Research Article

Extraction Techniques and Utilization of Natural Pigments

Nikhet Rafiq*, Neeraj Gupta, Anju Bhat, Jagmohan Singh, Julie D Bandral, Monika Sood, Mehnaza Bashir and Nikhil Thakur

Division of Food Science and Technology, Chatha, SKUAST Jammu

Abstract

Recently, there has been a huge global demand for natural plant-derived pigments, which are enriched with antioxidant potentiality and can replace artificial pigments, especially in the food, pharmaceutical and cosmeceutical-based industries. Majority of the petroleum based or synthetic pigments are reported for their ill-effects on human health, which can directly induce hyperactivity and allergenicity in children and other sensitive people. Modern day health-conscious consumers are demanding for the plant derived natural pigments, especially in food applications, which has led researches to exploit the vegetal wastes to isolate bioactive natural pigments. Consumer demand for natural pigments with possible health advantages, combined with consumer awareness of the importance of purchasing safe food, has resulted in efforts to replace synthetic colours with naturally produced bioactive pigments from plant sources. Hence, on a global scale, food industries are copiously interested to bring in natural pigments in the food chain as a better alternative for synthetic colourants, which is expected not only to meet the consumer's demand for safe and green food but also to meet the market challenges in relevance to regulatory policies. Fruit and vegetable wastes and by-products obtained from food-processing industries are consists of seeds, peels and pomace.

The extraction of numerous bioactive components, including basic constituents such as polysaccharides, proteins and peptides, dietary fibres, and other plant secondary metabolites such as pigments, polyphenols, antioxidants, antimicrobials, and so on, can be greatly aided by efficient use of these which have possible applications in food, pharma and nutraceutical industries. It could be used in the food, pharmaceutical, and nutraceutical industries. Natural pigments, for example, have potential applications since they are natural (plant origin), safe (non-toxic), and effective antioxidants, as well as having the ability to improve the aesthetic appeal of processed food items. Carotenoids, one of the major pigments, are copiously present in the food industrial wastes, and can be explored as natural colourants in food, pharma and cosmetic applications.

Keywords: Carotenoids, Pigments, Antioxidant, Polyphenols, Polysaccharides, Antimicrobials, Polysaccharides

***Correspondence**

Author: Nikhet Rafiq

Email: nikhet70@gmail.com

Introduction

Pigments make nature colorful and likable. Most pigments are colored. In general, the visible colors are the emission of certain wavelengths of light by colored pigments after they selectively absorb others specific to their molecules. Absorbed light may be captured by a few capable pigments as energy to fuel plant photosynthesis and biochemical reactions. These colored pigments not only visually attract animals for flower pollination and seed dispersal but also function in critical biological processes for plants and play essential coevolutionary roles in ecosystems. The biological, ecological, and evolutionary importance of plant pigments and the derived colors cannot be overstated. On the other hand, many pigment-rich fruits and vegetables are critical in the human and animal diet. Some pigments are essential nutrients, and others may serve as nutraceuticals with additional medical benefits, including the prevention and treatment of certain diseases. There are many different plant pigments, and they are found in different classes of organic compounds. Plant pigments give colour to leaves, flowers, and fruits and are also important in controlling photosynthesis, growth, and development.

Types of Pigments

Water soluble pigments

These are also known as lipophobic pigments. These pigments are not membrane-bound molecules but are dissolved in the cell sap of epidermal cells of these parts.

Fat soluble pigments

These are also known as lipophilic pigments. These are not soluble in water but soluble in fat. These pigments are fat-soluble due to the presence of a long unsaturated hydrocarbon chain. Carotenoids are found in plants (gives colour to the leaves, flowers, etc.), algae (red algae for example), and bacteria (for example Staphylococcus aureus).

Anthocyanins

Anthocyanins belong to a large group of secondary plant metabolites collectively known as flavonoids, a subclass of the polyphenol family. They are a group of very efficient bioactive compounds that are widely distributed in plant food. Anthocyanins occur in all plant tissues, including leaves, stems, roots, flowers, and fruits Anthocyanin pigments are responsible for the attractive red to purple to blue colors of many fruits, vegetables, flowers, and cereal grains. In plants they serve as attractants for pollination and seed dispersal, give protection against the harmful effects of UV irradiation, and provide antiviral and antimicrobial activities. Anthocyanin-based colorants are commercially manufactured for food use from horticultural crops and from processing wastes. Anthocyanins act as antioxidants both in the foodstuffs in which they are found and in the organism after intake of these foods.

