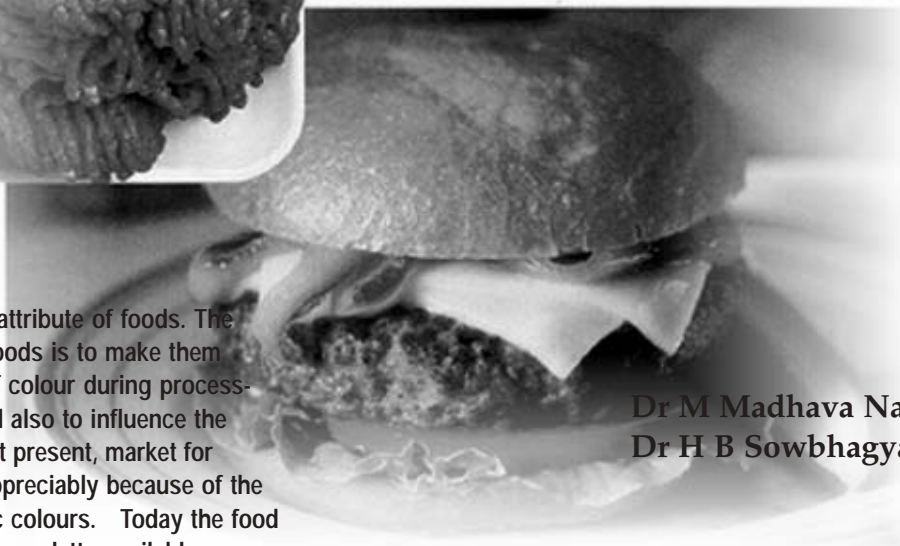
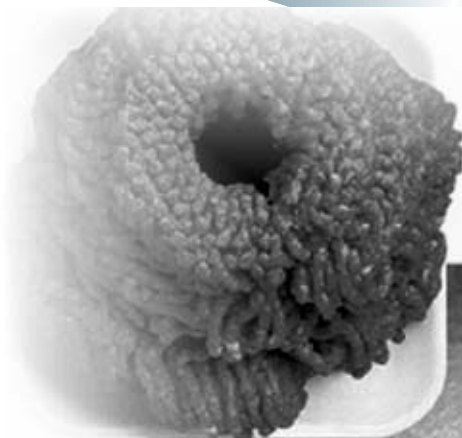


Technological Advances in Food Colours



Colour is an important quality attribute of foods. The objective of adding colour to foods is to make them appealing, augment the loss of colour during processing, to improve the quality and also to influence the consumer to buy a product. At present, market for natural colorants has grown appreciably because of the recognized toxicity of synthetic colours. Today the food industry has an extensive colour palette available, allowing the selection of the most suitable colour for their application requirements.

Colour suppliers are however constantly motivated to improve the technical and physical properties of their colour preparations. Development of cost-effective, viable technology for the preparation of a food colour and its application in foods is a challenge and the need of the day.

This article covers recent developments in technological advances of food colours with respect to natural colour application and stability in foods, compared to synthetic colours.

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Introduction

Colour is one of the most important qualities of foods, makes food appealing and affects every moment of our lives. Colour is added to food for the following reasons: 1) to replace colour lost during processing, 2) to enhance colour already present, 3) to minimize batch variations and 4) to color otherwise uncoloured food.

Food colours can be divided into four categories: (1) natural colours, (2) nature-identical colours, (3) synthetic colours and (4) inorganic colours.

Although the use of natural colours in food is an ancient practice, it is currently gaining increas-

