A REVIEW ON THE EXTRACTION METHODS OF FOOD COLOURS FROM NATURAL SOURCES

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Abstract
Colour plays a crucial role in the food production and processing sector as it contributes to the sensory attribute of food. It plays a major role in determining the freshness, nutritional value, safety, and aesthetic value of a food. Thus, in this way it directly affects the market value of the coloured food product. Natural colours are primarily derived from plants, insects, mineral ores or microbial sources. Pigments such as flavonoids, carotenoids, betanines, and chlorophyll are mainly applied to give colour to the products which do not have it, or have lost it in the course of processing. Research on natural food colours and their extraction techniques have become a key area in the food industry, particularly the discovery of new natural colorants. It is because natural colours are assumed safe compared to synthetic colours as they are non-allergic, non-toxic, non-carcinogenic, and biodegradable. Pigment extraction is one of the most important procedures in the manufacturing of natural colourants. It can be divided into conventional and non-conventional methods. Conventional methods include Soxhlet extraction, maceration, and hydrodistillation. With recent advancement in technology newer and more efficient methods have emerged such a ssupercritical fluid extraction, pressurized liquid extraction, microwave-assisted extraction and ultrasound-assisted extraction which not only extract pigments faster and effectively but are also environmentally friendly. This review article focuses on various methods utilised for extraction of these natural colour pigments.

Keywords: Conventional extraction methods, non-conventional extraction methods, natural colour pigments, food dyes, food additives.

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1. INTRODUCTION

Colour is one of the principle organoleptic properties in foods. It not only makes the food more appealing to the consumers but it also acts as a good indicator of the quality, safety and freshness of the food product. Thus, colour gives the first impression of the food product and influences customers' decision to buy the food product. However most of the natural colours present in various foods are lost in processing or there is formation of undesirable colour in food production. Sometimes, certain food items do not naturally have a bright colour. As a result, the majority of consumers dislike such items and choose not to consume them, which has a negative impact on their sales. Therefore, to increase the sales of various food products, food and beverage industries heavily rely on colour additives to enhance the colour of the food product, make it more attractive and provide uniformity to their products.

Food industries majorly depend on artificial colours derived from mineral or synthetic processes which imparts a vibrant colour to the food product, possess diverse hue and is very stable in different processing conditions. However, usage of artificial colours has been reported to be associated with various adverse health risks. It has been seen in various studies that regular consumption of products containing synthetic colour additives have increased creatinine, alkaline phosphatase, aspartate aminotransferase levels and oxidation of fatty acids. It has also shown to reduce endogenous antioxidant enzymes and causing acute inflammation and allergies. Colour dyes like Red 40, Yellow 5, and 6 contain benzidine which is a human and animal carcinogen. Although at low amounts they do not pose high cancer risk but continuous and regular intake may raise the risk of cancer. Thus, excess consumption of these synthetic colours can lead to cancer, renal and hepatic disorders (Okafor, et al., 2016&Potera, 2010).Thus, to prevent these untoward risks, consumers prefer natural colours which not only provide a brilliant colour to their foods but also do not pose any untoward health risk.

2. EXTRACTION OF NATURAL PIGMENTS

Natural food colours are derived from the various parts of plants such as leaves, flowers, barks, fruits, seeds etc. and from animal sources too. These colour components present in the plant and animal source is not present as a single chemical entity but occurs in a complex amalgamation with other non-colouring constituents such as water-insoluble fibres, colloids, protein, fat etc. Thus, to obtain the pure natural colorant or dye extract, the pigment needs to be obtained from the crude animal or plant matrix. (Mansour, et al., 2019). Therefore, to obtain the pure dye pigments by separating them from other non-dye constituents, various extraction methods are employed. Currently, technicians have different methods at their disposal to select from depending on the convenience, cost and yield from the procedure and pigment's nature and solubility characteristics. These extraction methods can be divided into conventional and non-conventional extraction methods.