Betalains

Anthocyanin pigments are absent in some families and are replaced by highly different compounds, betacyanins and betaxanthins. These two pigments together are known as betalains. High ratio of betacyanin to betaxanthin lead to violet, medium to red and low to orange tuber colours. A somewhat yellow pigment is contributed by the betaxanthins. Perhaps the most prominent use of betalains is their application as natural food colorants, although their use is limited to certain food products due to their tendency to degrade upon exposure to high temperatures and light. Betalains are nitrogenous, water-soluble, red-violet and yellow pigments that form one of the major pigment classes providing striking colors to plant organs, alongside chlorophylls, anthocyanins (and other flavonoids), and carotenoids [1]. They serve as a basis for numerous dietary supplement products, and have also been explored for potential use in dye-sensitized solar cells and as textile dyes. In science, their color properties can be utilized as chemical biosensors, protein- labeling fluorophores, and markers for genetic transformation. Betalains are thought to be synthesized in the cytoplasm and endoplasmic reticulum, based on subcellular localization of their key biosynthetic enzymes [2-4]

Anthoxanthins

These are colourless or pale-yellow pigments closely related to anthocyanins depanding on the pH, anthocyanins give color to cauliflower, onion and spinach or each leafy vegetable. These pigments are generally whiter in an acid medium and yellowed in an alkaline medium. They are very susceptible to color changes with minerals and metal ions, similar to anthocyanins. As with all flavonoids, they exhibit antioxidant properties, and are important in nutrition and are sometimes used as food additives. Example: Quercetin.

Organic Acids

Vegetables contain a number of organic acids, metabolic products of the cells. Formic, succinic, citric, acetic, malic, fumaric, tartaric, and benzoic acids are present in fruits and vegetables. The concentration of acid is lower in vegetables than in fruits. Tomatoes and vegetables with the concentration of acid have a pH Ranging from 4 to 4.6. Foods like lemon, mango green, tamarind (tartaric acid), gooseberries, raw citrus fruits and grapes have low PH. Most of the vegetables have pH 5.0 to 5.6. Potatoes and peas have pH 6.1 to 6.3, more neutral in taste. Organic acids are naturally occurring compounds, present in many foods of plant origin, as well as produced during the fermentation of foods.

Polyphenols and Tannins

These include anthocyanins, flavones, flavanols and tannins. They are responsible for the astringent taste of some vegetables and for the astringent taste of some vegetables and for the discolouration of some vegetables after cutting, cooking or processing. Polyphenols have strong antioxidant and anti-inflammatory effects, both of which can be beneficial for cancer prevention. The most commonly occurring dietary polyphenols are flavonoids and phenolic acids. In plants, polyphenols are generally involved in defense against different types of stress. Prevention of disease by polyphenols is mainly due to their antioxidative properties, however, reversal of epigenetic changes can have strong effects as well.

Phycobilins

It is a class of hot water-soluble pigments. Phycobilin are an important group of pigments that through complementary chromatic adaptation optimize the light-harvesting process in phytoplankton cells, exhibiting great potential as cyanobacteria species biomarkers Phycobilins consist of two kinds of pigments. These two types of pigments are Phycocyanin and Phycoerythrin. Phycobilins are concerned with the harvesting of light energy. They transfer the solar energy to the chlorophyll molecule. They are unique among the photosynthetic pigments in that they are bonded to certain water-soluble proteins, known as phycobiliproteins. Phycobiliproteins then pass the light energy to chlorophylls for photosynthesis. there.