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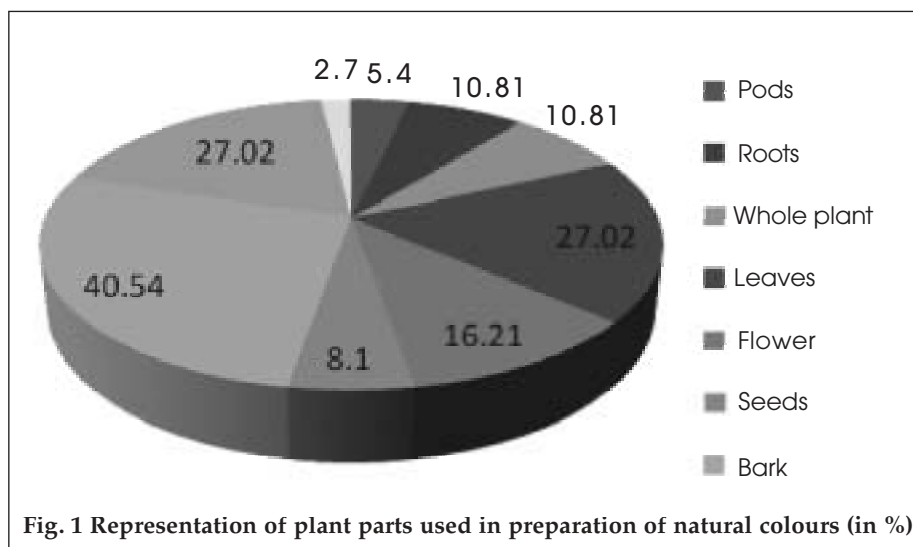


Fig. 1 Representation of plant parts used in preparation of natural colours (in %)

colours by food industries for application to a wide variety of foods including beverages and confectionery, eight synthetic colors, three for red shade (carmoisine, ponceau 4R, erythrosine), two for yellow shade (tartrazine and sunset yellow), one for green (Fast green FCF) and two for blue shade (brilliant blue FCF and indigotine) and eleven natural colors are available under current legislation in India.³⁷ Only seven synthetic colors are allowed in USA according to FDA (1995). Developments in the technology of food color preparation, improvement in the stability of colors and preparation

of color formulations including the search for new pigments are important aspects of study in the area of colours.

ing importance because consumers are becoming aware of the hazards of using synthetic colours. Synthetic colours are synthesized and not found in nature, whereas natural colors are obtained from plant and also animal sources. Various plant parts viz., leaves, fruits, seeds, bark, stem etc., (Fig.1) are traditionally used for extracting dyes by utilizing traditional extraction techniques.⁹

Pigments prepared by modification of natural materials such as caramel, vegetable carbon and Cucurbitacin (vide infra) are considered natural, although they are not found in nature as it is. Nature-identical colours are man-made pigments which are identical to the ones found in nature viz., carotene, canthaxanthin and riboflavin. The contribution of different parts of the plant in percentages used for the preparation of natural colours is represented in Fig. 1.

In the last two decades, the food market has changed rapidly with a larger proportion of processed foods and instant foods catering to the needs of working mothers, single parent families and older people. To make processed foods appealing and to attract the consumer, application of an appropriate food colour is necessary and selection of food colour is a great challenge in terms of cost, stability and feasibility of the colour.

History of Colours

The addition of colorants to foods is reported in Europe during the Bronze Age and the earliest written record of the use of natural dyes dates back to 2600 BC in China. It is reported that around 1500 BC, candy makers in Egyptian cities added natural extracts and wine to improve the product appearance.¹ Historical records show that injuries, even deaths, resulted from toxic colorants used to color candies and pickles. The first synthetic colour (mauve), was developed by Sir William Henry Perkin in 1856 (Walford, 1980) and, by the turn of the century, unmonitored colour additives had spread through the USA and Europe in a wide variety of foods, including ketchup, mustard, jellies and wine. More than 80 artificial colours, some of which were intended for dyeing textiles, were also used in foods as data on toxicity of the colorants was not available.

In the beginning of the 19th century, the bulk of synthetic colours were derived from the petroleum product aniline, therefore they were called 'coal-tar' colours because the starting materials were obtained from coal. Colours from plant, animal and mineral sources, which were used in earlier times, had their own drawbacks like heat, pH and light instability, and against oxidizing agents in food, which made synthetic colors gain popularity in food industry. Chemically synthesized colours were easier to produce, less expensive, and superior in colouring properties. They blended easily without imparting flavours of their own to foods. As the use of synthetic

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To meet the needs of food

colours in food increased, it raised safety concerns, which led to numerous regulations throughout the world and in the USA, the permitted list of synthetic colours was reduced from 700 to seven.⁴²

Market Trends

The global market for colorants is estimated to be \$940, which is distributed in various categories (Fig. 2) viz., synthetic colours (\$400), natural colours (\$250), nature identical (\$189) and caramel colours (\$100).² Future growth prospects for naturally derived colours are predicted to be optimistic, with an annual growth rate of 5-10%, while the synthetic colours market will grow at a much lower rate of 3- 5%. Consumer pressure, sociological changes, technological advances in the food processing industry and consumer's choice for "natural" have contributed to the increase in natural colour market significantly.

