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Biocolourants from Plants: Bio-Functional Additives for Food Applications

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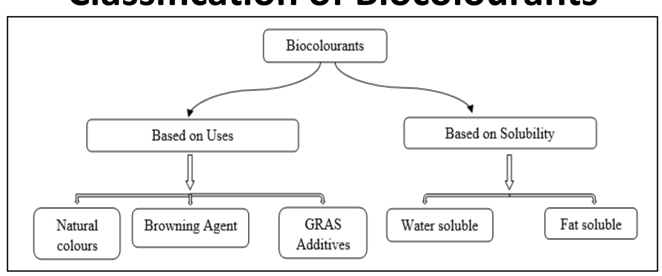
Abstract

Colour gives elegance to food item which attracts the consumer and thus increases the market value of the product. Because of several ill effects of artificial dyes such as hyperactivity in kids and kidney malfunctions, now a days, plant originated natural colourants are gaining popularity. The growing interest of consumers in the aesthetic, functional and safety aspects of food has increased the demand for natural pigments to be used as an alternative of synthetic colourants or additives in food products. With colour, antioxidant and antimicrobial potential of several biopigments, enhancing bio-functional status of foods and thus can be established as bio-functional additives for food applications. However, scanty work on standardized extraction protocols, lower yield percentage, higher initial investment, stability issue, pH dependency are the major constraints to establish biocolourants as an alternative of their artificial commercial counterparts.

Introduction

Biocolour are those coloring agents which are extracted from biological material. The biocolourants are extensively extracted from their rich sources such as pumpkin (carotenoids), citrus peel (flavonoids), black carrot (anthocyanins), beetroot (betalains) and spinach (chlorophylls) through various traditional and emerging technologies. Except saffron, they are generally extracted from low value horticultural crops and food industry waste. Due to several functions, biocolourants can be used as 'bio-functional additives' for various food applications such as for colour, antimicrobial and antioxidant benefits. Currently European Union has approved 43 biocolourants whereas United States has approved 30 biocolourants (Rymbai *et al.*, 2011). According to a survey, the global food colour market size will be of USD 2.97 billion by year 2025.

Classification of Biocolourants



There are mainly three types of biocolourants based on uses, which are (a) Natural colourants, (b) Browning agents, and (c) Additives.

i) Natural Colours

These are the organic colorants that are derived from natural edible sources and being utilized in various traditional food preparation methods from long.

Their respective source does not have GRAS (Generally Recognized as Safe) status due to lack of scientific evidences on toxicological effects, *e.g.*, Betalains from Cockscomb, Bougainvellia *etc*.

ii) Browning Colours

These are produced during cooking and processing *e.g.,* caramelized sugar can produce brown colour, interaction of reducing sugars and aminoacids leads to generation of melanoidins pigments during baking and frying.

iii) Additives

Food additive colours are approved by the European Union at specific level of doses. Their respective source must have the GRAS status. For example, many plants accumulate betalains, but only two source namely *B. vulgaris* L. and *Opuntia ficus-indica* are approved by the European Union to be used in food as natural colourants (labeled E-162). Maximum Permitted Limits (MPLs) of betanins (E 162) and Anthocyanins (E163) have been defined in commission regulation (EU) No. 1129/2011 as food additive in various products. Like, both the colourants, prepared by physical means from fruits and vegetables are authorized food colouring substances with a MPLs of 200 mg/Kg of fruit flavoured breakfast cereal alone or in combination with E 162/ E163 and E 120, respectively.

Based on solubility, there are mainly two types of biocolourants:

• Water soluble biocolourant eg. anthocyanins, betalains, flavanoids, caramalized sugar *etc*. However, co-existence of anthocyanins and betalains has never been reported anywhere.

• Fat soluble biocolourants: e.g. carotenoids, chlorophyll and lycopene *etc*.

Comparison of Biocolourants with Synthetic Colourants

Inspite of several ill effects and negative reports, artificial colourants are still in demand by food processors due to their low cost, easy storage, stability over wide range of pH, light, air and temperature. However, prevailing strict food regulations and guidelines, vigilance and consumers' inclination towards natural additives along with below mentioned additional benefits biocolourants are also gaining popularity now a days.

The main reasons for growing usage of biocolourants are-

• Providing natural tinge to the product.

• Some colourants possess antimicrobial effect, *e.g.* betanins.

• Most of bio-colourants, *e.g.* lycopene *etc*. are strong antioxidants.

• Few colourants exhibiting α-GIA (α-Glucosidase Inhibitory Activity) too, *e.g.* betalains and anthocyanins.

• Environment friendly.

• Mainly extracted from waste or low value horticultural commodities.

• Free from ill effects of overdoses.

Potential Sources of Different Biocolourants

• Carotenoids are the major source of natural colorants including alpha-carotene, beta-carotene, lutein, lycopene, *etc*. Carrots, orange, apricot, mango, peach *etc*. are good source of beta-carotene.

