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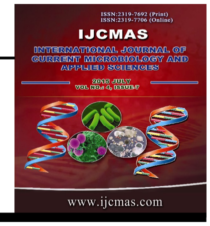


**Review Article**

# Biocolors: The New Generation Additives

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**A B S T R A C T**

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Biocolorants are those colouring agents, which are obtained from the biological sources**.** Biocolours can be classified into three main classes: natural colours, browning colours and additives. There are various microbial sources of biocolour as carotene production by a pigmented strain of bacterium *Bacillus* sp., carotenoid Astaxanthin produced by yeast *Phaffia xhodoxyma* which are considered as an important source of the natural pigment for colouring foods. Biocolours as additive can be used in various industries as in pharmaceutical industry, textile industry, dairy industry etc. Technological limitations are the major bottleneck for the commercial exploitation of the microbes for biocolour production while designing of proper bioreactors would help to ease out the situation.

## Introduction

Colour is one of the most important qualities of foods. In the past, consumers did not care about the kind of pigments used in food colouring (natural or synthetic). But with reference to food colorants recently there is an aversion towards synthetic pigments owing to the belief such as "synthetic pigments are associated with several illnesses" and "natural pigments have pharmacological benefits" (Clydesdale, 1993). Natural organic pigments are generally extracted from fruits, vegetables, seeds, roots and microorganisms and they are sometimes called biocolours because of their biological origin (Pattnaik et al., 1997). Biocolours that are permitted for human foods are very limited and there is difficulty in getting approval for new sources for the reason that the U.S. Food and Drug Administration (FDA) considers the pigments as additives and consequently pigments are under strict regulations (Wissgot and Bortlik, 1996; Wodicka, 1996). Recently there have been changes in the legislation also causing a significant reduction in number of synthetic colours used in foods (Downham and Collins, 2000).

According to a study on dyes and organic pigments, the worldwide demand for organic colorants is projected to increase from 4.9 % in 2003 to $10.6 billion in 2008. Future growth is going to be large for naturally derived colours with a predicted annual growth rate of 5-10 %. Synthetic colours are still forecast to grow but at a lower rate of between 3 and 5 % (Downham and Collins, 2000).

The demand for food colour in global market in 2000 was 2400 MT which increased to 3000 MT by the year 2005 and further to increase to 8000 MT by the year 2010 and is expected to increase to 15000 MT by the year 2015 (Lakshmi, 2014). The investment in natural food colour market across the globe has touched to US $ 1 billion and is continuously growing as there is demand for natural food colours against synthetic food colours (Magoulas, 2009). Because of consumer's choice for 'natural' food processing industry and have contributed to the increase in natural colour market significantly (Ree, 2006).

Only few natural pigments are available in sufficient quantities to be useful for industry because they are usually extracted from plants (Lauro, 1991). But there is an ever growing interest in microbial pigments due to several reasons like their natural character and safety to use, production being independent of seasons and geographical conditions, controllable and predictable yield (Francis, 1987).

**Classification:** Biocolours can be classified into three main classes (Sharma, 2014).

**1. Natural colours:** The principal natural colours 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 as it is converted in the body to vitamin A and has antioxidant properties. It has 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.*

**2. Browning colours:** These are produced during cooking and processing and thus may not be of any direct importance in foods. For e.g., 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 colorants in nineteenth century and early 1900's. Anthocyanins are polyphenolic group of compounds which have been named **'Vitamins of the 21'st Century'** due to their impressive medical and health benefits.

**Applications of biocolours as additives:** There are various applications of Biocolours in different industries as described below:

**A. Pharmaceutical industry:** Hepatitis C virus (HCV) infects approximately 170 million people worldwide, and is often associated with chronic hepatitis, leading to liver cirrhosis and hepatocellular carcinoma (Brown, 2005). Currently, interferon (IFN) and the nucleoside analogue ribavirin are used as the standard therapy to treat chronic HCV infection. However, IFN-α alone or in combination with ribavirin often leads to a range of side effects.

The 65 kDa HCV NS5B protein has RNA-dependent RNA polymerase (RdRp) activity and is key player in HCV RNA replication. *Moncascus purpureus* produces pigments that are responsible for inhibiting hepatitis C virus replication by interfering with viral

RNA polymerase activity (Sun et al., 2011). A group of Monascus orange pigment (MOP) derivatives effectively inhibited NS5B RdRp activity and interfered with the mevalonate synthesis pathway, thereby suppressing HCV replication in cells. A double-hit strategy, including inhibition of HCV RdRp activity and interference with the mevalonate synthetic pathway, to inhibit HCV amplification may provide the basis for successful antiviral therapy using the MOP AADs derived from this microbial secondary metabolite.

**B. Dairy industry:** Among the various pigment-producing microorganisms, *Monascus sp.* is reported to produce non-toxic pigments, which can be used as food colorant. Besides a colouring agent it enhances the flavour of the food and acts a food preservative. *Monascus ruber* has been used widely in the preparation of flavoured milk by utilization of rice carbohydrate for its metabolism and production of secondary metabolite namely pigment. Red, orange and yellow pigments are produced using solid-state fermentation and rice broken as a substrate. Monascus Fermented Rice (MFR) 1.2 % is used in the preparation of flavoured milk (Vidyalakshmi et al., 2009).