2.1 Conventional Extraction Methods

Conventional methods, such as Soxhlet extraction, maceration and hydro distillation, are simple, inexpensive and easy to handle procedures (Azmir, et al., 2013).These techniques have been extensively employed to extract essential oils, bioactive chemicals, and natural pigments from a diverse range of plant materials (Ngamwonglumlert, et al., 2017 & Nawaz, 2019). The following methods have been discussed below:

1. Soxhlet extraction: Soxhlet extraction method is named after the German agricultural chemist, Franz Ritter von Soxhlet. This method was initially designed for lipid extraction but lately it also finds its application in extracting colour pigments. It is a well-established conventional extraction technique which surpasses the performance of other conventional methods except in the extraction of thermo labile components. Soxhlet apparatus contains a specialized glass refluxing unit mainly used for organic solvent extractions. In this method, the powdered solid material is placed in a thimble made up of filter paper and is placed inside the Soxhlet apparatus. The apparatus is attached to a round bottomed flask containing the solvent and to a reflux condenser. The solvent in the round bottomed flask is boiled gently, the vapor passes up through the side tube, condensed by the condenser and falls into the thimble containing the material and slowly fills the Soxhlet. When the solvent reaches the top of the connected tube, it syphons over into the flask, removing the extracted portion of the material. The operation is repeated until complete extraction is achieved (Rasul, 2018).

This method can be utilised for the extraction of β-carotene from carrots using solvent mixture containing hexane, acetone and ethanol at a temperature of 58^{o}C for 3 hours. (Ngamwonglumlert, et al., 2017)

The Soxhlet extraction method is an automatic continuous method which integrates the advantages of the reflux extraction and percolation. This method utilizes the principle of reflux and siphoning to continuously extract the dye constituent with fresh solvent. The advantage of Soxhlet extraction method is that it has a very high extraction efficiency which requires less time and solvent consumption than other conventional methods like maceration (Zhang, et al., 2018). Moreover, this method does not require filtration after extraction (Rasul, 2018).However it is not suited to extract heat sensitive pigments as this process involves high temperature and long extraction time which increases the risk of thermal degradation of the pigments (Zhang, et al., 2018). Other disadvantages of this method include lengthy extraction time and it is also a very labour intensive procedure (Rasul, 2018).

2. Maceration: Maceration extraction procedure is one of the oldest and simplest conventional extraction methods. It is mainly employed for solid–liquid extraction procedures. Maceration involves three principal steps. Firstly, the plant materials are grinded into very small particles to form powder. This increases the surface area of the particles which enables them to be properly mixed with the solvent. In the second step, the solvent also known as menstruum is added to it in a closed vessel. In the next step, the liquid is strained off but the solid residue of this extraction process is pressed to recover large amount of occluded solutions. After straining, the resultant strained and the pressed out liquid solution is mixed and separated from impurities by filtration. The sample is subjected to occasional shaking to facilitate extraction by two ways by increasing diffusion and it also removes concentrated solution from the sample surface by bringing new solvent to the menstruum for more extraction yield (Azmir, et al., 2013&Rasul, 2018).

This method can be used to extract β-carotenes from red fruit (*Pandanus conoideus*) using acetone at 25^{o}C for 24 hours. It was covered in aluminium foil and kept in a dark condition (Purnomo et al., 2020).

The advantages of this method include that it is a very simple method which can be used for the extraction of thermo labile pigments. This process can be done using very simple and inexpensive equipment which does not require highly skilled labour. It is an energy saving process. Moreover, it is ideal for substances which are very less soluble in solvents and requires prolonged contact with the solvent for proper extraction of the dye pigment (Rasul, 2018; Zhang, et al., 2018).The major disadvantages of maceration extraction method include that it is a very time consuming procedure and the duration of extraction of dye pigments may sometimes extend to a couple of weeks. This method also has low extraction efficiency and a large amount of solvent is required for extraction (Rasul, 2018; Zhang, et al., 2018).

3. Aqueous Extraction: Aqueous extraction is another popular conventional method which is used to extract dyes from plants and other materials. In this method, the pigment containing material is initially broken into small pieces or powdered and sieved to improve extraction efficiency. It is then steeped in water in earthen, wooden, or metal vessels (ideally vessels made from copper or stainless steel) for an extended period of time, generally overnight, to loosen the cell structure before it is boiled to extract the dye solution, which is then filtered to remove non-dye plant residues. The process of boiling and filtering is repeated to remove as much dye as possible. During large scale extraction procedures stainless steel vessels are used and the time of soaking the materials in water may be reduced by boiling the solution for an extended time period to obtain highly purified dyes. Generally, centrifugation is used to separate residual matter. Trickling filters are used to ensure removal of fine plant material particles and ensure better solubility of the purified natural dye. (Mansour, et al., 2019).

This method can be utilised to obtain anthocyanin from jamun fruit using distilled water as a solvent at a temperature between 40-60^{o}C for 20-100 minutes. (Maran, et al., 2015a).

