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ANTHOCYANIN, THE NATURAL COLORANT AND ITS IMPLICATIONS IN HEALTH AND FOOD INDUSTRY: A SEARCH

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ABSTRACT

Anthocyanins are unique plant pigments since they are critical for most of the red, purple and blue pigmentation of flowers, fruits and vegetables. Meanwhile, they are reactive in nature, anthocyanins degrade easily, or react with other compounds such as reactive metals such as iron, aluminum, and tin in the media, to form colorless or brown colored by products. Anthocyanins are glycosides of anthocyanidins (aglycones) and sugars. Anthocyanidins are almost always glycosylated in the 3-position, though glycosylation in other positions and in more than one position at a time is also encountered. Furthermore, the sugar moiety may be acylated with aliphatic or aromatic acids. Anthocyanidins are less in number but anthocyanins show much diversity offered by glycosylation and acylation. 635 anthocyanins were identified in nature, featuring six common aglycones and various types of glycosylations and acylations. Reports suggest that dietary consumption of anthocyanins is good for health. Based upon many cell-line studies, animal models, and human clinical trials, it has been suggested that anthocyanins possess anti-inflammatory and anti-carcinogenic activity, cardiovascular disease prevention, obesity control, and diabetes alleviation properties, all of which are more or less associated with their potent antioxidant property. Evidence suggests that absorption of anthocyanins occurs in the stomach and small intestine. Epithelial tissue uptake seems to be highly efficient, yet transportation into circulation, tissue distribution, and urine excretion are very limited. The bioactivity of bioavailable anthocyanins should be a focus of future research regarding their putative health-promoting effects.

Keywords: Anthocyanin, pigments, colorant, dietary, economical, therapeutic roles.

1. INTRODUCTION

Anthocyanins are flavonoid group of phytochemicals common in teas, honey, wines, fruits, vegetables, nuts, olive oil, cocoa, and cereals. The flavonoids, perhaps the most important single group of phenolics in foods, comprise a group of over 4000 C_{15} aromatic plant compounds with multiple substitution types. The primary members in this group include the anthocyanins like cyanidin, pelargonidin, petunidin, the flavonols (quercetin, kaempferol), flavones (luteolin, apigenin), flavanones (myricetin, naringin, hesperetin, naringenin), flavan-3-ols (catechin, epicatechin, gallocatechin), and, although sometimes classified separately, the isoflavones (genistein, daidzein). They are commonly referred as bioflavonoids due to their multifaceted roles in human health maintenance and anthocyanins in food are typically ingested as components of complex mixtures of flavonoid components (Skibola and Smith, 2000).

The anthocyanins are anthocyanidins with sugar group(s) mostly 3-glucosides of the anthocyanidins. The anthocyanins are subdivided into the sugar free anthocyanidin aglycones and the anthocyanin glycosides. Anthocyanin pigments are assembled from two different streams of chemical raw materials in the cell: both starting from the C2 unit acetate (or acetic acid) derived from photosynthesis, one stream involves the shikimic acid pathway to produce the amino acid phenylalanine. The other stream (the acetic acid pathway) produces 3 molecules of malonyl-Coenzyme A, a C3 unit. These streams meet and are coupled together by the enzyme chalcone synthase (CHS), which forms an intermediate chalcone via a polyketide folding mechanism that is commonly found in plants. The chalcone is subsequently isomerized by the enzyme chalcone isomerase (CHI) to the prototype pigment naringenin, which is subsequently oxidized by enzymes like flavonoid hydroxylase and coupled to sugar molecules by enzymes like UDP-O-glucosyl transferase to yield the final anthocyanins. More than five enzymes are thus required to synthesize these pigments, each working in concert (Raghvendra *et al.*, 2011). The most common combination of side groups and their names are displayed in Table 1.

