

Understanding Biocolour- A Review

Dipti Sharma

Abstract: Colour of food is an integral part of our culture and enjoyment of life. Colour is also an important parameter for sensory analysis and consumer preference. However using synthetic colour could be harmful for health of a consumer. Therefore in today's progressive world a shift from synthetic to biocolour is observed. The present article will enable to understand the what are biocolour, how they can be extracted and where they can be used.

Keyword: Biocolour

Introduction:

The colour of food is an integral part of our culture and enjoyment of life. They have been used to enhance the aesthetic value of foods. Colour is the most important characteristic of food, since common consumers usually judge quality of the food from its colour. Overall objective for the addition of colour in food is to make the food more appealing and recognizable. Everyone is sensitive to the colour of the food as it can stimulate or suppress one's appetite. There are several reasons for the addition of colours to food as mentioned below:

1. To make up the colour loss due to light, air, temperature, moisture and storage
2. To correct natural variations in colour
3. To enhance the natural colours associated with a given product.
4. To provide a colourful identity to the colourless or dull looking foods.
5. To provide a colourful appearance to foods.
6. To protect flavors and vitamins that may be affected by sunlight.

In the decade of sixty, synthetic colours such as azo dyes became highly popular owing to their low cost and easy availability. However subsequent toxicological evidences and adverse physiological effects of many such synthetic food colours has resulted in their removal from the permitted colour list for food uses and even more are likely to be banned in near future. Some of them were found to be carcinogenic.

Consequently attention has been shifted towards the use of natural alternatives. Though natural colours enjoy the advantage of being safe but they have drawbacks also such as

- Their seasonal production,
- Variation in quality and purity from source to source,
- Availability in limited shades,
- Low concentration in source material,
- Difficulties in extraction from the source,
- Instability during storage and use,
- High cost etc.

What is Biocolour?

BIOCOLOUR word consists of two words BIO meaning natural & COLOUR meaning anything which is used for colouring purpose. Biocolour is any dye obtained from any vegetable, animal or mineral, that is capable of colouring food, drugs, cosmetics or any part of human body. These natural colours come from variety of sources such as seeds, fruits and vegetables, leaves, algae & insects. According to the application a suitable Natural Colour can be achieved by keeping in mind the factors such as pH, heat, light, storage conditions and interaction with other ingredients of the formula or recipe. The storage conditions for natural colours depend on the particular need of the product. A tight sealed container is best to store the product in a cool storage to preserve colour strength and quality, along with its degree of cooling point. As per FDA colour pigments having a natural origin are exempt from certification. It does not carry any categorization as natural or synthetic. The reason is that the source may be natural but it may or may not be natural to the food it is added to. There are 26 colours permitted to be used in food and 28 to be used in cosmetics and pharmaceuticals. A few commonly used natural colours are Annatto (seed), turmeric, beet juice (root), bell pepper, red cabbage (vegetable), spinach (leaf) etc. Table 1 shows the colour shade and EEC No. of the biocolours.

-
- Dipti Sharma Asst. Professor, Delhi University
 - Corresponding author: sharmadipti23@gmail.com

COLOUR	PRODUCTS	C I Nos	E E C. Nos
	Annatto Colour Oil Soluble	75120	E-160b
	Annatto Colour, Water Soluble	75120	E-160b
	Annatto Bixin Powder	75120	E-160b
	Annatto Nor Bixin Powder	75120	E-160b
	Annatto Colour, Acid Proof	75120	E-160b
	Beet Root Red colour	-	E-162b
	Curcumin Yellow Colour	75300	E-100
	Caramel Colour Liquid	-	-
	Caramel Colour Powder	-	E-150
	Paprika Colour	-	E-160c
	Anthocyanin Colour	-	E-163
	Chlorophyll Green Colour Water Soluble	75810	E-141
	Chlorophyll Green Colour Oil Soluble	75810	E-141
	Safflower Extract/Carthamus	75760	-

Table1. Showing the colour shade and EEC no of biocolour

History of Biocolours:

Colour is vital constituent of our food. Colour irrespective the form has been added to our foods from centuries. The colouring of candy by the Egyptians and colouring of wine dates back to as long ago as 400 BC. Saffron, turmeric and paprika etc were used as traditional food colourants. Butter has been coloured yellow as far back as the 1300s. The use of colourants in cosmetics can be traced to the early Egyptian transcripts. In nineteenth century, synthetic organic dyes were developed creating, a more economical and wider range of colourants. Since then their quality has been improved due extensive research and development. Ancient Romans used saffron and other spices to put a rich yellow colour into various foods. Other natural foods, such as carrots, pomegranates, grapes, mulberries, spinach, beets, parsley and flowers, were also used as food colouring agents. Our ancestors also used minerals and ores, such as azure (copper carbonate), gold and silver leaf, some of which were downright poisonous if used improperly. Elise Fleming researched cookbooks dating as far back as 1390 A.D. In the late 1800's the food industry had a vast array of available synthetic colours. After the discovery of Perkins mauve named after the developer Sir Williams Henry Perker in 1856 many new colour were synthesized. These were called coal tar colours as the starting material was obtained from bituminous coal. Chemically synthesized colours were used extensively as they were

- Easy to produce,
- Less expensive and
- Superior in quality and properties,
- They are easily blended without giving any off-flavour to foods

Food Colour Laws and Regulations:

Today all food colour additives are carefully regulated by federal authorities to ensure that foods are safe to eat and accurately labeled. The Food and Drug Act of 1906 in the

United States established a voluntary certification program regulating the addition of colours to our foods in the U.S. Mandatory certification came with the Federal Food, Drug & Cosmetic (FD&C) Act of 1938, regulating what colour enhancers could be added to not only foods, but also drugs and cosmetics. In 1960, the laws were further amended to require any colour additive be on the Federal Food & Drug Administration (FDA) approved list. And, most recently, the Nutrition Labeling and Education Act of 1990 now requires that any certifiable colour additive used in food must be listed in the ingredient statement by its common or usual name. All new colour additives must be tested and proved not to cause harmful effects when consumed, and are approved only by petition to the FDA to be added to the certified list. Once approved, the FDA may still restrict usage to only certain types of foods. In India, FSSAI is monitoring the regulation of addition of colouring additive to food.

Why Should Industry Use Biocolours?

With the advent of strict legislative regulations and growing awareness among the consumers about food safety, biocolour have become the choice in the foods as they are extracted from sources of biological origin and are much safer than their synthetic counterparts. Biocolour could be a dye, pigment or substance that can impart colour when added or applied to a food drug, cosmetic or human body but is of biological origin derived from plants, insects or microbes. There are a number of natural colours, but only few are available in a sufficient quantity to be useful for the industry because they are directly extracted from plant, flowers, fruits, leaves and roots. It is therefore advantageous to produce natural colours from different methods. The trend towards natural foods has led in recent years to the substitution of synthetic by natural or nature identical colours in many foods. Table 2 shows the salient features of some important biocolours.

Benefits of Biocolours :

1. They have a protective role against lethal photo oxidation.
2. They inhibit mutagenesis.
3. Use of biocolour may enhance immune systems.
4. They may also lead to inhibition of tumour developments.

named 'Vitamins of the 21st Century' due to their impressive medical and health benefits. The commercial production of anthocyanins through tissue culture is being exploited.

Classification of Biocolour:

Food colours have been classified in different ways such as natural/non synthetic/biocolours; synthetic colours & nature identical colours. However food colours essentially biocolours can be classified into three main classes viz. natural colours, browning colours and additives.