The advantages of this method are that cost of equipment is very less and it does not require technical labour. The disadvantages of this extraction procedure include a long duration of extraction, a considerable amount of water requirement, the usage of high temperatures, and a limited dye yield because only water-soluble colour components are removed, but many dyes are insoluble in water. Furthermore, along with the dye, other water-soluble substances such as sugars also get extracted. This has to be removed if the extract is to be concentrated and converted to a powder form. Yield of heat-sensitive dye substances such as betanin gets reduced at boiling temperature;

therefore a lower temperature should be used for extraction in such instances (Mansour, et al., 2019).
4. Hydro distillation: Hydro distillation is another traditional method for extraction of dye pigments. Moreover, this method does not utilise organic solvents as water or steam acts as the extraction medium. It is usually done before dehydration of plant materials. In hydro distillation, the plant materials are packed in a still compartment; in which sufficient amounts of water is added to it. After that the mixture is boiled. Alternatively, direct steam can also be injected into the plant sample. Hot water and steam act as the main influential factors which help in the extraction or separation of the dye pigments from the plant matrix. Water condenses the vapour mixture of water and oil during indirect cooling. Condensed mixture flows from condenser to a separator, where oil and pigments separate automatically from the water. Hydro distillation involves three main physicochemical processes i.e. hydro diffusion, hydrolysis and decomposition by heat. (Azmir, et al., 2013, Ngamwonglumlert, et. al, 2017)
This extraction procedure can be divided into three types: water distillation, water and steam distillation and direct steam distillation.
a. Water Distillation: In this process, the material is totally immersed in water, which is then boiled by using direct fire, steam jacket, closed steam jacket, and closed steam coil to apply heat.(Rasul, 2018).
b. Water and Steam Distillation: In water and steam distillation method, steam can be generated either in a satellite boiler or within the sill, although separated from the plant material. Moreover, it does not require extra capital expenditure than water distillation. Also, the equipment used is generally similar to that used in water distillation, but the plant material is supported above the boiling water on a perforated grid. In fact, it is common that water distillation extraction method eventually progresses to water and steam distillation (Rasul, 2018).
c. Direct Steam Distillation: In this process the plant material is distilled with steam generated in a steam generator generally referred to as a boiler. Similar to water and steam distillation, the plant material is supported on a perforated grid above the steam inlet. A real advantage of satellite steam generation is that the amount of steam can be readily controlled. As steam is generated in a satellite boiler, the plant material is heated at a temperature just below 100° C so that the sample does not undergo thermal degradation (Rasul, 2018).
The main advantage of hydro distillation method includes usage of cheap and less toxic solvent i.e. water. However, the main disadvantage of this method is that the utilisation of high extraction temperature, about 100°C can lead to loss of certain volatile and heat sensitive components. This drawback limits its use in extraction of thermo labile compounds (Ngamwonglumlert, et al., 2017; Rasul, 2018).
This method is used for the extraction of curcuminoids from turmeric. However, generally, it is used at laboratory level to screen the quality of the raw material. Hydrodistillation is not commonly practiced in industries because of the long distillation time and the resulting mass after hydrodistillation is not easily amenable for future use (Manzan et al., 2003).
5. Solvent Extraction: Natural colouring matter, depending upon their nature can also be extracted by using organic solvents such as acetone, petroleum ether, chloroform, ethanol, methanol, or a mixture of solvents such as mixture of ethanol and methanol, mixture of water with alcohol, etc. The water/alcohol extraction method is able to extract both water-soluble and water-insoluble substances from the plant sources. The extraction yield is thus higher as compared to the aqueous method as larger number of chemicals and colouring matter can be extracted. Acid or alkali can also be added to alcoholic solvents to facilitate hydrolysis of glycosides and release of colouring matter. Purification of extracted colour is easier as solvents can be easily removed by distillation and reused (Mansour, et al., 2018).

This method can be utilised to extract anthocyanin from berries using methanol, trifluoroacetic acid and water. It was sonicated for 5 minutes. After that it was centrifuged at 13,000rpm at 5^{o}C for 10 minutes (Sommer, et al., 2018).

Advantages of this method are that it can be used to extract heat sensitive pigments as this extraction technique is performed at a lower temperature thus chance of degradation of the pigment is few. The disadvantages of the method are the presence of toxic residual solvents which contribute to greenhouse effect leading to global warming. Another disadvantage of this method is that the extracted material is not readily soluble in water and the subsequent dyeing process has to be carried out in an aqueous medium (Mansour, et al., 2018).

