas compactin resistant mutants of *H. pluvialis* (compactin inhibits HMGR that strongly blocks cholesterol formation) showed 2 times enhanced yield[115]. Several species of marine micro algae such as *Dunaliella bardawil* and *Dunaliella salina* produce β-carotene as their main carotenoid[116].

The fungus *Blakeslea trispora* is known to produce β-carotene[1]. The cell growth and β-carotene production are enhanced in medium containing surfactants such as Span or Triton, except Triton X-100[117]. Another interesting fungus which contains colors is *Phycomyces blakesleeanus* for β-carotene production[118]. In *Blakeslea trispora*, sexual stimulation of carotene biosynthesis remains essential to increase yield up to 35 mg/g[119]. Several strains of *Monascus* are also being exploited for commercial production of red and/or yellow pigments[120].The red yeast, *Xanthophyllomyces dendrorhous* (formally *Phaffia rhodozyma*) synthesizes astaxanthin and zeaxanthin as its main carotenoids[121 & 13]. Commercial production of carotenoids using microorganism has been achieved in case of astaxanthin, by red yeast fermentation[1]. Yeasts of the genus *Rhodotorula* including species *R. glutinis, R. gracilis, R. rubra,* and *R. graminis* are synthesize carotenoids and studying regarding biocolorants production in undergoing[1, 122, 123 & 124].

The Czech Republic‘s Ascolor Biotech is awarded patents of compounds from new fungal strains that produce a red colorant which can be applied in the food and cosmetic industries[1]. The strain *Penicillium oxalicum* var. Armeniaca CCM 8242, obtained from soil, produces a chromophore of the anthraquinone type. After evaluation, the red colorant Arpink Red was recommended as 100 mg/kg in meat products are in non-alcoholic drinks, 200 mg/kg in alcoholic drinks, 150 mg/kg in milk products including ice creams and 300 mg/kg in confectionery items[125].

2. Plant cell culture for biocolorants production

Cells culture is the most common practice method for production of plant pigments, as culture ensures uniform quality and continuous production of pigments. Various studies on color production in plants are presented in Table 3.

Table. 3. Source, method and food grade colors in plants through biotechnological tools

Source	Biotechnology method	Food grade biocolorants	References
Vitis vinifera *Aralia cordata* *Aralia cordata* *Fragaria anansa*	Cells Culture	anthocyanin	Kakegawa,et.al.[144]; Zhang, et.al.[145]; Suvarnalatha, et.al.[146]
Perilla frutescens	Cells Culture	anthocyanin	Zhong et.al.[147]
Daucus carota	Cells Culture	anthocyanin	Vogelien, et.al.[148]
Crocus sativus	Somatic Embryogenesis	crocin	Chattopadhyay et al.[1]
Bixa orellana	Relationship between degree of genetic diversity, using isozymes.	carotenoid, bixin and norbixin	Siva & Krishnamurthy[126]
Beta vulgaris	Cell Cultures, Root Cultures	betalain, betacyanin, betaxanthins (portulaxanthin-II and vulgaxanthin-I), muscaauri-VII, dopaxanthin, and indicaxanthin	Leathers , et.al.[149]; Kino-Oka, et.al.[150]

GENETIC VARIATION AND COLORANTS CONTENT

The content, nature and quality of colorants vary with species and plant parts[35]. Siva & Krishnamurthy[126] studied an important colorants-yielding plant, *B. orellena*, for understanding the relationship between degree of genetic diversity (using isozymes) of various populations and their pigment content, and reported that Bixin ($C_{25}H_{30}O_4$) and norbixin ($C_{24}H_{28}O_4$) are carotenoid pigments that form the main components of *B. orellena*. Siva and Krishnamurthy[127] also estimated the total amount of these two pigments in seed materials collected from ten different geographical localities was using HPL, and reported that the lowest band frequency shows the least total pigment and bixin content. Similarly, greater band frequency (i.e. genetic diversity) showed greatest dye content. In other words, it is likely that individuals with greater genetic diversity may have high dye content. However, further critical study is needed to establish the relationship between the geographical localities with the dye content.