**C. Fish industry:** Aquaculture is a rapidly growing global industry, comprising cultivation of various freshwater and marine species of finfish, shellfish, molluscs and ornamental fish (Garcia and Maurilio, 2013). Pigmentation is one of the important quality attributes of the aquatic animal for consumer acceptability. As fishes cannot synthesize their own colouring pigments *de novo*, the colouring agents which are synthesized by some plants, algae and microorganisms, need to be incorporated in the diet (Johnson and An, 1991). Most promising pigment proved to be successful in enhancing skin colour is Astaxanthin. Commercially available products of astaxanthin (carotenoid) rich yeast *Phafia rhodozyma* and fermentation product of *Xanthophyllomyces dendrorhous* has been widely used. Microalgae *Chlorella vulgaris* imparts yellow – blue hues, yielding both muscle and skin pigmentation effects.

**D. Textile industry:** The textile industry discharges large proportion of effluent that mainly consists of synthetic dyes. Synthetic dyes have been extensively used in the textile industries due to their ease and cost effectiveness in synthesis, high stability towards light, temperature and technically advanced colours covering the whole colour spectrum. However, these synthetic dyes are often toxic, mutagenic and carcinogenic leading to several human health problems such as skin cancer and allergic reactions (Srikanlayanukul et al., 2006; Gurav et al., 2011). Thus, the worldwide demand for the dyes of natural origin is increasing rapidly in the textile industry.

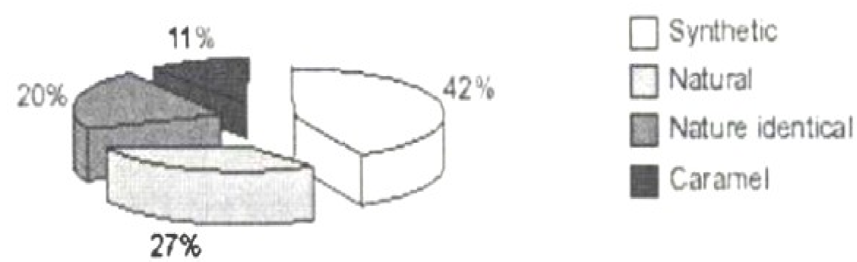
**E. Printing industry:** It has become important to reuse and recycle used papers in offices, etc. for the purpose of the conservation of forest resources and reduction of wastes. Reuse of papers in offices is preferable, but it requires that letters and images easily disappear from printed papers. Decolorable ink for inkjet printing contains a Monascus pigment. The Monascus pigments are easily discoloured and finally lose their colours by the irradiation of visible and/or ultraviolet light. (Tsuyoshi et al., 2004)

**Methods of extraction of pigments:** Solvent extraction is the conventional method that is followed to extract colours. Anthocyanin and betalain pigments, which are water soluble, are extracted from the raw material with water and sometimes with aqueous methanol.

**Table.1** 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 andcomprises of all the trans isomers and possesses pro vitamin A activity |
| Astaxanthins | *Haematococcus pluvialis* | Orange pink to red | Astaxanthin belong to the Carotenoids family. Astaxanthin can protect against chemically induced cancers and is very strong antioxidant. |
| Phycobiliproteins | Algae belonging to Rhodophyta and Chlorophyta | Red and blue | These have good long term stability when stored refrigerated (2-5°C) as ammonium sulphate precipitates. These 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. |

**Fig.1 Percentage market share of food colorants**



For carotenoids extraction, hexane is the solvent of choice. After thorough extraction, the extract is concentrated and subjected to purification steps by using column chromatography. Identification and quantification of the pigment is performed by spectrophotometry or by high pressure liquid chromatography (Naidu and Sowbhagya, 2012).

**The current advance techniques followed in colour extraction are as follows:**

**1. High Hydrostatic Pressure (HHP) and Pulsed Electric Field (PEF):** These methods are environment friendly and energy efficient technologies that enhance mass transfer processes within cellular tissues, as the permeability of cytoplasmic membranes can be increased which in turn enhances extraction of valuable cell components. PEF is reported to be an ideal method to enhance juice production, increase the extraction of valuable components better than the yields obtained by enzymatic maceration (Mason and Zhao, 1994).

**2. Sonication-assisted extraction:** It is one of the most commonly used methods to enhance mass transfer phenomena by cavitation forces, where bubbles in the liquid/solid extraction can explosively collapse and generate localized pressure, causing plant tissue rupture and improving the release of intracellular substances into the solvent. (Nayak et al., 2007).

**3. Gamma irradiation:** Gamma-irradiation, as a pre-treatment to a plant material, increases cell wall permeabilization, resulting in enhanced extraction of cell constituents in higher yield (Sowbhagya and Chitra, 2010).

**4. Enzymatic extraction:** Enzyme assisted extraction of pigments is another new technology. Enzyme pre-treatment cannot be a complete substitute for conventional solvent extraction, but can result in increased yield of value added cell components and a reduction in time of extraction and amount of solvent consumption (Rodriguez et al., 2001).

**5. Membrane technology:** Membrane processing is a fast and emerging technique for the concentration and separation of macro and micro molecules based on molecular size and shape in biotechnology and food processing industries (Downham and Collins, 2001). Advantages of membrane processing are many which include improved product quality with higher yield, utilization of by products, temperature and pH sensitive products can easily be extracted without alteration and lastly is environmental friendly as no harmful chemicals are being used and less energy is consumed. (Spence et al.,2010).

**Future prospects:** A giant leap forward in colour production could be achieved by combining genetic manipulation and fermentation. Technological limitations are the major bottleneck for the commercial exploitation of the microbes for biocolour production and designing of proper bioreactors would help to ease out the situation.

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