Fat soluble pigments

Chlorophyll

Along with some carotenes and xanthophylls. Two the green pigments of leaves and stem are usually held close to the cell wall in small bodies called chloroplasts chlorophylls have been isolated. Chlorophyll-a is intense blue green in colour and chlorophyll- b is dull yellow green in colour. They occur on 3a:1b ratio. It is mostly insoluble in water. These green pigment of leaf vegetables and other green-coloured vegetables like capsicum, beans, peas and chillies**.** Chlorophyll is found in virtually all photosynthetic organisms, including green plants, cyanobacteria, and algae. It absorbs energy from light; this energy is then used to convert carbon dioxide to carbohydrates.

Carotenoid

These are plant pigments responsible for bright red, yellow and orange in many fruits and vegetables. These pigments play an important role in plant health. People who eat foods containing carotenoids get protective health benefits as well. In plants, carotenoids are present as alpha, beta, gamma carotenoids, xanthophylls and cryptoxanthin. Beta-carotene is valuable in the synthesis of vitamin A. Foods: squash, carrots, grapefruit, oranges and apricots, yellow corn, tomatoes, red capsicum, green capsicum, carrots**.** They also provide photo-protection against excess light damage to the photosynthetic reaction center by quenching excited species such as singlet oxygen and free radicals or by other carotenoid enabled mechanisms.

Xanthophylls

Xanthophylls (originally Phyllanthus) are yellow pigments that occur widely in nature and form one of two major divisions of the carotenoid group; the other division is formed by the carotenes. Xanthophylls are found in all young leaves and in etiolated leaves. Examples of other rich sources include papaya, peaches, prunes, and squash, which contain lutein diesters. Dietary sources of xanthophylls include lutein and zeaxanthin in green leafy vegetables and corn, and β-cryptoxanthin in pumpkins, papayas, and peppers. Xanthophylls may be cancer preventative as scavengers of free radicals, quenchers of reactive oxygen species and reactive nitrogen species, and chain-breaking antioxidants.

Cryptoxanthin

Cryptoxanthin is a natural carotenoid pigment. It has been isolated from a variety of sources including the petals and flowers of plants in the genus Physalis, orange rind, papaya, egg yolk, butter, apples, and bovine blood serum.

Extraction

- Extraction is the method of removing active constituents from a solid or liquid by means of liquid solvent.
- The separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents.
- In this method the wanted components are dissolved by the use of selective

After the extraction unwanted matter is removed.

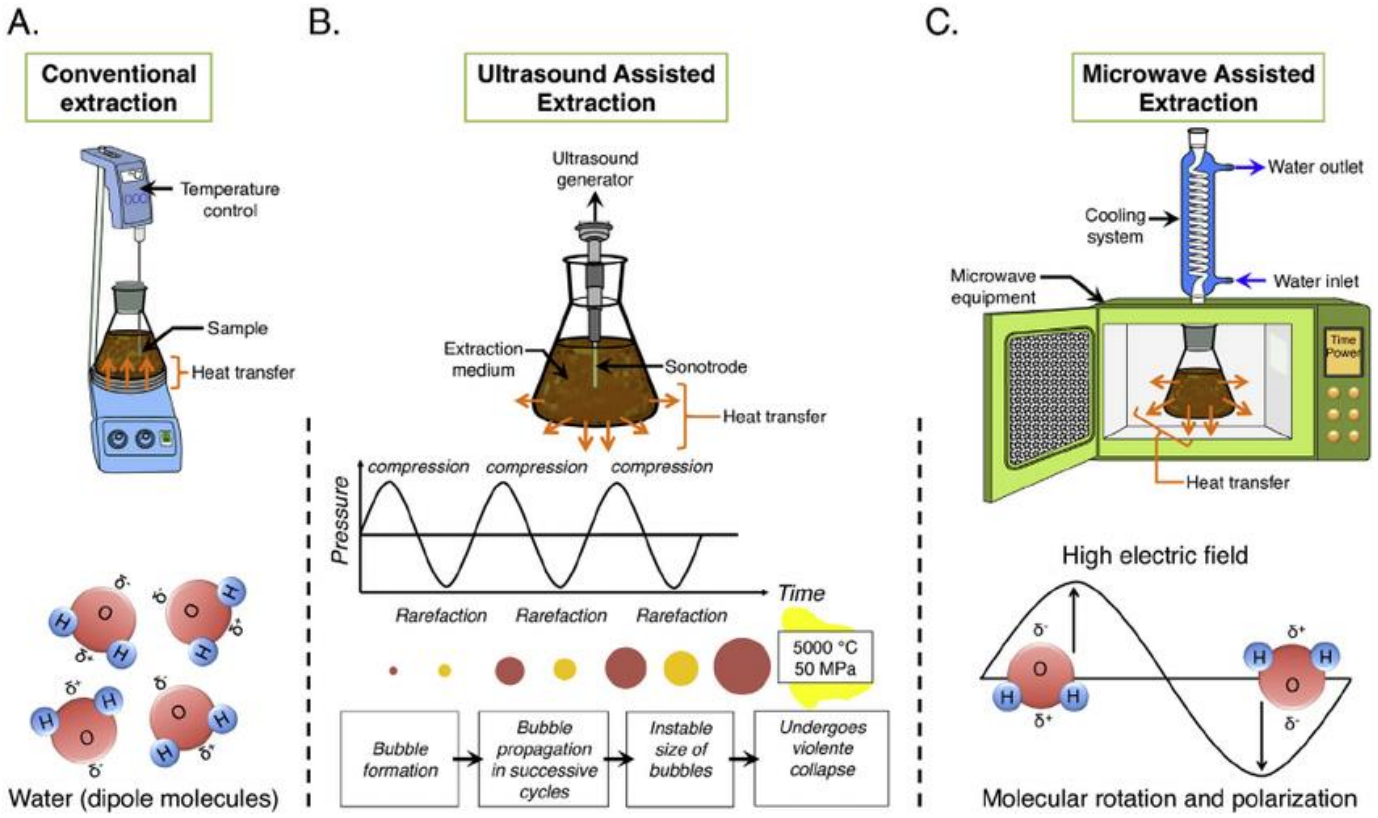
Extracts are prepared by using ethanol or other suitable solvent.