Anthocyanidin	R_1	R_2	R_3	R_4	R_5	R_6	R_7	main colour
Apigeninidin	-H	-OH	-H	-H	-OH	-H	-OH	orange
Aurantinidin	-H	-OH	-H	-OH	-OH	-OH	-OH	orange
Capensinidin	$-OCH_3$	-OH	$-OCH_3$	-OH	$-OCH_3$	-H	-OH	bluish-red
Cyanidin	-OH	-OH	-H	-OH	-OH	-H	-OH	magenta
Delphinidin	-OH	-OH	-OH	-OH	-OH	-H	-OH	purple, blue
Europinidin	$-OCH_3$	-OH	-OH	-OH	$-OCH_3$	-H	-OH	bluish red
Hirsutidin	$-OCH_3$	-OH	$-OCH_3$	-OH	-OH	-H	$-OCH_3$	bluish-red
Luteolinidin	-OH	-OH	-H	-H	-OH	-H	-OH	orange
Pelargonidin	-H	-OH	-H	-OH	-OH	-H	-OH	orange, salmon
Malvidin	$-OCH_3$	-OH	$-OCH_3$	-OH	-OH	-H	-OH	purple
Peonidin	$-OCH_3$	-OH	-H	-OH	-OH	-H	-OH	magenta
Petunidin	-OH	-OH	$-OCH_3$	-OH	-OH	-H	-OH	purple
Pulchellidin	-OH	-OH	-OH	-OH	$-OCH_3$	-H	-OH	bluish-red
Rosinidin	$-OCH_3$	-OH	-H	-OH	-OH	-H	$-OCH_3$	red
Triacetidin	-OH	-OH	-OH	-H	-OH	-H	-OH	red

2. DIVERSITY OF MAJOR PIGMENTS

2.1. Pigments in general

Pigments are chemical compounds that absorb light in the wavelength range of the visible region. Produced color is due to a molecule-specific structure (chromophore); this structure captures the energy and the excitation of an electron from an external orbital to a higher orbital is produced; the non absorbed energy is reflected and/or refracted to be captured by the eye, and generated neural impulses are transmitted to the brain where they could be interpreted as a color.

2.2. Classification

2.2.1. By their origin

Pigments can be classified by their origin as natural, synthetic, or inorganic. Natural pigments are produced by living organisms such as plants, animals, fungi, and microorganisms. Synthetic pigments are obtained from laboratories. Natural and synthetic pigments are organic compounds. Inorganic pigments can be found in nature or reproduced by synthesis.

2.2.2. By the chemical structure of the chromophore

Pigments can be classified by taking into account the chromophore chemical structure as:

Chromophores with conjugated systems: carotenoids, anthocyanins, betalains, caramel, synthetic pigments, and lakes.

Metal-coordinated porphyrins: myoglobin, chlorophyll, and their derivatives.

2.2.3. By the structural characteristics of the natural pigments

Tetrapyrrole derivatives: chlorophylls and heme colors.

Isoprenoid derivatives: carotenoids and iridoids. N-heterocyclic compounds different from tetrapyrroles: purines, pterins, flavins, phenazines, phenoxazines, and betalains.

Benzopyran derivatives (oxygenated heterocyclic compounds): anthocyanins and other flavonoid pigments.

Quinones: benzoquinone, naphthoquinone, anthraquinone.

Melanins

2.2.4. As food additives

By considering the pigments as food additives, their classification by the FDA is

Certifiable: These are manmade and subdivided as synthetic pigments and lakes.

Exempt from certification: This group includes pigments derived from natural sources such as vegetables, minerals, or animals, and manmade counterparts of natural derivatives.

The colorants that occur naturally in food plants have been the source of the traditional colorants of raw as well as the processed food. 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. Although, biocolorants are structurally much diversified and from a variety of sources, the three most important are: tetrapyrrols, tetraterpenoids, and flavonoids. The main pigments and their potential natural sources are discussed below.