1. **Natural Colours** : The principal natural colours, most of which in refined form used as additives are the green pigment chlorophyll, the carotenoids which give yellow to red colours; and the flavonoids with their principal subclass the anthocyanins, which impart red to blue colours to flowers and fruits. In recent years, there has been much interest in carotenoids, especially β -carotene. Besides being a natural orange pigment present in carrots, mango, papaya, tomato, winter squash, etc. it is converted in the body to vitamin A and has antioxidant properties. It is believed to have a beneficial effect in reducing the risk of some cancers and perhaps heart diseases. It can be produced commercially using microorganisms like *Dunaliella salina* and *Blakeslea trispora* as these microbes are easy to culture and produce β -carotene in fairly good quantities. The natural colour include: Paprika, Annatto Extract, Anthocyanins, Aronia / Red fruit, Spinach, Beet Juice Colours , β -Carotene , Beta APO 8 Carotenal , Black Currant, Burnt Sugar, Canthaxanthin, Caramel , Carmine, Carmine Blue , Carminic Acid , Carrot , Chlorophyll, Chlorophyllin , Cochineal Extract , Copper-Chlorophyll, Copper-Chlorophyllin, Curcumin , Curcumin/CU-Chloro, Elderberry , Grape , Hibiscus, Lutein, Mixed Carotenoids , Paprika Extract, Paprika Oleoresin , Riboflavin, Turmeric etc.
2. **Browning Colours** : These are produced during cooking and processing and thus may not be of any direct importance in foods. For ex. As produced during sugar caramelization, baking etc.
3. **Additives** : Food additive colours are based on anthocyanins derived from sources such as red grapes or beet but the first additive colour were synthetic dyes which were extensively used as food colourants in nineteenth century and early 1900's. Some sources of anthocyanins, besides grapes are elderberries, red cabbage, blood orange, the less familiar black chokeberry and sweet potato. Anthocyanins are highly dependent on acidity. As consumer demand for natural product grows, these natural colourants are replacing synthetic food colourants. The most important point about anthocyanins is their strong antioxidant abilities and other health promoting properties. Anthocyanins are polyphenolic group of compounds which have been

Salient Features of Some Important Biocolours:

PIGMENT	SOURCE	COLOUR SHADE	REMARKS
β -carotene	Dunaliella salina, Euglena, Blakeslea trispora	Yellow to orange depending upon colour formulations	It is sparingly oil soluble, it comprises of all the trans isomers and possesses pro vitamin A activity
Astaxanthins	Haematococcus pluvialis	Orange pink to red	Astaxanthin belong to the Carotenoids family and are responsible for the red colour of shrimps and salmon, Astaxanthin can protect against chemically induced cancers and is very strong antioxidant
Phycobiliproteins	Algae belonging to Rhodophyta and Chlorophyta	Red and blue	Phycobiliproteins have good long term stability when stored refrigerated (2-5C) as ammonium sulfate precipitates. Purified biliprotein may dissociate into subunits under acidic or basic conditions, but are relatively stable at room temperature and neutral pH.
Monascus pigments	Monascus purpureus and M anka	Yellow, orange and red	Pigment production and quality is good when the organism is provided with carbon source such as maltose, fructose and glucose and yeast extracts as nitrogen source. Pigments are stable to pH change in temperatures.
Carminic acid	Cochineal insect	Orange-red	Water dispersible pigment with colour becoming more red with increased pH
Curcumin	Turmeric	Bright lemon yellow	A non soluble pigment that is light sensitive once solubilized and in the presence of water. It is widely used in desserts and confectionary.

Table 2: Salient features of some important biocolours**Production of Biocolour:**

The biocolours are used in every type of industry whether it is a food or pharma industry. These colour can be used everywhere. Biocolour are used in almost all sort of food industry like beverages, confectionery, processed foods, bakery products, dairy products, pet foods, coloured sugar. Naturally the colour can be obtained by following:

- By biotechnological approach,
- By microorganism,
- By using certain enzymes etc.

1. Biotechnology – A Boon For Natural Colour Production

Biotechnology is an integrated multi-disiplinary science and has a profound impact in every industrial sector and in the field of natural food colours. Biotechnology has answered many problems of natural food colours including :

1. Genetic modification for pigment production.
2. Improving the traditional methods for extraction of pigments
3. Microbial production of pigments
4. In vivo pigment production by plant tissue culture

Biotechnology holds a good promise. Plant cell and tissue culture, microbial fermentation and gene manipulation are being explored with respect to pigment production for development of new biocolours and improvement of the existing ones. It can help in altering the metabolic pathways in the micro organism so that the colour characteristics can be enhanced, resulting in a product with good appearance. Many food colours can be produced through microbial fermentation with greater efficiency. The genes controlling the production and excretion of indigo have been transferred into bacteria in order to produce the dye by fermentation rather than extraction. Plant tissues are often considered to be an effective alternative method for the production of natural pigments. Carotenoids, anthocyanins and betalins have already been produced in plant cell cultures. Continuous production using currently available techniques appears to be impossible because most pigments are not excreted by the cells but are stored within them so the continuous commercial production of such colours through microorganism appear to be a viable proposition.