Synthetic Colours

Synthetic colours are obtained by chemical synthesis. In last three decades, consumers have become increasingly health conscious and careful of the ingredients of foods and prefer 'natural' against synthetics. The indiscriminate use of food colours is perceived to pose health concerns^{5, 31} and hence these are subjected to increasing legislative control, restricting them to selective commodities along with specified levels.^{16, 37, 49} Colours such as green FCF, sunset yellow, tartrazine and ponceau 4R are reported to incite allergic reactions including urticaria, dermatitis, angioedema and exacerbation of asthma in sensitive individuals. Tartrazine and sunset yellow, green FCF have also been implicated to cause irritability, restlessness, sleep disturbance and hyperactivity in children.³⁴

The Prevention of Food Adulteration Act of India permits the use of eight synthetic colours in specified food commodities at a uniform level of 100 mg kg⁻¹ or mg l⁻¹.³⁷ The acceptable daily intakes (ADI) of the food colours currently approved in India²⁴ vary from 0.1 to 25 mgkg⁻¹ body weight day⁻¹, i.e. a difference of 250-fold. Since there is a lack of dietary intake data with respect to colours in the Indian context, the actual intake of colours from various

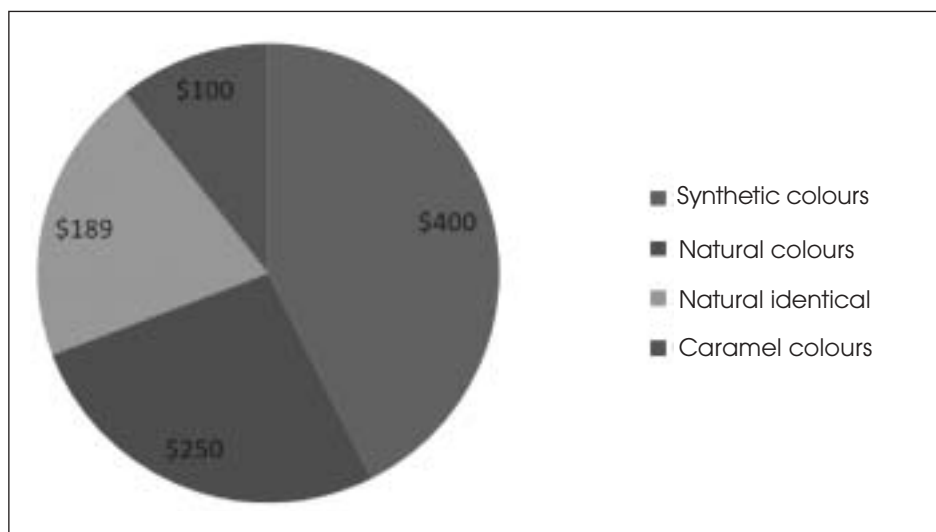


Fig. 2: Usage of colours

coloured foods has to be monitored, so as to evolve a scientific yardstick to fix levels of colours in commodities based on technological and safety requirement.¹²

Natural Colours

Natural colours are extracted from fruits, vegetables, seeds, roots and also form microorganisms which are sometimes called biocolours. Plant pigments, by virtue of their natural occurrence in edible plants, are generally considered to be harmless. Nature produces a variety of brilliant coloured pigments viz., water soluble anthocyanins, betanins and fat soluble pigments carotenoids and chlorophylls used for colouring foods.

Anthocyanins, a group of water soluble pigments, are responsible for the attractive red to blue colours in many fruits and vegetables including cherries, grapes, black berries and have high colour intensity at pH values less than 4.0. Anthocyanins absorb UV and visible light in a range 250–650 nm with a λ_{max} at 535 nm.²⁰ They are used worldwide as a source of food colours in foods which have an acidic pH, such as beverages and desserts.

The anthocyanin and betalain groups are mutually exclusive in the plant kingdom. Betalain, found in red beet, is a water-soluble pigment similar to anthocyanin, having high tinctorial strength with nitrogen in its struc-

Although the use of natural colours in food is an ancient practice, it is currently gaining increasing importance because consumers are becoming aware of the hazards of using synthetic colours. Various plant parts are traditionally used for extracting dyes by utilizing traditional extraction techniques.