2.2 Non-Conventional Methods

Although conventional extraction methods have many advantages, these methods require large amount of solvent, long extraction time, and may lead to significant pigment degradation. Most of the conventional methods heavily rely on the use of organic solvents such as acetone, petroleum ether, chloroform etc. for the extraction of pigments. These solvents are considered toxic and may increase the risk of human health and environmental hazards (Naidu & Sowbhagya, 2012). Thus, non-conventional methods or green extraction methods are considered better alternatives to conventional methods as they have been proposed to alleviate limitations posed by conventional extraction procedures (Cheok et al., 2014). Some of the non-conventional extraction methods are discussed below:

1. Supercritical fluid extraction: The supercritical phenomenon of fluids was first discovered by the Frenchman Baron Charles Cagniard de la Tour. (Phelps et al., 1996). This extraction method is one of the new emerging techniques for extraction and purification of dye pigments. This method utilises supercritical fluid for extraction of dye pigments. A gas functions as a supercritical fluid beyond its critical values of temperature and pressure. This type of a fluid has physical properties that lie between those of a liquid and a gas. Supercritical fluid possesses gas-like properties of diffusion, viscosity, and surface tension, and liquid-like properties of density and solvation power. These physical properties allow them to spread out along a surface more easily when compared to a true liquid because they have much lower surface tension than liquids. Due to their low viscosity, they have an excellent diffusivity and consequently have a better interaction with the substrate. Moreover, a supercritical fluid may dissolve many substances like liquids because a substance's solubility in any solvent increases with pressure and temperature, and such parameters are required to keep a gas in the supercritical state. Thus, these properties make it suitable for extracting compounds in a short time with higher yields (Mansour, et. al., 2019; Azmir,et. al., 2013).

This method can be used to extract carotenes from pumpkin using carbon dioxide as the solvent at a temperature of 70^{o}C, pressure 35MPa and at a flow rate of 1.5ml/min for 40 minutes.(Shi et al., 2010)

A supercritical fluid extraction system includes the following components: a tank of mobile phase, typically CO_2, a pump to pressurise the gas, a co-solvent vessel and pump, an oven containing the extraction vessel, a controller to maintain a high pressure inside the system, and a trapping vessel. Various types of metres, such as flow metres and dry/wet gas metres, are typically fitted to the system. Supercritical fluid extraction using carbon dioxide (CO_2) is a good alternative to solvent extraction as it is nontoxic, cheap, easily available, and does not leave residues. Critical temperature and pressure values for carbon dioxide are 31.4^{o}C and 1,070 pounds per square inch (psi) or 73.8 bars, respectively. Supercritical extractions using CO_2 typically operate at temperatures between 32 and 49^{o}C and pressures between 1,070 and 3,500 psi. As CO_2 is a nonpolar molecule it acts as a nonpolar organic solvent and helps in the extraction of nonpolar compounds. For the extraction of slightly polar

solutes, a cosolvent or a modifier may be added to improve the solubility of polar solutes (Mansour, et. al., 2019; Azmir, et. al., 2013).
The advantage of the process is that the extract is free from residual solvent traces and heavy metals and is light coloured due to the absence of polar polymerizing substances. Moreover good solvation of non-polar analytes and low toxicity of CO_2 which can be easily evaporated make this technique more advantageous. Hence this process has gained popularity in extraction of purified natural products for food and pharmaceutical applications. The disadvantage of the method is the high cost of the equipment and poor extraction of polar substances (Mansoor, et. al., 2019; Tzanova, et. al., 2020).
2. Pressurised liquid extraction method: Pressurized liquid extraction method is also known as pressurized fluid extraction, accelerated fluid extraction, enhanced solvent extraction and high pressure solvent extraction. This method was developed by Dionex which was owned by Thermo Fisher Scientific. This method utilizes a liquid solvent at elevated pressure (10.3–13.8 MPa) and temperature (40–200^{o}C) for extraction. It mainly involves the application of high pressure and high temperature which facilitates faster extraction. High pressure keeps solvents in a liquid state above their boiling point resulting in a high solubility and high diffusion rate of lipid solutes in the solvent, and a high penetration of the solvent in the matrix. Thus, the high pressure forces the solvent into matrix pores and hence allows better contact between the solvent and compounds to be extracted. High temperature results in better diffusion of solvent into sample matrix and also helps disrupt plant cells, resulting in a more effective release of pigments from the cells, and hence more effective extraction. The higher extraction temperature can promote higher analyte solubility by increasing both solubility and mass transfer rate. It also decreases the viscosity and surface tension of solvents, thereby improving extraction rate (Ngamwonglumlert, 2017, Zhang, 2018).
Anthocyanins from blackberry can be extracted using this method at a temperature of 100^{o}C, presure of 75 bar, using ethanol at a flow rate of 3.35 mL/min for 30 minutes (Machado et al., 2015).
The main advantage of pressurized liquid extraction technique is that it is an automated technique that requires less extraction time and consumes less solvent compared to other extraction techniques. It can also extract both water- and oil based pigments, depending on the selection of an extraction solvent. However, the main disadvantage of this method is that it cannot effectively extract heat-sensitive pigments since the method involves the use of high temperature (Ngamwonglumlert, et al., 2017, Tzanova, et al., 2020).
3. Ohmic Heating Assisted Extraction: Ohmic heating also known as electroconductive heating, utilizes the inherent electrical resistance of food materials to generate heat. James Prescott Joule initially discovered that the transfer of electric current produces heat. Hence, ohmic heating is also known as Joule heating. Most food materials contain ionic constituents, such as salts and acids, allowing the conduction of electrical current. This process can be used to generate heat within the product, transforming the electrical energy into thermal energy and thereby extracting the pigments from the plant matrix. Furthermore, it does not require additional heating medium or surface for heating materials at exceptionally rapid rates. (Loypimai, et al., 2015, Nawaz, 2019).
Purple anthocyanin pigments from black rice bran can be obtained using ohmic heating assisted extraction method (Loypimai, et al., 2015).
Ohmic heating is more advantageous than other conventional methods as it reduces thermal damage of heat sensitive substances (Nawaz, 2019).
4. Ultrasound Assisted Extraction Method: The origins of ultrasounds used for extraction can be traced back to 1998, when the team of Luque-Garcia and de Castro created the first Ultrasound assisted Soxhlet extractor. With