Pigment	Coloring principle from plant origin
Purple	Anthocyanins - Red Cabbage
Blue	Anthocyanins - Red Cabbage
Turquoise	Anthocyanins - Red Cabbage
Red	Beet Red
Yellow	Curcuma (Turmeric)
Orange	Annatto - Bixin
Green	Cu-Chlorophyllin
Light Green	Red Cabbage and Curcuma
Dark Brown (charcoal)	Caramel Color
Medium Brown (slight red tint)	Caramel Color
Light Brown (slight yellow tint)	Caramel Color

Conjugated systems for electron bond chemistry that causes these molecules to have pigment. On the basis of this pigments may be classified as

1. **Heme/porphyrin-based**: chlorophyll, bilirubin, hemocyanin, hemo globin, myoglobin

2. **Light-emitting:** luciferin
3. **Carotenoids:**

a. Hematochromes (algal pigments, mixes of carotenoids and their derivates)

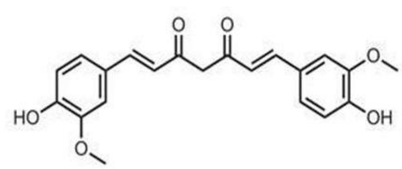
b. Carotenes: alpha and beta carotene, lycopene, rhodopsin

c. Xanthophylls: canthaxanthin, zeaxanthin, lutein

4. **Proteinaceous:** phytochrome, phycobiliproteins
5. **Polyene enolates:** a class of red pigments unique to parrots
6. **Other:** melanin, urochrome, flavonoids

Carotenoids

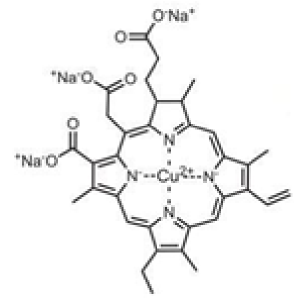
Carotenoids are familiar as food ingredient such as beta-carotene, apocarotenal, lycopene, annatto, paprika and lutein. They can deliver shades from weak yellow to a reddish colour, and anything in between.



They all decay by oxidation, losing their colour, so incorporating antioxidant ingredients is the key to stability and a good shelf-life in the warehouse, during processing and over time on the supermarket shelf. This is commonly done with ascorbic acid, ascorbyl palmitate or tocopherol. Temperature, pH, air and light are also important. Food production often includes heat treatment to control bacteria, which can affect the colour. Orange-red annatto is derived from the seeds of the *Bixa orellana*. The main coloured component is the oil-soluble carotenoid bixin- a carboxylic acid group at one end of the conjugated chain, and a methyl ester at the other. Norbixin is the de-esterified diacid, which is water soluble. It's in widespread use in dairy products such as cheddar, colby and red leicester cheeses, where it has been used for centuries to impart a characteristic orange colour.

2.4. Canthaxanthin

Canthaxanthin, meanwhile produces a bright deep red colour and when people taking canthaxanthin capsules as a sun-tanning aid developed reversible deposits of canthaxanthin crystals in their retinas.



Anthocyanin

The largest group of water-soluble pigments is the anthocyanins, whose colour tends to change with pH. They're basically indicators. Anthocyanin can be anywhere from red to a purple to blueish at neutral pH, and if pH increased further it will go green or brown, and ultimately colourless. This pH sensitivity makes food applications a real challenge. Synthetic colours remain a constant shade regardless of pH. Anthocyanins are also often light sensitive. In contrast to the carotenoids, which need ascorbic acid to stabilise them, they will be destroyed by ascorbic acid.

Curcumin

Another water-soluble pigment, curcumin, is extracted from turmeric. Its vibrant lemon-yellow colouration fades very rapidly in beverages as it is not light stable. Yellow sweets are commonly coloured with curcumin, and it performs brilliantly in confectionery, with a fantastic shelf-life and maintaining its vibrancy. But in a beverage or anywhere else with an excess of free water, it will fade very rapidly (Kulkarni *et al.*,2012).

Carmine

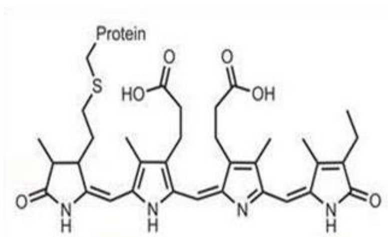
One natural pigment that many food manufacturers are moving away from is carmine, which, as it is derived from the cochineal beetle, is not vegetarian, kosher or halal. Carmine is a very stable red, and while anthocyanins are a successful replacement in beverages. Here, the colour of choice is often one derived from beetroot, which contains the indole-based pigment betanin. Beetroot used for commercial colour production are selectively bred to contain more betanin.

