



## Strategy for Enhanced Stability of Betalain Pigments: Effect of Encapsulating Agents

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### Abstract

In recent years, food coloring with artificial colorants has been increasingly disapproved by consumers. In return, the application of coloring foodstuffs, among them betalain-containing fruits and vegetables, has gained importance for the food industry. Betalain has several bioactivities. As commonly true for natural pigments, betalains are afflicted with inferior stability compared to synthetic dyes. Especially temperature, oxygen, and light are known to exhibit detrimental effects on betalain integrity, while certain antioxidants and chelating agents may act as stabilizers. Encapsulation is an excellent process to enhance bioaccessibility, digestibility, and controlled release. During food fortification, efficient encapsulation technologies are needed to prevent the degradation of pigments and reserve their bioavailability in the human gastrointestinal system. The development of cost-effective and viable technologies for the preparation of natural food color and its application in foods is a great challenge and a major need of the day.

**Keywords-** betalain, bioactivity, stability, encapsulation, controlled release

### Introduction

In nature, there exists a huge number of pigments that can be utilized as bio-colorants. But one of the major constraints is instability. Hence for enhancing the stability of natural pigments during processing and storage, different methods of stabilization have to be considered combining more than one stabilization technique is also found useful in enhancing the stability of many natural pigments including betalains (Castro-Munoz et al., 2015).

Betalains are nitrogen-containing natural pigments that provide bright coloration to fruits, flowers, and roots of plants belonging to the order Caryophyllales. They are divided into two groups: the violet betacyanins, with absorbance spectra centered at wavelengths around  $\lambda_m = 536$  nm, and the yellow betaxanthins, with absorbance spectra centered at wavelengths around  $\lambda_m = 480$  nm. Both groups share betalamic acid as the structural and chromophoric unit, which is condensed with cyclo-DOPA in the betacyanins and with amines and amino acids in the betaxanthins (Fu et al., 2020).

### Bioactivity of betalain

- **Anti-inflammatory:** Betalain found to possess anti-inflammatory activity through inhibition of cyclooxygenase, hypochlorous acid scavenging,
- **Antioxidant activity:** The excellent antioxidant activity of betalains is due to their unique molecular structures, which is reflected by their capability to donate hydrogen to reactive species.

- **Antimicrobial activity:** Betalains, which could suppress gram-negative bacteria infection. It could affect structure, function and permeability of microbial cell membranes, which eventually resulted in death of the microorganism.
- **Cancer chemopreservative property:** Betalains strong free radical scavenging ability, which can activate the key transcription factor (Nrf2) and phase II enzymes to induce the antioxidant defense mechanism of endogenous cells (Otalora et al., 2015).

### Factor affecting the stability of betalain

- **Water activity:** Water activity can act as an important factor in evaluating the susceptibility of betalain toward aldimine bond cleavage. The lower water activity could improve betalain stability, and water activity below 0.63 has been found the most effective.
- **pH ranging:** pH from 3 to 7, there is no shift in the maximum absorption of betalain (537 nm), and the spectrum was identical without a color change. However, when the pH is lower than 3, the absorption maximum shifts slightly toward a shorter wavelength (pH 2.0, 535 nm) and the absorption intensity decreases. At the same time, the absorption degree can be slightly increased in the range of 575–650 nm, and the solution color was converted from red to purple. When the pH increases, the maximum absorption wavelength changes (Fernando et al., 2013).
- **Metallic cation:** Some metal cations, such as  $\text{Sn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Al}^{3+}$  and  $\text{Cr}^{3+}$  also exert certain adverse impacts on stability by accelerating degradation of betalain.
- **Temperature:** Betalains are heat sensitive to degradation when the temperature is above 50 °C, which is a severe drawback for their application as food colorants. If a betalain solution is heated at 100 °C, the red color gradually fades and turns into yellowish-brown.
- **Light:** Light could increase the degradation rate of betalain. The degradation of betalain can be provoked by light and is susceptible to light absorption between the ultraviolet and visible spectra, which could promote a transition from an electron-excited state to a more active state of the chromophore of betalain (Ghosh et al., 2022).
- **Aerobic condition:** Storage of betalain solutions under low oxygen levels can lead to the decreased degradation of pigment than under an air atmosphere.