Extract: Extracts can be defined as preparations of crude drugs which contain all the constituents which are soluble in the solvent.
Marc: Solid residue obtain after extraction.

Menstruum: Solvent used for extraction

Extraction techniques

1. Conventional Extraction
2. Ultrasound assisted Extraction
3. Microwave Extraction
4. Enzyme Based Extraction



Conventional extraction

Conventional extraction technologies are used to recover value-added products from plant materials.

The conventional extraction methods, including:

1. Maceration
2. Percolation and
3. Reflux extraction

- These methods usually use organic solvents and require a large volume of solvents and long extraction time.
- All of the extraction techniques have advantages and drawbacks that should be carefully evaluated for each raw material, as the best extraction technique for one residue may not be the best solution for another.

Maceration

Maceration was a popular and inexpensive homemade technique for the preparation of tonic since a long time. In this process solid ingredients are placed in a stoppered container with the whole of the solvent and allowed to stand for a period of at least 3 days (3 - 7 days) with frequent agitation, until soluble matter is dissolved. The mixture is then strained (through sieves / nets), the marc pressed and the combined liquids clarified (cleaned by filtration) or by decantation, after standing. Moreover, this technique was used for the extraction of essential oils and active compounds from plant materials. Generally, the maceration procedure consists of multiple steps in extraction. The whole or coarsely powdered crude drug undergoes grinding to increase the surface area for proper mixing of powdered materials with the solvent. This process is done in a closed vessel where an appropriate solvent

(menstruum) is added. Next, the solvent is strained off followed by pressing the solid residue of the extraction process known as marc to recover an optimum amount of occluded solution. Both the obtained pressed out liquid and the strained solvent are mixed together and separated from unwanted materials by filtration. Frequent agitation during maceration facilitates extraction by two processes: (1) promotes diffusion, (2) separates concentrated solution from the sample surface by adding new solvent to the menstruum for increasing the extraction yield.

Percolation

- It is continuous downward displacement of the solvent through the bed of crude drug material to get extract.
- Most frequently used to extract active ingredients in the preparation of tinctures and fluid extracts.
- It is the method of short successive maceration or process of displacement.
- A percolator (a narrow, cone-shaped vessel open at both ends) is generally used.

