Stabilizing Betalains: A Review
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Abstract

This review analyzes different methods for stabilizing the natural plant pigment category of betalains. Due to recent consumer demand for natural ingredients in commercially produced food products, it has become pertinent to research methods to stabilize betalains because of their unstable nature in many food systems. Some of the most practical and well-research methods for stabilizing betalains include complex formation (primarily through a cyclodextrin complex), copigmentation, and encapsulation, all of which will be discussed in this review. Research concerning betalains and their subsequent incorporation into the food industry will provide consumers with natural options for colored food products and the betalains in these food products will provide additional health benefits that appeal to many consumers. In all, research involving stabilizing betalains provides many benefits to the food industry and consumer alike.

Keywords: betalains, anthocyanins, plant pigments, stabilization, beet pigment, natural food dye, betacyanins, encapsulation, chelating, betanin, beetroot red, preservation, natural pigment, bioactive pigment, and pigment chemistry
Introduction

Consumers eat with their eyes. Would we enjoy strawberry yogurt as much if it weren’t a perfect pink, or cranberry-apple juice if it weren’t a rosy red? Stable and attractive colors are valuable in the competitive food and beverage market (Fernandez-Lopez, Agosto, Jimenez et. al, 2013). Being so valuable, color acts as a quality indicator for consumers and food producers alike. Due to color’s importance, the food industry has long used color additives and other stabilizing methods to preserve the color of countless food products. Recent consumer demand pushes towards the use of naturally derived pigments as colorants because of the common perception that natural is better. In addition to this new trend, Gokhale & Lele (2014) state that the increased interest in natural colorants is in part attributed to the appeal naturally derived food pigments have in offering health benefits like antioxidant activity. Other health properties offered by natural pigments sought after by consumers, nutritionists, and food scientists alike include antimicrobial and anti-cancer activities. According to Cebi & Yangilar (2016), these properties have driven food scientists to explore methods of replacing synthetic colors with naturally derived pigments. Because trends indicate that it indeed matters to consumers whether their strawberry yogurt is colored with Red #40 or beet extract, it is pertinent to discuss a class of natural pigment that has great potential to add vibrancy and color to our foods in a natural way.

Betalains are natural pigments found in some plants that produce vibrant colors primarily in the purple, red, and yellow categories. They appear ideal due to their abundance, ease of extraction, and pleasing color properties. Despite these benefits, many factors contribute to the instability of betalains as food colorants. Delgado-Vargas et al. (2000) discuss this in their research by explaining the major factors that affect the stability of natural colorants in foods. They describe that the concentration of pigments, pH and water activity of the food, oxygen,
light, metallic ions, enzymes, temperature and time of processing, and storage conditions contribute to the instability of natural pigments in a food system. Because food systems often involve many of these factors, the usage of betalains as food pigments proves difficult. Because of consumer demand and the health benefits betalains may provide, it is important to research and apply methods for stabilizing betalains for use in food systems. In this review, an overview of the usages and properties of betalains will be provide, and a discussion of three betalain stabilization techniques will follow. These techniques include complex formation, copigmentation, and encapsulation and each have great potential to alter the way we use natural pigments in our food supply.

**Betalain Overview**

In order to fully understand the various techniques used to stabilize betalains that will be discussed, it is essential to have a background on the nature, chemical properties, and applications of betalains. Betalains are water-soluble nitrogen-containing pigments that have two structural groups, which are the red-violet betacyanins and the yellow-orange betaxanthins (Azeredo, 2009). The most common betalain in food applications is betanin, which reflects a wavelength of visible light that is a pleasing and vibrant red color. These plant-based pigments are sourced for commercial purposes primarily from red beet root, though there are alternative sources found in plants such as the prickly pear, hibiscus, and some mushrooms. Betalains are found in abundance in the vacuoles of such plants. Betalains are used in a wide range of food applications, including dairy products like yogurt and flavored milks, processed meats, salad dressings, confections, drink mixes, and frozen desserts (Cebi & Yangilar, 2016).