### Stabilization techniques

There are three stabilization techniques including, copigmentation, complex formation and encapsulation. Encapsulation is a technique by which liquid droplets, solid particles or gas bubbles of core material is coated with a thin film of protective materials. The coating film or wall protects the core against deterioration, reduces the evaporation of volatile compounds, and releases the core under desired conditions. The material to be encapsulated may be referred to as the internal phase, core material, fill, payload phase or active agent and encapsulating material may be referred to as membrane, carrier material, coating, shell, matrix, external phase or wall material (Kumar et al., 2020).

### Encapsulating agents

The suitability of encapsulating agents used in coating the core material should be strictly considered. The important criteria of an encapsulating agent that should be considered are high water solubility, high stability, superior drying, and emulsifying properties, and tend to construct a fine and dense network during drying and exhibit a good surface activity for stabilization, having a suitable drying profile and form a protective coating, surrounding the core.

- **Water soluble resin:** Gelatin, gum arabic, cmc, methyl cellulose, arabinogalactan, polyvinyl acrylate, polyacrylic acid, etc.
- **Water insoluble resin:** Ethyl cellulose, polyethylene, cellulose nitrate, silicones, etc.

- Wax & lipid: Paraffin, carnauba wax, bees wax, stearic acid, stearyl alcohol, etc.

### Methods of encapsulation

**Spray Drying:** Spray drying is an economical and effective method for protecting materials and specialized equipment is not required. For encapsulation purposes, modified starch, maltodextrin, gum or others are hydrated to be used as the carrier or wall material the encapsulation material is homogenized with the carrier material usually at a ratio of 1: 4. The mixture is then fed into a spray dryer and atomized with a nozzle or spinning wheel. Water is evaporated by the hot air contacting the atomized material. The capsules are then collected after falling to the drier's bottom.

**Spray Cooling:** The material to be encapsulated is mixed with the carrier and atomized by cooled or chilled air. The outer material is usually vegetable oil. The disadvantage of the method is that special handling and storage conditions could be required.

**Extrusion Coating:** Core material is incorporated into molten carbohydrate mass. Then the mixture is forced through a series of dies and collected in a bath of dehydrating solution. Common coating materials used include glucose, glucose syrup, sucrose, maltodextrin and glycerine which can be used as a single or a mixture of compounds. The primary advantage of this method is the protection of the core compounds from oxygen as they are completely isolated from the air by the wall material.

**Fluidized Bed Coating:** Solid particles are suspended in a temperature and humidity-controlled chamber of high-velocity air where the coating material is atomized. Optimal results are obtained with particle sizes between 50 and 500 microns. The amount of material that coats the particles is dependent on the length of time that the particles are in the chamber.

**Liposome entrapment:** One type of capsule with more versatile properties and less fragility than those made of fat is liposomes. Permeability, stability, surface activity and affinity can be varied through size and lipid composition variations. Liposomes are vesicles made of bilayers mostly composed of phospholipids.

**Inclusion complexation:** In this technique-beta-cyclodextrin is used as coating material. The center is hydrophobic while the outer surface is hydrophilic. In the center of the cyclodextrin, water molecules are replaced by less polar molecules. The complex then precipitates out of the solution only water can serve as the suspension medium.

**Rotational suspension separation:** Mixing the core and wall materials and then adding them to a rotating disk. The core materials then leave the disk with a coating of residual liquid.

### Release mechanism

Although the main purpose of microencapsulation is to protect the core materials from environmental elements, it is meaningless if the cores cannot be released or are released at the wrong time or place. Therefore, the release mechanism of the core at the appropriate place and time is as important as the protection capacity for microencapsulation. The main factor to the release rate is associated with interactions between the carrier and the core materials (Indrawati et al., 2015).