Copper chlorophyllin

Copper chlorophyllin has a vibrant bluish green colour. Green can be achieved using chlorophyll and copper chlorophyllin, a more stable derivative of chlorophyll with a more vibrant blue-green shade, and a more lime-green shade is possible when mixed with curcumin. It's not acid stable (Alison and Paul, 2000).

Phycocyanin

Blue is difficult to achieve with natural colours, and the only way is spirulina blue from spirulina blue–green algae. 'The colouring portion is phycocyanin, which is a pigment–protein complex, Its proteinaceous nature means it's limited to pH neutral applications. It's not a pure chromophore, and often contains a small amount of a reddish phycorubin component that can lend brown undertones when used with yellow to make a green. Phycocyanin, a pigment protein complex, is concentrated from algae.



GLOBAL VALUE

Biocolourant 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 global market for natural carotenes has reduced after the introduction of synthetic colour. Moreover, biocolourant are more costly and unstable in nature. The leading markets for natural colours are the UK, Germany, France, Italy and Spain. Currently, there is also a flourishing market in China, India and South Korea. The demand for natural colours are increasing regularly because of the following factors such as

- Health-promoting features of food with natural colours
- Biocolourant has been the public priority
- Low lipid content preferred for improved food formulations, replacing high fats or other synthetic food additives
- Demand of consumer for organic food
- Variety and international preference of natural food colour and flavours.

The market for natural food colours is estimated to increase by approximately 12% annually. Fig. 1 and 2 display the global scenario about the usage of biocolourant. Many of the raw materials for colours and flavours require growing conditions which are more favourable in countries outside Europe. So they imports natural colours and flavours estimated to **€** 2,765 million or 789 thousand tonnes. 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 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 due to inadequate strategies and 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, i.e., 31% of the world's current agricultural land, 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 standardization on modern scientific lines (Glover and Pierce,1993).

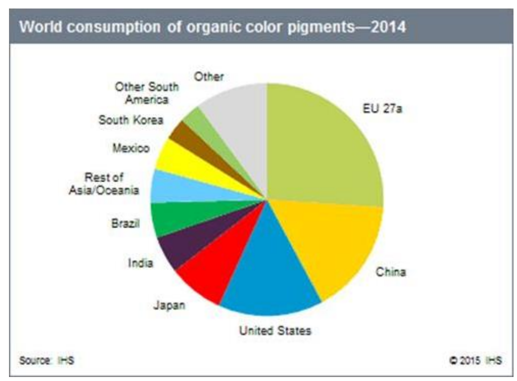


Fig. 1. World consumption of organic colour pigments

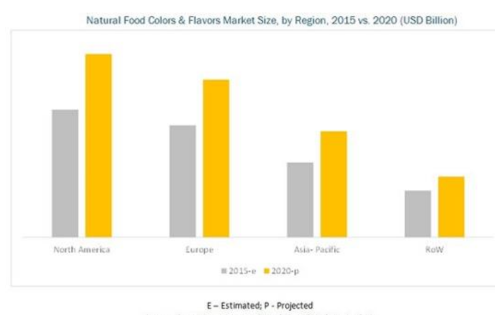


Fig. 2 Natural colour usage in different continents

COLOUR CHOICE

Commonly single natural coloring agents may not give the desired effect, the background color and of neighboring colored substances make a large impact in the color outlook. 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. Annatto or turmeric tends to represent a cheese color or have an eggy tone compared with the bright color produced with the

FD and C-yellow. Now-a-days, fluorescent colors are also getting importance in food industry as consumers favor foods to glow under conditions. Turmeric is highly fluorescent, thus it is commonly used in food (Martin *et al.*, 2007).