The search for low-cost raw materials and environment-friendly technologies has led to newer technologies for extraction and identification of natural pigments from existing and new sources of plant materials. Extraction by hydrostatic pressure and pulsed electric fields highlights the benefits of moderate temperatures (60°C–70°C) for an optimal extraction.

ture. Betalains are a group of red and yellow pigments, betacyanin and betaxanthin respectively. Betalain absorbs visible radiation over the range of 476–600 nm with maxima at 537 nm at pH 5.¹⁴ The colour intensity is relatively unchanged from pH 3 to 7 but at pH values lower than 3 it turns more violet. At pH values higher than 7, the colour becomes more yellowish-brown

because of the loss of betanin. Both the red and yellow pigments are thermo-labile, either with or without the presence of oxygen and are degraded by light. Metal cations, such as iron, copper, tin and aluminium accelerate their degradation. Francis (2000) reports the chemistry and commercial preparation of betalain from red beets.¹⁸ Beet powders containing 0.4–1.0% betanin pigment, 80% sugar, 8% ash and 10% protein together with citric acid and ascorbic acid as a preservative are commercially available.

Carotenoid group of pigments are responsible for the yellow, orange and red colours of many plants and are used extensively as natural and nature-identical colours in foods. The major representative of carotenoids is β -carotene. Carotenoids are hydrocarbons with 40 carbon atoms formed by linkage of C5 isoprenoid units in a series of conjugated diene bonds and their oxygenated analogues are called xanthophylls.

Carotenes tend to be soluble in non-polar solvents such as hexane, while xanthophylls are more freely soluble in polar solvents such as methanol and ethanol. Carotenoids are largely unstable, especially in isolation, to light, heat and oxygen, so special precautions are necessary during handling. The isoprenoid structure of carotenoids, a series of conjugated diene bonds, exhibits characteristic absorption spectra in the UV-VIS range that have diagnostic features (such as cis-/trans-isomerism), which are useful for analytical detection and measurement. A significant advance has been made by the food industry to use carotenoids as food color as a replacement for artificial colours, which also has an extra advantage of their associated health benefits. As a result, a large number of published articles are available on the extraction and analysis of carotenoids and on identification of new carotenoids from new sources. Information

on nutritional profile of foods including cis-and trans-isomers of carotenoids, health consequences of carotenoid consumption and use of carotenoids as food colorants are reported.^{10,38}

Chlorophylls, a group of fat soluble natural pigments, are obtained by solvent extraction of grass material, grass, lucerne and nettle. The principal colouring matters are the phaeophytins and magnesium chlorophylls, which are highly unstable to light. The green colour is due to the pigments chlorophyll a (blue-green) and chlorophyll b (yellow-green) that occur together in a ratio of about 3:1.²² Chlorophyll is converted to chlorophyllins in presence of alkali, which renders it water soluble. The technological advance in chlorophyll pigment is that the magnesium atom in the structure is replaced by zinc or copper, which improves its stability to light.

Caramel, another natural colour, is obtained by heating carbohydrates, alone or in the presence of food-grade acids, alkalis and/or salts, produced from commercially available, food-grade nutritive sweeteners consisting of fructose, dextrose (glucose), invert sugar, sucrose, malt syrup, molasses and/or starch hydrolysates and fractions thereof. The acids used are food-grade sulfuric, sulfurous, phosphoric, acetic and citric acids; the alkalis are ammonium, sodium, potassium and calcium hydroxides; and the salts are ammonium, sodium and potassium carbonate, bicarbonate, phosphate (including mono- and dibasic), sulfate and bisulfite. Caramel, a dark brown to black liquid, is a complex mixture of compounds. Caramel is soluble in water and is used to impart a range of brown colours to foods. Caramel colour preparations can also be converted to powder form and are suitable for application in a wide range of foods and beverages.

Colour Extraction Methods

Conventionally, solvents are used to extract colorants from plant materials. Anthocyanin and betalain pigments, which are water soluble, are extracted from the raw material with water and sometimes with aqueous methanol at a ratio of 60:40 or 80:20 (v/v). With water, pigments readily leach out from the matrix and methanol denatures the protein structures of the enzymes present, thus inhibiting degradation reactions by polyphenol oxidases, peroxidases, and α -glucosidases. For the extraction of carotenoids, hexane is the solvent of choice and acetone is good choice of solvent for the initial extraction of pigment from the plant material.