2. Genetic Modification for Pigment Production:

In genetic modification hereditary apparatus of animal, plant or bacterial cell is altered so as to produce more different chemicals or perform new function. The alternation may be brought about by:

- Appropriate mutation through physical and/or chemical means
- Genetic engineering through recombinant DNA technique.

Typically pigment is produced intracellularly in organism. However, by cloning of genes responsible for pigment production, it is possible to obtain hyperpigment producers. These hyperpigment producers excrete pigment into the growth medium, thus making the process more economical. One of the example of such extracellular secretion of the pigment by a mutant of *Monascus*.

Improving Traditional Methods for Extraction of Pigment :

The traditional methods for extraction of colouring agents usually involve disruption of the source material followed by acidified aqueous extraction for anthocyanins, beet red, cochineal etc. or by solvent extraction for chlorophyll, Carotenoids, annatto etc. Various efforts are being made to eliminate problems encountered in extraction and preparation of the natural colouring materials by use of enzymes and/or microorganisms aided extraction from their conventional sources.

2a. Enzymes Aided Extraction: Enzymes are used to facilitate the extraction, increase pigment yield, improve quality and stability of the result and colour preparation. For the commercial extraction of astaxanthin from *Phaffia rhodozyma*, extracellular enzymes produced by *Bacillus circulans* are used. These enzymes digest the yeast cell wall and allow easy extraction of the pigment by acetone or ethanol. Endopolygalacturonase prepared from *Aspergillus* Sp. is used for the extraction of beta carotene from tissues and juices of various vegetables such as spinach, lettuce, squash, green pepper, carrot etc. Glycosidic and proteolytic enzymes such as beta galactosidase and bromelain are used in extracting pigment. In the case of algal biliproteins, protease is used to release the protein bound pigment. Proteinase treatment of cochineal extract produces better quality cochineal. Similarly, treatment of pulped grape gives satisfactory extraction of anthocyanin and fermentation time is reduced considerably. Addition of invertase to pectinase treated beet juice increases betalains content by three fold when the juice is purified and concentrated by membranes processing. Use of these enzymes in extraction of anthocyanin from strawberries increases the pigment content upto 80%. The maximum liquefaction of carrot can be achieved by cellulose plus pectinase treatment of the carrot, which gives 25% enhancement of Carotenoids concentration in suspension. When comminuted orange peels are pretreated with enzyme papain, cellulose or pectinase, then it enhances the recovery of pigment.

2b. Microorganism Aided Extraction: The natural colouring extracts, especially those obtained through

aqueous extraction or direct pressing of plant materials, although it contain required pigments but it also contain high amount of unwanted dissolved solids like salts, organic acids, phenolics, sugars etc. Such high solid extract on concentration produces hygroscopic material which can have several limitations for its use. For example, powder of beetroot extract has a characteristic odour, high nitrate and nitrite content, which limits its application in foods. Similarly sugars in anthocyanin extract produce furfural during concentration and drying of the extracts, which then contribute to accelerated pigment degradation. The corrective measure of such problems usually involves application of fermentation, using appropriate microorganisms during and /or after extraction. Approximately 80% of beet root juice solids are carbohydrates and nitrogenous compounds. Fermentation of the juice using yeasts (*Saccharomyces* sp.) and moulds (*Aspergillus* sp.) reduces the amount of solids substantially giving 5-7 fold increase in the yield of pigment on dry weight basis. The dried product is free from beet root odour and has reduced nitrate content. In application of such fermentation process yeast cells and ethanol are the important by-products obtained during the process. The yeast cells may be harvested and used as a source of biomass for the animal food supplement. The ethanol has economic and industrial value of its own.

3. Microbial Production of Colour Compounds:

Fermentation is a process of obtaining products of commercial significance in large quantities by the use of microorganisms owing to their fast multiplication rate. It is therefore advantageous to produce natural colours from microorganisms and it has been established that various pigments are produced from *Monascus*, *Blakeslea*, *Cordyceps*, *Euglena* etc. and many other microbes such as algae. Production of materials by culturing microorganisms has several advantages. The rapid growth of microbes cuts production time to matter of days and the process leads itself to continuous operation compared to plant or animal sources. Moreover, production can be obtained by bacteria, yeast or moulds, the process is flexible and can be controlled very easily. Biocolours produced by microorganisms include Astaxanthin, Prodigiosin, Naphthoquinones, Carotenoids, *Monascus* pigments, Phycobiliproteins

3a. Bacteria: Carotenoids are very well known and highly popular as food colourants. The carotene production by a pigmented strain of bacteria *Bacillus* (alkaliphilic yellow) has been documented.