3. APPLICATIONS IN FOOD AND PHARMACEUTICAL INDUSTRY

3.1. Food preservatives

Generally, natural biocolorants possess antagonistic activity against certain bacteria, viruses and fungi for protecting the food from microbial spoilage. (Chattopadhyay *et al.*, 2008) reported that dyes were active against protozoans such as *Leishmania brasiliensis*, and insects like *Calliphora erythrrocephala.* Carotenoids are also known to act as sun screen for maintaining the quality of food by protecting them from intense light. Norton (1997) reported that corn carotenoids inhibit the synthesis of aflatoxin by *Aspergillus flavus* (90%) and by most of the *A. parasiticus* (30%) strains.

3.2. 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 (Boyles and Wrolstad, 1993). Anthocyanin profiles have been used to determine the quality of fruit jams. Adulteration of blackberry jam with strawberries can also be detected efficiently by the analysis of pelargonidin and cyanidin-3-glucoside content (Garcia-Viguera *et al.*, 1997).

3.3. Nutritional supplements

Natural colours includes phytochemicals produced by plant cells, which are known as the vegetal active principles. These are sources for obtaining biologically active drug substances and many other natural compounds used in various industries such as food, pharmaceuticals, cosmetics, with important commercial value (Filimon, 2010). Carotenoids are also used as vitamin supplements, 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 (Counsell *et al.*, 1979 and Nagaraj *et al.*, 2000). Yellow β- xanthins, are potential as food colorant and also may be used as a means of introducing essential dietary amino acids into foodstuffs.

3.4. Medicinal

Plant dyes play remarkable roles in human health as they contain biologically active chemicals, which possess a number of pharmacological features like strong antioxidant, antimutagenic, anti-inflammatory and antiartheritic effect (Hari *et al.*, 1998; Saleem *et al.*, 2004). 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 (Zeb and Mehmood, 2004). Lycopene is particularly effective at quenching the destructive potential of singlet oxygen. Lutein, zeaxanthin and xanthophylls are believed to function as protective antioxidants in the mascular region of the human retina (Landrum *et al.*, 1997). These compounds also act against aging, mascular degeneration, and senile cataracts. 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. It has been established that flavonoids present in different plant products show good antioxidant activity, sometimes better than the commercially available antioxidants. 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. Canthaxanthin also shows antioxidant property. Astaxanthin is another naturally occurring xanthophyll with potent antioxidant properties. Other health benefits of biocolorants include enhancement of immune system function, protection from sunburn, and inhibition of the development of certain types of cancers (Bendich, 1989; Mathews-Roth, 1990). Lycopene prevents oxidation of low-density lipoprotein (LDL) cholesterol and reduces the risk of developing atherosclerosis and coronary heart disease. Epidemiological studies revealed that there is a positive correlation between the consumption of chlorophylls and decreased risk of colon and other cancers.

4. MODERN TECHNOLOGY VS NATURAL DYES

Even though there are many natural dye yielding plants only a few are reported and explored for the same. Therefore, modern technology could be a solution for biopharming coloring compounds which are difficult to synthesize by traditional methods of extraction. Biotechnology and cell line culture are alternatives for this process. Biotechnological production of such colorants, plants and microorganisms are more suitable due to understanding of proper cultural techniques and processing.