After thorough extraction of the plant material, 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 (HPLC). Spectrophotometry is used to estimate the total anthocyanin and betalain content, while by HPLC individual anthocyanin can be identified and quantified.

Currently, reversed phase solid phase extraction (SPE) cartridges are used to separate anthocyanins from betalains. A sample extract is applied to cartridges pre-equilibrated with methanol and through excessive washing with purified water of neutral pH. After application of samples containing both betacyanins and anthocyanins, the betacyanins are eluted with neutral water, while the anthocyanins are successively eluted with methanol.

Earlier, betacyanin and betaxanthin, the red and yellow pigment present in beetroot, were quantified using the extinction coefficient of the pure pigments by the spectrophotometric method. As in advancement over the above method, Stintzing et al. (2002) identified betaxanthins, the yellowish orange water-soluble pigments from yellow beet, using HPLC linked with mass spectrometry. To enable the concentration of betalain samples, solid phase extraction was used. With this technique, betacyanins could be separated from the betaxanthins by using the pH-dependent retention characteristics of red and yellow betalains.

Advances in Extraction Technologies

The search for low-cost raw materials and environment-friendly technologies has led to newer technologies for extraction and identification of natural pigments from existing and new sources of plant materials. Extraction by hydrostatic pressure and pulsed electric fields highlights the benefits of moderate temperatures (60°C–70°C) for an optimal extraction, since high temperature enhances the mass transfer phenomena, increasing the internal liquid phase, which raises the pressure, causing centrifugal circulation of the solutes through plant membranes. Thus, the use of novel technologies that are able to enhance the cell disruption combined with a temperature of 70°C may represent an economical and environment friendly technique, wherein grape by-products could be recycled for the food industry as value-added ingredients or additives.

High hydrostatic pressure (HHP), pulsed electric fields (PEF) and sonication belong to the category of environment-friendly and energy efficient technologies that enhance mass transfer processes within plant or animal cellular tissues, as the permeability of cytoplasmic membranes can be increased which in turn enhances extraction of valuable cell components.⁴⁸

High Hydrostatic Pressure (HHP)

It is reported that HHP has an effect on cell permeability, increasing secondary metabolite diffusion according to changes in phase transitions, resulting in enhanced mass transfer rates. The potential application of HHP for extraction of

flavanoids from propolis has been reported.⁴³ The increased extraction yields with HHP are attributed to its ability to deprotonate charged groups and disrupt salt bridges and hydrophobic bonds in cell membranes, which leads to a higher permeability. In addition, the decrease in the dielectric constant of water under HHP combined with temperature leads to a decrease in the polarity of the media, contributing to the higher yield of total phenolics and other antioxidants. A better colour retention of jams and grape juices when treated by HHP is also reported.²¹

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Pulsed Electric Field (PEF)

PEF is reported to enhance mass transfer rates by electroporation of plant cell membranes, improving tissue softness and thus influencing the textural properties. 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.¹⁵ Carrots, potatoes and apples treated with PEF lost their water content more rapidly during osmotic drying.²⁸ Pulsed electric field treatment was used for the extraction of pigment from grape skin using a Pure Pulse (exponential decay pulse generator with a maximum voltage of 10 kV and a maximum average power of 8 kW). The peak pulse voltage used was 9 kV, resulting in an electric field strength of 3 kV cm⁻¹.⁸ A series of 30 pulses were applied at ambient temperature to obtain a specific energy input of 10 kJ kg⁻¹ with an increase in temperature of less than 3°C. Subsequent extraction was performed at 70°C. PEF treatment resulted in an increased yield of anthocyanin (10%) in comparison with HHP and 17% compared to the conventional extraction.

Similar results were reported by Toepfl (2006), wherein

a higher extraction of anthocyanins from PEF treated purple fleshed potatoes was obtained. PEF remarkably enhanced the extraction of anthocyanin monoglucosides compared to the amount of acylated glucosides extracted, as they are entrapped within the matrix, or form hydrogen bonds with cell wall polysaccharides, which are not easily extracted. The highest recovery of anthocyanin was obtained by PEF corresponding to 60% of the maximal recovery with a solid/liquid ratio of 1:20. Estiaghi and Knorr (2000) reported that when an external electrical field in a range of 1–3 kVcm⁻¹ was applied to grape extraction during wine production, the nutritional content of fruit juices and the number of intracellular compounds from grapes during wine production was enhanced.