LEGISLATION AND ASSESSMENT OF BIOCOLORANTS

The use of food additives (including colorants) is governed by strict regulation, in many countries. The legislation specifies which colorant may be used; the source(s) of the colorant, the purity of the colorant, to which foods the colorant may be added, and at what level the colorant may be added to a specific food[2]. Food colorants are tested for biosafety before its promotion and are controlled by various regulatory bodies around the world and regulation varies with countries[128]. In US, FD&C (Food, Drug and Cosmetic) numbers are given to synthetic food colorants approved by FDA that do not exist in nature, while in EU, E numbers are used for all additives of food applications. Thus, the approved list of food colors varies along the countries, it means each country has its own approved list, including limit of maximum daily intake. Out of them, some other regulatory agencies are there like PMDA (Pharmaceuticals and Medicinal Devices Agency) in Japan, SFDA (State Food and Drug Administration) in China, CDSCO (The organization and function of the Medicines Agency) in India and KFDA (Korea Food and Drug Administration) in South Korea etc. Yet, most of the food grade biocolorants approved by FDA or EU are also approved by other agencies. For example, in India, Rule 26 of The Prevention of Food Adulteration Act (PFA) permits 11 colors for food use: Lactoflavin, Caramel, Annato, Saffron, Curcumin etc., also approved by EU and FDA[1].

Under FDA regulations[22], a colorant added to a food product cannot be considered natural, no matter what the source is. Unless the colorant is natural to the food product itself, for example; strawberry juice or red beet color is used to make the ice cream a pink hue for strawberry ice cream, it would not be considered as naturally colored, because the colorant from strawberry or beet are not a natural component of ice cream. FDA considered only few colorants as food additives[22]. In 1958, additives were redefined and classified with three categories, appeared as:

- Substances approved by the FDA or the USDA (United States Department of Agriculture) during 1938 to 1958;
- GRAS (Generally Recognized as Safe) substances do not require FDA evaluation, and
- All other substances used in food are evaluated to fit with the recommendations of FDA before commercialization[3, 11 & 129]. The natural colors under section 205.606 of FDA list are only allowed in organic foods.

FDA uses the term indirect additives to group those additives which are used in the coloration for animal feeds and in course the animals are used as human food[24]. It may happen that, some of the natural colorants of non biological origin are decertified (Red Dye #2, amaranth). All these evidences suggest that, the food grade substances have to follow strict regulations. Further among the food additives, color additives are in no condition be considered as GRAS substances. But the accepted natural pigments, or colors (from red beet, carrot, fruits, pepper etc.) are grouped under 'exempt of certification'category[27]. Further, legislation is often based on local, traditional usage of coloring matter. Thus, lac, monascus, gardenia, and spirulina are important colorants in some parts of Asia, but none of them are allowed in the EU or in the USA, where there is no traditional use of the raw materials[2].

TOLERANCE AND ALLERGIES TO BIOCOLORANTS

The plant pigments are the most important precursor of several nutrients (e.g. β- carotene is the precursor of vitamin A, as well as many other carotenoids) and they have always been present in the diet of man. As interest in food allergy and intolerance has increased in recent years, efforts to identify foods and food constituents that may cause reactions have also increased. Thus a variety of foods and food constituents have been identified which cause reactions[130]. The consensus adopted by the Codex

Alimentarius Commission of the World Health Organization in 1998[131] experts for investigating food colourants, they consider eight foods or food groups to be the major causes of food allergy. Natural color additives are justifiably not included among the foods and food groups identified by the Codex. Lucas, Hallagan & Taylor[132], critically evaluated of the available information and demonstrated that reactions to natural color additives are rare. Studies of turmeric and carotenoid pigments administered in mixtures with other food colourings failed to definitely identify reactions to either color additive and also found no reports of sensitivities to grape skin extract or grape color extract[132] and hence concluded that the ingestion of natural color additives presents a very low risk of provoking adverse reactions.