time different innovative extraction techniques were developed using ultrasound. (Khadhraoui et al., 2021). Thus, ultrasound assisted extraction method is one of the most recent and advanced method and it is also known as sonication. This method utilises ultrasound waves, which are electromagnetic waves that have a frequency ranging from 20 kHz to 100 MHz. Ultrasound waves pass through a medium by creating compression and expansion. This process causes a phenomenon known as cavitation, which refers to the formation, growth, and collapse of bubbles. Cavitation accelerates the dissolution and diffusion of cell ingredients. At sufficiently high strength, the ultrasonic wave propagates through the medium's molecules, forming cavitation bubbles.The disintegration of these bubbles generates energy, and the jets of solvent towards herbal particles extract the target compounds from them more efficiently (Chemat, et al., 2017).

Betacyanin and betaxanthin from *Bougainvillea glabra* flower can be extracted using this method. In this process methanol is used as solvent. It is subjected to ultrasound waves at 20 kHz frequency and 88W power for 37 minutes (Maran et al., 2015b).

The application of ultrasound assisted extraction method is very advantageous as it results in better extract yields, utilises low solvent and less energy consumption and can extract efficiently in low extraction temperatures and less time when compared to other conventional extraction methods (Ranjha et al., 2021).

5. Microwave Assisted Extraction Method: Microwave-assisted extraction method is another emerging novel technique used for extracting both water- and oil-based plant pigments. This technique was invented by J.R. Jocelyn Parè, Michel Sigouin and Jacques Lapointe (Pare et. al., 1991). Microwaves are electromagnetic fields that operate at frequencies ranging from 300 MHz to 300 GHz. They are made up of two perpendicular oscillating fields, i.e., the electric and magnetic fields. This technique utilises microwaves which generate heat by interacting with polar compounds such as water and some organic components in the plant matrix. Microwave radiation works on the dipoles of the polar and polarizable materials causing heating via ionic conduction and dipole rotation mechanisms. This rapid heating by microwave radiation results in an expansion of plant cell structure with subsequent rupture of plant cell walls. Thus, compounds, including pigments, can easily migrate out of cells into the solvent medium, resulting in an enhanced extraction rate (Destandau et al., 2013). Moreover, in this technique there is transfer of heat and mass, in the same direction which generates a synergistic effect to accelerate extraction and improve extraction yield. (Zhang, et al., 2018).

Anthocyanin from purple corn can be extracted using this method. In this process ethanol and hydrochloric acid is used. It is subjected to microwaves at 555W power for 19 minutes (Yang & Zhai, 2010).

This method is very advantageous as it utilises less time and solvent, reduces extraction temperatures but provides a better yield when compared with other conventional extraction methods (Zhang, et al., 2018; Tzanova, et al., 2020).

6. Enzyme Assisted Extraction Method: Enzyme-assisted extraction is a non-conventional method which employs the use of enzymes to enhance the extraction of bioactive compounds, including pigments, from a plant material. Enzymes, such as pectinase, cellulase, and hemicellulase, are used to hydrolyze plant cellulosic cell walls resulting in easier release of cellular constituents, which help in the extraction of plant pigments. These enzymes are also used in certain organic reactions such as cleavage of ester bonds, separation of racemic mixtures to obtain a single optically active compound (Puri, et al., 2012, Sowbhagya, et al., 2010).