Sonication-assisted Extraction

Sonication is one of the most commonly used methods to enhance mass transfer phenomena. Its feasibility for the extraction of secondary metabolites such as tea, mint, chamomile and ginseng has been highlighted in many research studies.³² Ultrasound enhances mass transfer rates 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. A few studies on application of sonication to extraction of food colours have been reported.

The extraction of grape (Dornfelder var.) anthocyanins and other antioxidant compounds can be described as a mass transport phenomenon where solids in plant structures migrate into the solvent up to equilibrium. Mass transfer phenomenon can be increased by heating, changes in concentration gradients and with the influence of technologies such as sonication, HHP and PEF. The optimal extraction conditions for extraction of anthocyanin from grapes with 50% ethanol as extraction solvent and conditions of ultrasonication extraction were optimized.^{8,28} Spigno, *et al.*, (2006) highlighted the advantages of a mixture of ethanol/water for anthocyanin recovery from grape skins.⁴⁶ Sonication, HHP and PEF techniques led to a higher recovery of secondary metabolites viz., phenolic compounds approximately two-fold higher than the control extraction at 70°C. Sivakumar *et al.*, (2009) have reported the ultrasound-assisted extraction of natural dye from beet root in higher yield for industrial application.⁴⁴

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. Nayak *et al.*, (2006) studied the effect of gamma irradiation as a pre-treatment prior to the solid–liquid extraction on betanin extraction in comparison with control beetroot extraction.³⁵ Beet roots subjected to gamma irradiation in doses of 2.5, 5.0, 7.5 and 10.0 kGy and control (without irradiation treatment) were dipped in an acetic acid medium (1% v/v) to extract betanin. The diffusion coefficients for betanin as well as ionic component were estimated considering Fickian diffusion. The results indicated an increase in the diffusion coefficient of betanin (0.302×10^{-9} – 0.463×10^{-9} m²/s) and ionic component (0.248×10^{-9} – 0.453×10^{-9} m²/s) as the dose of irradiation increased (from 2.5 to 10.0 kGy).

The degradation constant was found to increase (0.050–0.079 min⁻¹) with an increase in gamma-irradiation doses (2.5–10.0 kGy). Effect of gamma irradiation on histological and textural properties of carrot, potato and beetroot were also studied.³⁶ The exposure to irradiation pre-treatment (3.0–12.0 kGy) resulted in increase in cell wall permeabilization, leading to softening of tissue, thereby affecting the textural and histological properties supported by the histological examination. Textural properties such as hardness, cohesiveness, springiness, gumminess and chewiness were found to decrease with an increase in irradiation doses up to 12.0 kGy. Pre-treatment of the plant material with calcium before irradiation was found to reduce the damage in texture.

Enzymatic Extraction

Enzyme assisted extraction of value added products from plant materials viz., pigments, antioxidants, flavours and phytochemicals is another new technology. Enzyme pretreatment 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 reduction in amount of solvent consumption. A review on enzyme-assisted extraction of flavorings and colorants from plant materials has been published.⁴⁵ Hydrolytic enzymes, used singly or in different combinations, will act on cell walls, breaking down the structural integrity, in turn increasing the permeability of the intracellular components more easily available for extraction by solvent or by mechanical force.

Based on this approach, enzymes have been explored as a means to enhance the extraction of carotenoids in marigold flowers. Matoushek (1974) described a process in which fresh flowers in water (10% w/v) were pre-treated with cellulase enzyme for 16 h (pH 4–6, 45°C) prior to chloroform or hexane extraction, resulting in 36%

increase in carotenoid yield compared to control without enzyme pretreatment.³³ Delgado-Vargas and Paredes-Lo'pez (1992) have studied the effect of an aqueous enzymatic treatment of marigold flowers meal (not subjected to silage) on the extraction yield with a mixture of hexane-ethanol-acetone- toluene (10:6:7:7 v/v).¹¹