• Lycopene acts as precursor in carotene biosynthesis which is present in tomato, red pepper, onion and water melon *(Citrullus lunatus*) *etc*.

• Lutein, a profound nutraceutical for eyes, is commonly present in marigold and green vegetables.

• Quercetin is majorly present in onion, apple, peapods and even in broccoli leaves.

• Chlorophyll is present in green tissues and can be extracted from leafy vegetables, alfa alfa grass and even from mulberry leaves.

• Betalains or Betacyanins are mainly extracted from beetroot, opuntia and malabar spinach.

• Anthocyanins are present in black grapes, black carrot, red cabbage, purple cauliflower *etc*. Black carrot athocyanins are heat stable and known for their remarkable heat stability (Silva *et al*., 2017).

Different Methods of Biocolourant Extraction

1. Solvent Extraction

Solvent extraction also called liquid-liquid extraction (LLE) is a method to separate compounds based on their relative solubility in two immiscible liquids. These liquids are usually water and an organic solvent. Commonly used solvents are polar, including methanol, ethanol, acetone and water acidified with both organic and inorganic acids. Effect of different solvent (methanol, ethanol and water) and acid (HCl, citric, acetic, propionic, tartaric and formic acid) combinations on the extraction of anthocyanins and other biocolourants has been studied. Methanol was found the best performing solvent, extracting 20 and 73% more anthocyanins than ethanol and water. For anthocyanins methanol acidified with citric acid gives the best results and for betanins ascorbic acid proved better for stabilizing and regeneration of betanins pigments.

2. High Hydrostatic Pressure (HHP)

Several energy efficient technologies that affect mass transfer processes within cellular tissues by increasing the permeability of cytoplasmic membranes and in turn enhance extraction of valuable cell components are emerging. For example, lycopene, using 75% ethanol as solvent, was extracted from tomato waste (92% recovery) at 1 minute exposure of 500 MPa pressure. Similarly through HHP, anthocyanins were extracted from blueberry (pressure-443 MPa, using ethanol 63%).

3. Pulsed Electric Field (PEF)

Short pulses of high electric fields with duration of microseconds to milliseconds and intensity in the order of 10-80 kV/cm create cavitations pressure on cell boundaries of tissue and thus enhances the extract yield. For example, enhanced yield of betalains was obtained from beetroot at 4.38 kV/cm of strength.

4. Ultra Sonication-Assisted Extraction

The procedure involves the use of ultrasound with the frequency ranging from 20 Khz. Here 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.

5. Enzyme Assisted Extraction Methods

Enzymatic pre-treatment is the best option for inherent hard, firm and less juicy matrix such as for carrot, pomace *etc*. For example, viscozyme (a combination of several cell degrading enzymes such as cellulase, hemicellulase, arabanase, beta-glucanase and xylanase is used for extraction of anthocyanins from black carrot. Enzymes also limit the use of solvent. However, cost, pH and temperature dependency of enzymatic action cannot substitute conventional solvent extraction method completely.

Applications of Biocolourants

- Biocolourants can provide functional benefit to foods.
- Use as natural colourant for beverage industry, dairy and bakery industry such as curcuminoids enriched coconut slices, pineapple slices and apple slices, betacyanins enriched yoghurt, milk and flavoured milk *etc*.
- Some innovative shelf stable products are developed by ICAR-IARI Pusa are as follows:
  - Anthocyanins enriched aonla candy (Pusa Nutra Candy).
  - Betanins enriched papaya candy (Joshi *et al*., 2019).
  - Anthocyanins and Betanins enriched microwaved potato chips (Joshi *et al*., 2019).
  - Red capsicum colourant for bread.

Limitations of Biocolourants

- Tedious extraction process from raw materials.
- Expensive than synthetic dyes.
- Seasonal dependency on raw material.
- There is lack of standardization of extraction techniques and technical knowledge.
- Sensitive for light/ air/ temperature/ pH *e.g.* curcumin is susceptible to photobleaching and anthocyanin has very low pH stability.
- Scanty reports on storage requirement.

Conclusion

Since most of the biocolourants are extracted from underutilized horticultural crops or from their waste suffering from the problem of glut therefore, challenges associated with cost in comparison to its commercial counterparts can be neglected, provided the extracted colourants would be stable enough to ensure uniform colour stability/ pattern during the expected shelf life in the targeted/ selected food matrix. Low cost extraction protocols for higher yield, pigment stabilization protocols, stability studies, matrix selection as per the biocolorant behaviours and strategise for enhancement of colour stability through derivatization and copigmentation are key areas for practical use of biocolourants. These factors need to be worked out and documented for easy and true reference of food industry so that a biocolourant can be used not only as colourant but as a biofunctional additive in food products.

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