Enzymes have been used in the food processing industries since the dawn of civilization. It has also found its application in extraction of food colour pigments. The following are some of the earliest documented uses of enzymes in the extraction of colour pigments. In 1979, Mandzhukov and Velichkov reported that treating pulped Cabernet sauvignon grapes with the commercial enzyme preparation Irgazyme M-10 resulted in effective extraction of anthocyanins and leucoanthocyanins (Mandzhukov, et al., 1979).Nippon-Terpeien patented a method for obtaining high quality colour concentrate from paprika oleoresin in 1987-88 by pretreating it with lipase enzyme.(Nippon-Terpeien, 1988).In 1993, Saito developed a procedure in which pre-treatment of safflower with β-glucosidase for efficient extraction of colour pigment precarthamine (Saito, 1993). In 1997, Nicolini and Mattivi reported that addition of pectinase enzymes increased and accelerated the extraction of anthocyanins and tannins from grapes (Nicolini, et al., 1997)

This method is very advantageous as it is non-thermal in nature and hence can be used to extract heat sensitive pigments and consumes little to moderate amounts of solvents. However the disadvantages of this method are that it takes longer time for extraction of compounds and filtration is required (Sowbhagya, et al., 2010).

7. Membrane technologies: Membrane-based technologies, such as microfiltration, ultrafiltration, and nanofiltration, are valid alternatives for efficient extraction and purification of pigments from natural sources. The membrane separation technology uses a selective permeable membrane as a separation medium and is driven by a certain driving force so that a solution containing an active component or a mixed gas is cross flowed through the medium thereby achieving the purpose of separation and purification. The ultrafiltration membrane has a pore size ranging from 1000 Da to 50nm, so that macromolecule impurities such as protein tannin, cellulose, colloid and other similar impurities in the pigment extract can be retained and the colourant may be separated and purified. The nanofiltration membrane has a pour size ranging from 150Da to 1000 Da which effectively retains the macromolecules of the pigment and thus concentrates the pigment. This method is very advantageous as it produces a higher yield in a very short extraction time (Castro-Muñoz, 2020).

Extraction of anthocyanin from cranberry can be done using acidified alcohol for extraction and ultrafiltration was done for purification. Another method for extraction of anthocyanin from cranberry is by using nanofilters with $0.36m^2$ filtration area. The juice feed flow rate was 1to 12L min^{-1} at transmembrane processes were between 20-40 bar (Ah-Hen et al., 2017).

3. NATURAL COLOUR PIGMENTS

The aforementioned extraction methods are used for extraction of natural food colour pigments from plants and animal sources. The following tables lists down various natural colour pigments, their sources and application in the food industry.

Table 1. Sources, composition, colour and application of natural colourants for food

Sources	Color	Chemical Constituent	Application Uses
ANTHOCYANINS			
Black Currant	Purple black	Anthocyanin (cyanidin, delhinindin)	Beverages, confectionaries, products prepared from fruits, soft drinks, preserves.
Black Grapes	Red	Anthocyanin	Confectionary, red wine, and dessert products.
Grape	Green	Anthocyanin (malvidin, peonidin, delphinidin, cyanidin, pelargonidin)	Confectionary, dessert products, sorbets, sauces and all fruit based beverages.
Raspberries	Red and purple	Anthocyanin (cyanidin)	Confectionary and dessert products.
Blueberries	Blue	Anthocyanin (malvidin, peonidin, delphinidin, cyanidin, pelargonidin)	Confectionary, dessert products and folk medicines.
Aronia or Chokeberry	Bright red orange	Anthocyanins and carotenes	Jelly, candies, pies, cookie fillings, yoghurt, sorbet and flavored milk.
Strawberries	Red	Anthocyanin (pelargonidin, cyanidin)	Confectionary, juices, sorbets and dessert