4.1. Microbial cell culture for biocolorants production

Bradyrhizobium sp. strain are known to produce canthaxanthin (4, 4‘-diketo-β- carotene) and the carotenoid gene cluster was fully sequenced (Asker and Ohta, 1999). This keto-carotenoid was also found in *Halobacterium.* Culture of *Flavobacterium* sp. (Shepherd *et al.*, 1976) in 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. *Haematococcus lacustris* is commercially used for the production of astaxanthin using bioreactor. Besides, echineone and canthaxanthin are also identified in *Haematococcus* cultures. *In vivo* and *in vitro* 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 (Yuan *et al.*, 1997; Yamane *et al.*, 1997; Chumpolkulwong *et al.*, 1997a). 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 (Chumpolkulwong *et al.*, 1997b). *Dunaliella bardawil* and *Dunaliella salina* produce β-carotene as their main carotenoid (Phillips, 1995). *Blakeslea trispora* is known to produce β-carotene. The cell growth and β-carotene production are enhanced in medium containing surfactants such as Span or Triton, except Triton X-100 (Kim *et al.*, 1997). *Phycomyces blakesleeanus* is known for β-carotene production (Ootaki *et al.*, 1996). In *Blakeslea trispora*, sexual stimulation of carotene biosynthesis remains essential to increase yield significantly (Mehta *et al.*, 1997). Several strains of *Monascus* are also being exploited for commercial production of red and/or yellow pigments. The red yeast, *Xanthophyllomyces dendrorhous* synthesizes astaxanthin and zeaxanthin as its main carotenoids. Commercial production of carotenoids using microorganism has been achieved in case of astaxanthin, by red yeast fermentation. *Rhodotorula* including species *R. glutinis, R. gracilis, R. rubra,* and *R. graminis* synthesize carotenoids (Sakaki *et al.*, 2000; Simova *et al.*, 2004; Tinoi *et al.*, 2005).

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. The strain *Penicillium oxalicum* var. armeniaca CCM, obtained from soil, produces a chromophore of the anthraquinone type.

4.2. Plant cell culture for biocolorants production

Cells culture is the most common practice for production of plant pigments, as culture ensures uniform quality and continuous production of pigments. *Vitis vinifera*, *Aralia cordata*, *Aralia cordata*, *Fragaria anansa, Perilla frutescens*, *Daucus carota*, *Crocus sativus, Bixa Orellana* and *Beta vulgaris* produce via cell culture produce anthocyanin, crocin, carotenoid, bixin and norbixin betalain, betacyanin, betaxanthins (portulaxanthin-II and vulgaxanthin-I), muscaauri-VII, dopaxanthin, and indicaxanthin and other similar compounds (Rymbai *et al.*, 2011).

5. SIDE EFFECTS OF BIOCOLOURANT

Natural pigments are the most important precursor of several nutrients (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. Food allergy and intolerance among human beings 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 allergies. The consensus adopted by the Codex Alimentarius Commission of the WHO (1998) 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 and Taylor (1998) 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 and hence concluded that the ingestion of natural color additives presents a very low risk of provoking adverse reactions.

6. IMPORTANCE

The use of natural colours may show benefits over synthetic colours. Natural dyes are less toxic, less polluting, less health hazardous, non-carcinogenic and non-poisonous and prevent chronic diseases such as prostrate cancer. 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. Above all, they are environment friendly and can be recycled after use. 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. Anthocyanins also possess antineoplastic, radiation-protective, vasotonic, vasoprotective, anti-inflammtory, chemo- and hepatoprotective potentialities.

7. PITFALLS OF PIGMENTS

Natural colours in spite of having many 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. 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 and pH. They can also be decolourised or degraded during storage. Anthocyanin degradation and brown pigment formation cause color loss in food products. Curcumin is very prone to photobleaching and beetroot color has low heat stability. However, stability of biocolourant can be maintained by adding dextrins additives extracted from tart cherries or maltodextrin extracted from Roselle as a stabilizer. It has been demonstrated that increased glycosidic substitution, and in particular, acylation of sugar residues with cinnamic acids and reduced water activity will enhance stability and anthocyanin pigments in dried forms can exhibit high stability.

8. CONCLUSION

The review highlights the need to tap existing indigenous knowledge and understanding of plants to promote art education and technical skills development so that traditional values can be incorporated in the schools and colleges curricula towards the creative information particularly in the vocational subjects. This exercise also offers opportunity for common man about the nature of the plants, their characteristics, local and botanic names, medicinal value, dye-yielding quality, and their uses across the country. Since the dye extraction and application project involves visits to nature reserves and the indigenous textiles production centers where plant dyes are traditionally used on a large scale, the students will be knowledgeable in using dyes extracted from plant source for use as colourants for food and textiles to sustain life and generate employable skills. This will also offer opportunity for people to learn skills in tie-dye, batik, printing, dyeing of yarns for macramé, and crocheting at little or no cost and only buy synthetic dyes and food colourants for examination purposes since practically does not recognize the use of natural dyes and pigments for this purpose.