3b. Yeast: The Carotenoids astaxanthin, produced by yeast *Phaffia Xhodozyma*, is considered as an important source of the natural pigment fro colouring foods. The growth of *Phaffia rhodozyma* on 7-10% B or C grade molasses gives 2-3 times more astaxanthin than with glucose or sugar blend. Use of grape juice may also be a useful raw material especially where surplus of juice is available. Temperature is an important factor in the production of type of pigment. At 20 °C mainly astaxanthin (80-85%) is synthesized. In addition to *Phaffia rhodozyma* even food spoiling xerophytic yeast produces highly pigmented colonies, which may be

also likely to contribute in the natural colouring for food in near future.

3c. Algae: The Microalgae constitute a reservoir of substances of commercial value. The production is based on exploiting their highly efficient photosynthetic machinery. Red algae (Rhodophyta) and blue-green algae (Cyanophyta) produce a group of highly light absorbing pigments based on bilin or tetrapyrrole skeleton. These phycobilin proteins have potential as natural colourants for use in food, cosmetics and pharmaceuticals particularly as substitutes for synthetic dyes. The protein bound pigments are separated by protease treatment and extracted into dilute alkali. Pigment preparations either in water or alcohol are recommended for use in ice candies, frozen confections, sherbets, confectioneries and chewing gum.

3d. Mould: The pigment production by a mould of *Monascus* group, especially, *Monascus purpurus* and *Monascus anka*, for use as a food colour is well known. Its crude pigment is red and marketed as red mould rice in powder form for use as household as well as industrial food colouring. The pigment is extensively used in oriental countries like China, Indonesia, Japan, etc., as a general food colourant or as a colour additive in wine and also as preservative for meat. The crude pigment is a mixture of red, yellow and purple pigmented polyketides. It can be made water soluble as well as oil soluble. The pigment is heat stable and can be autoclaved. These properties

together with a colour range from yellow to red make the pigment a good candidate for use as food colourant. The production is a two stage process, first stage is for optimizing organism for growth, and second is for pigment production.

4. Plant Tissue Culture (PTC) For Pigment Production:

PTC in fermentation has the potential for the production of secondary metabolites such as pigments, flavoring compounds etc. Attempts have been made to produce anthocyanin, betalains, Carotenoids, saffron and other pigment through PTC. This technique offers several advantages over the conventional plant sources which includes:

- More reliable and predictable, since it is independent of weather, season, plant variability etc.
- Continuous harvesting rather than once or twice in a year.
- Simple and easy extraction due to highly aqueous aggregates of cells instead of complex tissues of plant organs.
- Reduction or even elimination of co extracted material thus minimizing or avoiding problems of the co-extracted material. Table 3 lists few organizations involved in commercial production of biocolour.

S.No.	Name of the organization	Product
1	Roche Products Pvt. Ltd., Australia	Carotenoids
2	Roche Vitamins fine Chemicals, Canada	B-carotene
3	Synthetic industrial chemicals Ltd., India	Xanthophylls
4	Gist Brocades, The Netherlands	B-carotene
5	Bush Boake allen co, USA	Natural colours
6	Cyanotech Corporation and Aquasearch Inc, USA	Astaxanthin & Phycobiliproteins from spirulina
7	Overseal colour Inc	Curcumin and Anthocyanins
8	BASF, Germany	B-carotene
9	Overseal Foods Ltd, UK	Natural carotenes, lutein and paprika
10	Chr. Hansen	Natural colours
11	Burman Laboratories Pvt Ltd, India	Spirulina capsules

Table 3: Organizations involved in commercial production of biocolours

REFERENCES:

- [1]. Biotechnology-A boon for production of natural food colours Beverage and food world, July 2005.
- [2]. Application of natural colours in surimi seafood's- Beverage and food world, July, 2005.
- [3]. Microbial production of colours- Processed food industry, May, 2005.
- [4]. Biocolours: New Generation Additives for food, Indian food Industry, sept-oct, 1997, Vol 16(5).
- [5]. www.google.com
- [6]. Food Additives by Branen
- [7]. www.foodadditives.com