Sources	Color	Chemical Constituent	Application Uses
			products.
Hibiscus	Bright red and purple or bluish red	Anthocyanins	Bakery products, tea based products to enhance the brown tint.
Cacao	Brown	Anthocyanin (cyanidin monoglucoside cyanidin arabinose glucoside)	Candies, cake, chocolate, biscuits and dairy products.
Black sesame seeds	Black	Anthocyanin and flavone	Marination of meats, breads, ice cream, mocha or rice cake, Indian desserts such as ladoo and chikki.
Cornflower	Dark blue to purplish	Anthocyanin and flavone	Sugar confectionaries, tea, medicines, cornbread muffins.
CAROTENOIDS			
Carrots	Orange	β carotene	Dietary supplements, margarine, sorbet, chutneys, beverages, confectionaries and sauces.
Annatto seeds	Yellowish-orange	Norbixin, Bixin	Cheese, ice cream, butter, margarines, and low-fat spreads, breadcrumbs, flour confectionery, cream fillings in biscuits and other snack foods, custard powder, citrus juices, concentrates and candies.
Saffron	Yellow and orange	Crocin and crocetin	Milk desserts, rice preparations like Spanish paella, Scandinavian baked products, Indian biryani, candies, dietary supplements, medicines and meat products.
Paprika and Red Peppers	Red	Oleoresin, cryprtoxanthin, capsorbin, capsanthin	Soups, sausage, cheese, snacks, salad dressings, sauces, pizza, beverages and medicines.
Tomato	Reddish orange	Lycopene	Non-alcoholic flavored drinks, candied fruits and vegetables, preserves of red fruits, confectionery, decorations and coatings, viennoiserie, biscuits, cakes and wafers, edible ices, flavored processed cheese, desserts including flavored milk products, sauces, seasonings, pickles, relishes, chutney and piccalilli, jam, jellies, marmalades, fish paste, crustacean paste, pre-cooked crustaceans, extruded or expanded savory snack products and coated nuts, edible casings, soups, meat and fish analogues based on vegetable proteins, spirituous beverages, aromatized wines and other alcoholic beverages, aromatized fruit wines, cider and perry.
BETALAINS			
Beet root	Red and red-violet	Betanines (betanidin, betacyanin and betaxanthin)	Frozen foods, low temperature dairy products, yoghurts, ice cream and short shelf life food products.
Opuntia	Blush red or violet red	Betalains (betacyanins)	Beverages, frozen foods, fruit fillings, candies, baked products, condiments sauces, fillings, chewing gum, medicinal products, soyabean product and candies.
CURCUMINOIDS			
Turmeric	Yellowish orange	Curcumin	Dairy products, frozen desserts, beverages, cereal, pickles, sausages, confectionaries, ice cream, bakery, savory products, mustards and meal concentrates.

Sources	Color	Chemical Constituent	Application Uses
CHLOROPHYLL			
Alfa alfa grass	Green	Chlorophyll or chlorophyllin	Beverages, sweets, chewing gums, soup concentrates, cheese spreads, preserves and vegetable pickles.
Peppermint	Green	Chlorophyll	Beverages, sweets, chewing gums soup concentrates, cheese spreads, preserves and vegetable pickles.
Stinging Nettle	Green or Greenish yellow	Chlorophyll, chlorophyllin xanthophyll and carotene	Ice cream, delicatessen, baked goods and fruit preparations such as nettle soup and cheese.
Red Cabbage	Reddish purple	Chlorophyll and anthocyanin	Chewing gum, vegetable juice and beverages.
CARAMEL			
Caramel	Brown	Melanoidins	Multi-grain bread, breakfast cereal, cookies, biscuits, ice cream cones, muffin, chocolate, nutrition bar and rice cake.
OTHERS			
Cochineal (*Dactylopius coccus)*	Dark Red	Caraminic acid / Carmine	Milk desserts (ice creams and yoghurts), candies, alcoholic and soft drinks, jams, tomato preserves, confectionary and bakery products.
Genipap fruit	Blue	Iridods	Candy, ice-cream, condiments, beverages, chocolates, gums, jellies, toppings and cakes
Cephalopod Ink	Black	Melanin	Pasta and sauces.
(Azeez, 2007), (Verma, 2018), (Chaitaniya, 2014), (Mohamed, 2019), (Janiszewska-Turak, 2016), (Qaisar, 2019), (Sengar, 2014), (Nathia-Neves, 2018).			

3.1 Case Studies of Extraction of Colourants from Cochineal Insects

Carminic acid present in cochineal insects yields a red coloured colorant. It is used to colour dairy products like Cheddar cheese, ice cream and yoghurt, candies, alcoholic drinks, juice beverages, soft drinks, jams, tomato preserves, meat and meat products, sausages, processed poultry products, surimi, marinades, confectionary and bakery products, cookies, desserts, icings, pie fillings, jams, preserves, gelatine desserts and sauces (Chaitaniya, et al., 2014; Ranaweera, et al., 2020).

Carminic acid can be extracted from cochineal insects using various conventional methods such as solvent extraction and nonconventional methods such as supercritical fluid extraction and pressurised liquid extraction methods.