BENEFITS OF BIOCOLOURANTS

The use of bio-colorants may show benefits over synthetic colours**.** Natural dyes are less toxic, less polluting, less health hazardous, non-carcinogenic and non-poisonous[21] and prevent chronic diseases such as prostrate cancer[133]. In addition to this, they are harmonizing colours, gentle, soft and subtle, and create a restful effect. Most of them are water-soluble (anthocyanins), which facilitates their incorporation into aqueous food systems. These qualities make natural food colorants attractive[72]. Above all, they are environment friendly and can be recycled after use[21]. Thus, they attribute to food-both for aesthetic value and for quality judgement and also they tend to yield potential positive health effects, as they possess potent antioxidant and improve visual acuity properties[134 & 135]. They have also been observed to possess antineoplastic, radiation-protective, vasotonic, vasoprotective, anti-inflammtory, chemo- and hepato-protective activities[136].

LIMITATIONS OF BIOCOLORANTS

Biocolorants inspite of having several potential benefits, natural dyes have some limitations as well. Tedious extraction procedures of colouring component from the raw material, low colour value and longer time make the cost of dyeing with natural dyes considerably higher than synthetic dyes. Some of the natural dyes are fugitive and need a mordant for enhancement of their fastness properties while some of the metallic mordents are hazardous[21]. Besides, there are problems like difficulty in the collection of plants, lack of standardization, lack of availability of precise technical knowledge of extracting and dyeing technique and species availability. The use of these colorants in food products may also face some problems due to their instability during processing due to their sensitivity to temperature, oxygen, light[23] and pH[128]. They can also be decolourised or degraded during storage[81]. Anthocyanin degradation and brown pigment formation cause color loss in food products[137 & 138]. Curcumin is very prone to photobleaching and beetroot color has low heat stability[2]. However, stability of these dyes can be maintained by adding dextrins additives extracted from tart cherries[139] or maltodextrin extracted from Roselle as a stabiliser[81]. It has been demonstrated that increased glycosidic substitution, and in particular, acylation of sugar residues with cinnamic acids, reduced water activity will enhance stability and anthocyanin pigments in dried forms can exhibit remarkable stability[140].

PROSPECTS AND CONCLUSION

The earth is bestowed with plants containing a wide range of healthful and attractive pigments. But, worldwide, 70% of all plants have not been investigated at all, and the chemical composition of only 0.5% has been exhaustively studied[11]. Despite centuries of interest in natural pigments, our knowledge of their sources from plants, distribution, availability and properties is limited. The increasing market demand for dyes and the dwindling number of dye-yielding plants forced the emergence of several synthetic dyes like aniline and coal-tar, which threatened total replacement of natural dyes. Also, low colour value and longer time make the cost of dyeing with natural dyes considerably higher than synthetic dyes. Therefore, novel plant pigments must be searched for in unprospected land or in the sea for their proper collection, documentation, assessment and characterization. Natural dyes are confronted with the various drawbacks of commercial natural food colorants (instability to light, heat or adverse pH). Thus, more detailed studies and scientific investigations are needed to assess the real potential and availability of natural dye-yielding resources, including using of modern horticultural systems in order to realise their potentials. New sources of plant pigments need to be available in sufficient quantities for industrial extraction, large-scale cultivation, harvesting and storage facilities and new ways of formulating existing pigments, improvement of existing sources through breeding and selection of high yielding strains and application of biotechnological tools including plant cell and tissue cultures, genetic engineering and other modern techniques are required to improve the quality and quantity of dye production and to enhance their stability during processing and storing. Fermentative biocolorants production has a number of advantages; cheaper production, possibly easier extraction, higher yields, no lack of raw materials and no seasonal variations. Some fermented colors are already used today: *D. salina*, *B. trispora*, spirulina and monascus. Its is not unlikely that new, fermented colors such as lycopene from *B. trispora* will become allowed in the near future. A giant leap forward in color production could be achieved by combining genetic manipulation and fermentation. Finally, new bio-colorants would have to be approved by the authorities, which are very costly because of the various toxicological studies needed to confirm the safety and also the economics of a new food additive.

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