Barzana et al., (2002) reported an alternative extraction process for carotenoids consisting of a simultaneous enzymatic pretreatment followed by solvent extraction.⁴ The process employs fresh flowers directly as raw material, without silage and drying operations, which is a traditional process. The process was developed at 80 L scale, wherein carotenoid recovery of 97% was obtained. Pre-treatment of marigold flower with hydrolytic enzyme solution increased the diffusion coefficient from $1.56 \times 10^{-9} \text{ m}^2/\text{s}$ to $4.02 \times 10^{-9} \text{ m}^2/\text{s}$ and mass transfer coefficient from 0.14 h^{-1} to 0.36 h^{-1} with increased dry yield, resin yield and pigment yield compared to convention solvent extraction.⁴⁷

Enzymatic extraction of capsaicinoids and carotenoids from chili guajillo "puya" flour was studied.⁴¹ The chilli flour pre-treated with cell wall degrading enzymes, dried and extracted with ethanol showed an increased extraction of carotenoid and capsaicinoids by 11% and 7%, respectively. Sampathu et al., (2006) have reported that, treating chilli powder/ flakes with a multi enzyme preparation and extracting using a solvent mixture results in chilli oleoresin with enriched pungency and color.⁴⁰

Membrane Technology

Membrane processing is a fast emerging technique for the concentration and separation of macro and micro molecules based on molecular size and shape used in the field of chemical engineering, biotechnology and food processing. Advantages of membrane process are improved product quality with higher yield, utilization of byproducts and a solution to some environmental problems. Natural colours are sensitive to temperature and concentration by conventional methods of evaporation, resulting in deterioration of pigment. Alternative processes, such as freeze concentration, have a drawback with respect to the maximum achievable concentration.

In recent years, membrane processes such as microfiltration, ultrafiltration and reverse osmosis have gained importance for the concentration of natural colours. These existing membrane processes have limitations of concentration polarization, membrane fouling, and maximum achievable concentration (only up to 25° brix). Technological advances related to the development of thermal membrane process such as osmotic membrane

distillation and direct osmosis have shown potential to overcome the above limitations. The above processes can be employed as a pre-concentration step to reduce water load on subsequent processing steps and can be easily scaled up.

Osmotic Membrane Distillation (OMD) can be employed as a pre-concentration step prior to relatively costlier processes such as lyophilization, in case of thermally sensitive natural pigments. The use of pressure driven membrane processes such as ultrafiltration, reverse osmosis and nano filtration for the concentration of phycocyanin (natural food colourant/protein) result in shear damage to the product as well as membrane fouling.²³ The concentration of C-phycocyanin increased by around 220% without product damage (confirmed by spectrophotometric analysis) employing the OMD process in a flat membrane module using a hydrophobic polypropylene membrane. The concentrated C-phycocyanin was lyophilized to convert to powder form, which can be readily used for food applications. Anthocyanin extracted from red radish extract was concentrated by conventional evaporative technology followed by direct osmosis.³⁹

Application of Nanotechnology to Colorants

Nanotechnology has been applied to natural colorants to convert fat soluble pigments into water-soluble formulations. Since application of fat soluble pigment to foods can cause problems like non-uniform distribution and spots of colours in the processed foods, it is desirable to convert fat soluble pigments to water soluble formulations. To use a lipophilic natural pigment (β -carotene) in water-based foods, the colorant was entrapped in a matrix of Ca^+ cross-linked alginic acid. Effect of different parameters viz., solvent, alginic acid, calcium chloride concentration on nano particle morphology was evaluated.³ The nano particle stability was assessed by measuring aggregation against pH, oxidation and particle precipitation as a function of time. The synthesized particle measured 120-180 nm when formed with chloroform and 500-950 nm when synthesized with ethyl acetate. The particles were negatively charged -70 to -80 mV and were stable at pH 3 to 7. Addition of calcium increased nano particle density and improved β -carotene protection against oxidation.

Stability of Natural Pigments

The amount of anthocyanins in solution is related to their chemical structure and stability in conditions of processing. The stability of anthocyanins was shown to be dependent on the OH and OCH_3 - groups at position R1

in C3' and R2 in C5' of the B ring, as well as the sugar moieties, and phenolic acyl groups of the C ring. The higher content in H- and OCH₃- and acyl groups, the higher the stability, Mv being the most stable one followed by Pd, Pt, Cy and Dl. These significant chemical differences explain the higher yields of Pt, Pd and Mv, obtained by PEF and HHP extractions. HHP has the ability to reduce the pH of the solvent during the extraction not only because of the higher release of phenolics into the solvent but also because of the deprotonation of molecules present in the extracts. This decrease in the pH might also enhance the extraction of acylated anthocyanins since they are more stable at pH 4 where the flavylium cations are predominant. At this pH, aromatic acyl groups of acylated anthocyanins are known to stack on the flavylium nucleus and thereby protect the pyrylium ring from the nucleophilic addition of water, which leads to their colourless forms (chalcones).⁶