Betalains appear to be an excellent food ingredient due to their ability to provide natural vibrant colors to food products and also because they have properties that are sought after as a
food additives (such as excellent water solubility and strong fluorescence). However, they present many challenges due to their ready degradation in the presence of certain intrinsic and extrinsic factors. As previously mentioned, the major factors affecting the stability of natural colorants in foods are the concentration of pigments, pH and water activity of the food, oxygen, light, metallic ions, enzymes, temperature and time of processing, and storage conditions (Delgado-Vargas et al., 2000). Betalains, catalyzed by the aforementioned factors, can experience many changes during degradation including breakdown of the aldimine bond, dehydrogenation, deglycosylation, decarboxylation and isomerization (Manchali et. al, 2013). All of these chemical changes affect the betalain’s functionality as colorants. Since many foods with potential to use betalains as a color source are processed with thermal processing, pH adjustments, and storage conditions that often present exposure to oxygen, light, and water activity changes, in most food systems, it is unlikely that unstabilized betalains would maintain acceptable color properties.

In their thorough study on betalain stability, Herbach, Stintzing, & Carle (2006) consider the stability of betalains to be a measure of their retention of antioxidant and pigment properties. They add that color and antioxidant changes in betalainic foods due to different degradation pathways can be analyzed and assigned values primarily though high-performance liquid chromatography (HPLC), which is an important analytical method used to test the effectiveness of the different stabilization techniques. Having a method for measuring the stability and effectiveness of these compounds in different conditions proves essential to any study concerning the stability of betalains. These and other similar analytical methods have provided many of the stabilization techniques discussed forthwith with validation and a way of obtaining reliable data.
Stabilization Techniques

As mentioned, chemical changes (like pH, oxygen, water activity, ions, etc.) have an impact on the wavelength of light reflected by the pigment and also its antioxidant activity, two factors sought after in betalainic foods. In order to preserve its chemical structure, and therefore its functionality, different methods for the stabilization of these pigments must be considered. Mohammed I. Khan (2015) proposes three general methods by which betalains can be preserved and stabilized, which are through complex formation, copigmentation, and encapsulation.

Complex Formation

Complex formation suggests using molecules with specific chemical properties like charge or hydrophobic regions that allow a noncovalent complex to form between the molecule in question (in our case, the betalain pigment) and the molecule or metal ion being used. This noncovalent reaction between the complex and the betalain would stabilize the betalains in preparation for use in a food system. There are many different methods of complex formation that have been studied in regards to their ability to stabilize betalains so they can more readily be used in food systems. Such methods include metal-chelates, cyclodextrin complexes, various matrices, and also organic acids provide enhanced betalain stability.

Of all the complexes used to stabilize betalain molecules, cyclodextrins have shown the most promise. According to studies conducted by Hamburg (1992) and Drunkler, Fett, & Luiz et. al (2006), cyclodextrins protect the betalains from UV rays and can also prevent many chemical factors from reacting with the pigments. Cyclodextrins are unique molecules because the interior portion of the cyclic polysaccharide provides a more hydrophobic environment in which molecules with hydrophobic portions (like betalains) can interact and be shielded and contained within the cyclodextrin. The outside portion of the cyclic structure of cyclodextrins have more
hydrophilic regions and are therefore soluble in water. Betanin can form an inclusion complex with a molecule of cyclodextrin, which means the betanin is inserted into the hollow of the cyclodextrin. Betalain food colorants that have formed a complex with cyclodextrin, especially beta-cyclodextrin, have intensified color, increased water solubility, and improved light stability, all of which are important when using betalains in a food system.

Other non-cyclodextrin substances are able to form complexes that preserve the integrity of betalains, but do not have as much practical food industry application due to various factors. For example, TEOS (tetraethyl orthosilicate) has been shown to dramatically increase the stability of betalains, according to Molina, Cordez-Valadez, et. al (2014), as it was able to protect the cyclodextrins at varying pH levels, dissipate temperature affects, and quench UV radiation. However, this ingredient is not approved for use in food products due to its potential toxicity, but has the potential to be used in such applications as storing betalains. While these stored betalains could never be used in food applications, they can be held and later analyzed for research purposes. There have also been many studies conducted concerning complex formation to preserve the functional properties of betalains that have shown little to no success. For example, acids like gallic acids have unreliably been able to support the preservation of betalains in food systems (Khan & Giridhar, 2014), while metal chelates, such as Fe$^{2+}$ and Cu$^{2+}$, have been shown to actually reduce color stability of betalains (Pasch and von Elbe, 1979).