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REFERENCES

Alison, D. and Paul Collins, (2000). Colouring our foods in the last and next millennium. *IJFST* **35**: 5-22.

Asker, D. and Y. Ohta, (1999). Production of canthaxanthin by extremely halophilic bacteria. *J. Biosci. Bioeng.* **88**: 617-621.

Bendich, A. (1989). Carotenoids and the immune response. *J. Nutr.* **119**: 112-115.

Boyles, M.J. and R.E. Wrolstad, (1993). Anthocyanin composition of red raspberry juice: influences of cultivar, processing, and environmental factors. *J. Food Sci.* **58**: 1135-1141.

Chattopadhyay, P., S. Chatterjee and S.K. Sen, (2008). Biotechnological potential of natural food grade biocolorants. *Afr. J. Biotech.* **7**(17): 2972-2985.

Chumpolkulwong, N., T. Kakizono, T. Handa, and N. Nishio, (1997b). Isolation and characterization of compactin resistant mutants of an astaxanthin synthesizing green alga *Haematococcus pluvialis*. *Biotechnol. Letter.* **19**: 299- 302.

Chumpolkulwong, N., T. Kakizono, H. Ishii, and N. Nishio, (1997a). Enzymatic conversion of β-arotene to astaxanthin by cell extracts of a green alga *Haematococcus pluvialis. Biotechnol. Letter.* **19**: 443-446.

Codex Alimentarius, (1998). ALINORM 99/22 Draft Recommendations for the labelling of foods that can cause hypersensitivity (Draft amendment to the general standard for the labelling of repackaged foods) at Step 8 of the procedure. Appendix III.

Counsell, J.N., G.S. Jeffries and C.J. Knewstubb, (1979). Some other natural colors and their applications.

In: Counsell JN, Dunastable JA, Eds. Natural Colors for Foods and Other Uses. *Appl. Sci*. 122-151.

Filimon, R., (2010).Plants pigments with therapeutic potential from horticultural products. *Seria. Agro.* **52**: 668- 673.

Garcia-Viguera, C., P. Zafrilla and F.A. Tomas- Barberan. (1993). Determination of authenticity of fruit jams by HPLC analysis of anthocyanins. *J. Sci. Food Agric.* **73**:207-213.

Glover, B. and J.H. Pierce. Are natural colourants good for your health? *J. Soc. of Dyers Colourists*. **109**:5–14.

Hari, R.K., T.R.Patel and A.M. Martin. (1994). An overview of pigment production in biological systems: functions, biosynthesis, and applications in food industry. *Food Rev. Int*. **10**: 49-70.

Kim, S.W., W.T. Seo and Y.H. Park, (1997). Enhanced production of β-carotene from *Blakeslea trispora* with span 20. *Biotechnol Letters*. **19**: 561-562.

Kulkarni, S.J., K.N. Maske, M.P. Budre and R.P. Mahajan, (2012) Extraction and purification of curcuminoids from Turmeric (*Curcuma longa* L.) *IJPPT* **1**:81-84.

Landrum, J.T., R.A. Bone and M.D. Kilburn, (1997).The macular pigment: a possible role in protection from age-related macular degeneration. *Adv Pharm* 38: 537-556.

Lucas, C.D., J.B. Hallagan and S.L. Taylor, (1998). The role of natural color additives in food allergy. *Adv Food Nutr. Res.* **43**:195- 216.

Martin, M.L.G., W.J.R. Luo, J. Hutchings and F.J Heredia, (2007). Measuring colour appearance of red wines. *Food Quality and Pref*. **18**: 862-871.

Mathews-Roth, M.M. (1990). Plasma concentration of carotenoids after large doses of beta-carotene. *Am. J. Clin. Nutr*. **52**: 500-501.

Mehta, B.J., L.M. Salgado, E.R. Bejarano and E. Cerda-Olmedo, (1997). New mutants of *Phycomyces blakesleeanus* for β-carotene production. *Appl. Environ. Microbiol.* **63**:3657-3661.