- **Diffusion:** Occurs especially when the microcapsule shell is intact. Dissolution fluid dissolves the core by penetrating the wall material leading to leakage through the pores. The final release relies on the penetration rate of the microcapsule by dissolution fluid, the dissolving rate of the core into the dissolution fluid, and the leakage and dispersion rate of the core.
- **Osmosis:** The polymer shell of the microcapsule resembles a semi-permeable membrane. The osmotic pressure difference between the inside and outside of the microcapsule leads to the movement of the core through small pores in the shell (Muhamad et al., 2018)



- **Dissolution:** When the coating gradually dissolves, the cores are released. Therefore, the release rate is determined by the dissolution rate which is influenced by the thickness of the coating and its solubility in the dissolution fluid.
- **Biodegradation:** Enzymes such as proteases and lipases hydrolyze proteins and lipids respectively leading to release of the core.

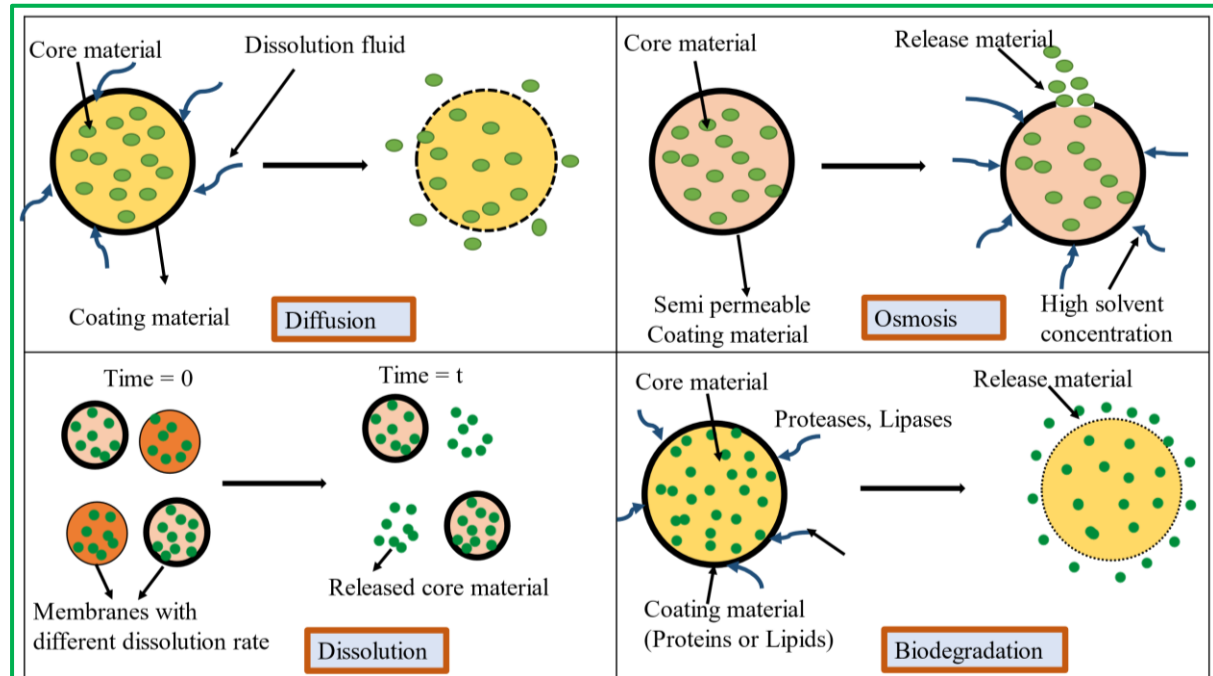


Fig 1. Microencapsulation Release Mechanisms: Diffusion, Osmosis, Dissolution, and Biodegradation

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