Byproduct Utilization

Grape by-products, consisting of peels, contain a high amount of secondary metabolites including phenolic acids, flavanols and are rich in anthocyanins,³⁰ which are reported to possess antibacterial, antiviral, antioxidant, anti-inflammatory, anti-carcinogenic properties and reported to prevent cardiovascular diseases.¹⁹ These compounds exist in plants enclosed in insoluble structures such as the vacuoles of plant cells and lipoproteins bilayers which are not easily extractable. Thus, different newer extraction methods viz., subcritical water, polymeric adsorbent resins and pressurised liquid extraction have been reported to enhance secondary metabolites extraction from grape by-products.²⁵

Encapsulation

Encapsulation is a technique wherein light and heat sensitive compounds like flavor, colour are covered (encapsulated) with a layer of inert wall material like gums or starches by a technique viz. spray drying. The process of encapsulation gives protection to the core material from external factors like light and heat and oxygen, preventing degradation of the compound. The process of spray drying of betalain from red beet roots, a natural colour using different carriers viz., maltodextrin, gum acacia and soluble starch and effect of these carriers, in varying percentages, with freshly extracted beet root juice was studied during spray drying by Koul et al (2002).²⁷ The chamber temperature of spray drier was maintained in the range of 150°-165 °C. These studies showed that with decrease in percentage of carriers in the juice, the percent

yield of betalain increased. The shelf life studies of the spray dried betalain dry powder, over 180 days showed that the spray dried product is stable at temperatures between - 4 °C and 20 °C.

Biotechnology

Biotechnology could be an effective technology for the efficient mass production of colorants from plant tissues. Plant cell and tissue culture, microbial fermentation and gene manipulation have been investigated with respect to pigment production. However, extensive safety testing of such products would be required along with clearance from regulatory agencies before they are accepted as safe. Production of carotenoids, anthocyanins and betalains by plant cell cultures has been reported but they cannot compete with the amount of pigment obtained by extraction of plant materials. Continuous production of colorants by cell culture has its own limitation because most pigments are not easily excreted by the cells but stored within them.

To date, no standardized methods are reported for the production of natural colour on large scale by plant cell culture methods. A lot of work is required in this direction. The pigments isolated from cell cultures also display the same instability as those isolated from naturally grown plants. Single cell algae and fungi are better options for new biotechnologically derived colorants. One recent development has been with the β-carotene from the fungus *Blakeslea trispora*. Carotenes are produced by fermentation in a reactor. This is currently being marketed as a natural food colour by DSM Gist Brocades Delft, Heerlen, Netherlands.

If the biotechnology method can be worked out/ standardized for large scale preparation of colorants, it would be a major breakthrough in terms of cost effectiveness and also avoiding the usage of toxic solvents for extraction.

Future outlook

Application of appropriate colour to food is very challenging and wrong selection of colour can lead to lot of problems viz., lack of consumer appeal and potential new product failure. Colour suppliers will have a challenge of providing a variety of cost effective food colours with good stability, easy and uniform dispersion in food matrix, which can come with a good effective technology for the production of colours. In this direction, new technologies are welcome with improved benefits over existing technologies.

Conclusion

Emerging technologies such as high hydrostatic pressure, pulsed electric fields and sonication could be potential methods for the enhanced extraction of pigments and bioactive compounds from plant materials in future. Still, all the above mentioned newer technologies cannot compete with the conventional method of extraction at an industrial level as these techniques require standardization in terms of huge quantity of raw material required to be handled, equipment at industrial scale application and also evaluation of the end product obtained by these techniques in comparison with the product obtained by conventional method. Though reports on application of newer technologies for the production of natural colorants, preparation of colour formulations by nano technology and application of the same for finer distribution of colour in food matrix are available in literature, the scale of operation is only on academic scale, which is appreciable in terms of innovations and as future technologies, but requires a lot of data generation on large scale applications. Due to the ever increasing cost of energy, it can be easily anticipated that athermal membrane processes could be the technology of the future in the food and allied industries.

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