Nagaraj, M., S. Sunitha and P. Varalakshmi, (2000). Effect of Lupeol, a Pentacyclic Triterpene, on the Lipid Peroxidation and Antioxidant Status in Rat Kidney after Chronic Cadmium Exposure. *J. Pl. Toxicol.* **20**: 413–417.

Norton, R.A., (1997). Effect of carotenoids on aflatoxin B1 synthesis by *Aspergillus flavus*. *Phytopathol*. **87**: 814-821.

Ootaki, T., Y. Yamazaki., T. Noshita and S.Takahashi, (1996). Excess carotenoids disturb prospective cellto- cell recognition system in mating responses of *Phycomyces blakes leanus*. *Mycoscience.* **37**:427-435.

Phillips, L.G., A.K. Cowan, P.D. Rose and M.R.R. Logie, (1995). Operation of the xanthophylls cycle in non stressed and stressed cells of *Dunaliella salina* Teod. in response to diurnal changes in incident irradiation: a correlation with intracellular β-carotene content. *J. Pl. Physiol.* **146**: 547-553.

Raghvendra, Vipin Sharma., Ambika Shakya, Hedaytullah, Ganesh Shankar Arya, Amlan Mishra, Anshu Deo Gupta, Amol P. Pachpute and Dharmendra Patel, (2011). Chemical and potential aspects of anthocyanins – a water-soluble vacuolar flavonoid pigments: a review. *IJPSRR.* **6**:28-33.

Rymbai, H., R.R. Sharma and Manish Srivastav, (2011). Biocolorants and its implications in Health and Food Industry - A Review. *Int.J. Pharm. Tech. Res*. **3**(4): 228-2244.

Sakaki, H., T. Nakanishi, K.Y. Satonaka, W. Miki, T. Fujita and S. Komemushi, (2000).Properties of a high-torularhodin mutant of *Rhodotorula glutinis* cultivated under oxidative stress. *J. Biosci. Bioeng.* **89**:203- 205.

Saleem M., F. Afaq, V.M. Adhami and H. Mukhtar. Lupeol Modulates NF-(kappa) B and PI3K/ Akt, (2004). Pathways and Inhibits Skin Cancer in CD-1 Mice. *Oncogene*. **23**: 5203–5214.

Shepherd, D., J. Dasek, M. Suzanne and C.Carels (1976). Production of zeaxanthin, US Patent. *Int.J. Pharm. Tech. Res*. **3**: 951- 743.

Simova, E.D., G.I. Frengova, D.M Beshkova, (2004). Synthesis of carotenoids by *Rhodotorula rubra* GED8 co-cultured with yogurt starter cultures in whey ultrafiltrates. *J. Indian Microbiol. Biotechnol.* **31**:115-121.

Skibola, C. and M. Smith, (2000).Potential health impacts of excessive flavonoid intake. *Free Radic Biol. Med.* **29**(3-4):375–383.

Tinoi, J., N. Rakariyatham and R.L. Deming, (2005). Simplex optimization of carotenoid production by *Rhodotorula glutinis* using hydrolyzed mung bean waste flour as substrate. *Process Biochem.* **40**:2551-2557.

Yamane, Y.I., K. Higashida, Y. Nakashimada, T Kakizono and N. Nishio, (1997). Influence of oxygen and glucose on primary metabolism and astaxanthin production by *Phaffia rhodozyma* in batch and fed-batch cultures: kinetic and stoichiometric analysis. *Appl. Environ.Microbiol*. **63**:4471-4478.

Yuan, J.P., X.D. Gong and F.Chen, (1997). Separation and analysis of carotenoids and chlorophylls in *Haematococcus lacustris* by high performance liquid chromatography photodiode array detection. *J. Agric. Food Chem*. **45**:1952-1956.

Zeb, A., and S. Mehmood, (2004). Carotenoids Contents from Various Sources and Their Potential Health Applications. *Pak. J. Nutr*. **3** (3): 199-204.