Solvent extraction was done to extract the dye from cochineal insects. The dried insects were finely grounded in a ceramic mortar and mixed with methanol solvent. The mixture was homogenised and the pigments present in the sample were extracted for 30 minutes in a water bath at several temperatures in a sealed vessel. The sample was cooled at 4^{o}C and centrifuged at 3000 rpm for 5 minutes to separate the colour pigment from the cochineal remains (Borges et al., 2012).

Supercritical fluid extraction was used to extract carminic acid from cochineal insect. This method uses carbon dioxide for extraction of the colour pigments from cochineal insect. Grounded cochineal insect and sea sand was used. Sea sand was added to efficiently disperse the sample. The extraction pressure in this procedure was between 150 and 300 bar and temperature was 40^{o}C. The time taken for extraction was 4 hours (Borges et al., 2012).

Extractions of carminic acid were performed using a pressurised liquid extractor. The cochineal insect was taken in a pressurised fluid extraction cell. Then ethanol was filled in it. The extraction was performed at a temperature of 200^{o}C and pressure of 10.5MPa for 30 minutes. After that, the cell was rinsed with the solvent, it was purged from the cell and depressurised. To minimise the loss of volatiles and to avoid sample degradation, the extracts were quickly cooled to freezing temperatures by placing the vials in a water-ice bath (Borges et al., 2012).

3.2Case Studies of Extraction of Colourants from Grapes

Anthocyanins present in grapes acts as a colouring agent. It is used to colour confectionary products, dessert products, red wine, kefir, sorbets, jams, jellies, preserves, sauces, dietary supplements and all fruit based beverages. (Verma, et al., 2018; Azeez, et al., 2007). Anthocyanin present in foods is used in traditional and herbal medicines as they possess antidiabetic, anticancer, anti-inflammatory, antimicrobial properties and help in prevention of cardiovascular and neurodegenerative diseases. It is also used to make medicines that treat hypertension, pyrexia, liver disorders, dysentery, diarrhoea, urinary problems and common cold (Khoo, et al., 2017).

Various extraction methods can be used to obtain anthocyanin from grapes. The conventional extraction methods for extracting anthocyanins include maceration and the nonconventional methods include the microwave assisted extraction, ultrasound assisted extraction and pressurized liquid extraction technique.

Maceration is a conventional technique used for extraction of anthocyanin from grapes. In this method, ethanol was mixed with the grape sample. It was extracted at a temperature of 70^{o}C for 3 hours. (Corrales et al., 2008).

Anthocyanin from grapes can be extracted using microwave assisted extraction technique. In this method, grapes were mashed and put in a plastic tube. Ethanol was added to it and the tube was closed with a lid. The mixture was placed in a microwave having 700W output. It was microwaved for 2.5 minutes with short interruptions for stirring after every full minute and after completion. Extract was then decanted under low manual pressure and centrifuged at 4200 rpm for 10 min at 5°C (Sommer, et al., 2018).

Another, nonconventional extraction technique used to extract anthocyanin from grapes is the ultrasound assisted extraction technique. In this method again, grapes were mashed and put in a plastic tube. Ethanol was added to it and the tube was closed with a lid. The mixture was placed in a sonicator containing ultrasounds having a frequency of 40 kHz for 60 minutes while stirring the mash every 10 min. Extract was then decanted under low manual pressure and centrifuged at 4200 rpm for 10 min at 5°C (Sommer, et al., 2018).

Pressurised liquid extraction can also be used for extraction of anthocyanin from lyophilized red grape skins using acidified methanol as solvent. Extraction using pressurised liquid extraction technique was carried out at a temperature of 333.15K and pressure of 10.1 MPa for a time duration of 3 minutes (Tzanova, et al., 2020).

4. CONCLUSION

Since consumers are becoming more conscious of the dangers of taking synthetic dyes, natural dyes have proven to be a boon to food processors by serving as a viable alternative to synthetic colorants. Furthermore, with the development of non-conventional or green extraction technologies, natural pigment extraction can be completed in a matter of minutes with great reproducibility, minimising solvent usage, simplifying manipulation and work-up, and resulting in higher purity of the finished product. These extraction procedures maximise target compound recovery from a sample matrix while conserving the integrity of the molecules of interest and reducing coextraction of unwanted contaminants or undesirable compounds. In addition to being an efficient extraction process, as compared to conventional extraction technologies, they consume less fossil energy and need less waste water post-treatment. As a result, non-traditional extraction methods give outstanding extraction efficiency while also being ecologically friendly.

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