Reflux extraction

Reflux extraction is more efficient than percolation or maceration and requires less extraction time and solvent. It cannot be used for the extraction of thermolabile natural products.

A mixture of reactants and solvent is placed in a suitable vessel, such as a round bottom flask. This vessel is connected to a water-cooled Liebig or Vigreux condenser, which is typically open to the atmosphere at the top.

The reaction vessel is heated in order to boil the reaction mixture; vapours produced from the mixture are condensed by the condenser, and return to the vessel through gravity.

The purpose is to thermally accelerate the reaction by conducting it at an elevated, controlled temperature (i.e., the solvent's boiling point) and ambient pressure.

Enzyme based extraction

- EAE is a rapid and promising method for the extraction of FPSs with maximum yield compared to conventional techniques.
- EAE is based on the specificity of enzyme reactions that can break down specific cell wall membranes with desirable quantities of solvent utilization.
- In EAE the plant material is pretreated with the enzymes such as protease, pectinase, pectinesterase, cellulase, hemicellulase, cellobiase, α-amylase and fructosyl transferase to hydrolyze the cell walls and release the phytochemicals bound to lipid and carbohydrate chains inside the cell [5].
- This pretreatment is followed by solvent extraction or pressurized hot water extraction for the extraction of volatile compounds, hydrophilic and hydrophobic pigments, phenolic compounds, and other bioactive compounds from plant samples.

Microwave Assisted Extraction

MAE is a conventional technique for the extraction of active components from medicinal plants, using microwave energy to heat solvents containing samples, thereby partitioning analytes from a sample matrix into the solvent. Microwave-assisted extraction is an efficient method which involves deriving natural compounds from raw plants. Microwave extraction allows organic compounds to be extracted more rapidly, with similar or better yield as compared to conventional extraction methods. High microwave power might increase the product temperature overly high, and decrease the extraction yield through product damage or compound breakdown [6]. Also, a decrease in the flavonoid yields with increase in power has been found to be significant when the extraction period was extended [7]. The main advantage of MAE is its ability to rapidly heat the sample solvent mixture, resulting in its wide applicability for the rapid extraction of analytes, including thermally unstable substances. Microwave-assisted extraction has remarkable outputs of yield, quality, and selectivity which have helped in retaining its utility. With continued modification of equipment and use of microwave, developments have contributed in establishing it as one of the leading analytical extractions.

Factors affecting microwave assisted extraction

Extraction time

By increasing extraction time, quantity of analytes is increased but there is the risk of degradation.

Microwave power

The power must be correctly chosen to minimize the time needed for extraction. However, increased power may cause solvent loss by evaporation. Maximum power used ranges between 600W and 1000W for closed systems and around 250W for open systems.

Matrix characteristics

The plant particle size and the status in which it is presented for MAE can affect the recoveries of compounds. The particle sizes of the extracted materials are generally in the range of 100 m – 2mm. Fine powders enhance extraction as they provide a larger surface area

Temperature

Temperature should be sufficient to ensure good solubility of compounds and a good penetration of solvent in the plant matrix to enhance extraction yield. However, it should not be too high enough to degrade the target compounds.

Ultrasound assisted extraction

Ultrasound assisted extraction (USAE) is an interesting process to obtain high valuable compounds and could contribute to the increase the value of some food by-products when used as sources of natural compounds. Ultrasonic extraction is used in the food, nutritional supplement and pharmaceutical industry to release bioactive compounds such as vitamins, polyphenols, polysaccharides, cannabinoids and other phytochemicals from botanicals. The ultra-assisted extraction is based on the working principle of acoustic or ultrasonic cavitation.

The main benefits will be a more effective extraction, thus saving energy, and also to the use of moderate temperatures, which is beneficial for heat sensitive compounds. For a successful application of the USAE, it is necessary to consider the influence of several process variables, the main ones being the applied ultrasonic power, the frequency, the extraction temperature, the reactor characteristics and the solvent-sample interaction. The highest extraction rate is usually achieved in the first few minutes, which is the most profitable period.

Conclusion

- Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures.
- The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use.
- There are several techniques for the separation and identification of natural products. Selection of method is important in result.

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