Despite the unsuccessful food applications of some complex formation methods, the cyclodextrin complex approach provides a promising future for betalains as a natural colorant in our food supply. Since cyclodextrins are already approved for food use and are currently being used in caffeine and cholesterol applications, we can assume that, through further research and
FDA approval, reliable methods will be established to stabilize and preserve the pigment properties of betalains for food use.

**Copigmentation**

Another promising method to stabilize betalains and preserve their pigment and antioxidant properties is a process called copigmentation. Copigmentation occurs when there are direct or weak interactions that occur between anthocyanins (like betanin) and other naturally occurring compounds. The term “copigment” refers to these naturally occurring compounds that interact with betalains. This interaction can cause a different expression of color, depending on the type of interaction. According to Mazza & Brouillard (1990), many substances can act as copigments, including phenols, alkaloids, amino acids, organic acids, nucleotides, polysaccharides, and anthocyanins themselves. All of these substances are naturally found in foods and in many instances are approved ingredients for food use. Betalains that have undergone copigmentation have an increased color intensity due to a change in the spectral position of the molecule, which causes a shift in light emission to a longer wavelength. When ascorbic acid or citric acid are used as copigments with betalains, their property of removing oxygen from the solution reduces the polarity of the N-1 position on the betanin, which makes the molecule of pigment less susceptible to an attack by water (Kearsley & Katsaboxakis, 1980). **Figure 1** indicates the site of hydrolysis. If water is unable to attack at this position due to copigmentation, the

![Figure 1: Chemical structure of betanin (Kearsley & Katsaboxakis, 1980)](image-url)
molecule will remain intact, as the N1 position is the most susceptible to cleavage. If this site is preserved, the molecule’s integrity is also preserved.

Other copigmentation procedures performed on betalains, like glycosylation, acylation, the addition of selenium, and esterification with aromatic acids, enhance its chromatic properties. These processes to manipulate, enhance, and preserve the properties of betalains are very applicable to the food industry, especially because many ingredients used as copigments are seen as beneficial in food products. For example, ascorbic acid (mentioned earlier), known commonly as vitamin C, is an ingredient that enhances appeal to consumers when it appears on a food label. Other beneficial copigments include selenium, which is an essential mineral, and certain amino acids, which are the building blocks of proteins in our bodies.

Due to the “natural” status of many of these copigments, in some ways this stabilization method shows more promise for use in the food industry than the previously mentioned method of complex formation. In fact, cyclodextrins are not commonly accepted as being a natural ingredient, so consumers that seek natural colorants in their foods most likely would be unsupportive of an unnatural ingredient stabilizing their sought-after natural pigment. Though copigmentation is a promising option for stabilizing betalains, there exists an even more practical method, called encapsulation, that is the most likely of all the stabilization methods to normalize betalains as a food ingredient.

**Encapsulation**

The most commonly used application of betalain stabilization in the food industry is encapsulation. The most accepted and used method of encapsulation is facilitated by spray-drying, which is very economical, flexible, and yields good results. In the spray drying process, a homogenous mixture of a coating material (often maltodextrin) and the pigment are fed through
a spray dryer. In their most basic form, spray dryers consist of a heated system, a nozzle called an atomizer, and a vortex. The mixture is homogenized and heated in a piping system until it reaches the atomizer. What emerges from the atomizer of the spray dryer are tiny particles (10-100 μm) containing the pigment encapsulated in the coating material (Fang & Bhandari, 2010). The particles then dry as they fall through a heated vortex of air and are collected as a dry powder.

Maltodextrin is not the only coating material that is used in this process, as other polysaccharides, including pectin, guar gum, or gum Arabic, are also practical. These ingredients are functional in supporting the preservation of betalains because they reduce hygroscopicity, or the absorption of water, and thus prevent the betalains from being hydrolyzed over time (Lejeune, Pouget, & Porrat, 1983). However, both Azeredo et. al (2007) and Obón et. al (2009) report the superior storage capacity of betalains through the encapsulation by means of maltodextrin, which is inexpensive and a widely available food ingredient, thus the most practical of all coating agents. Though many of the mentioned studies on encapsulation of betalains via maltodextrin reported an initial increase of betalain degradation due to the heat involved in the spray-drying process, one study (Obón et. al, 2009) reports only a 10-20% loss of betalain pigment properties over a 6-month extended storage study. These losses are not significant in the overall functionality of betalains for food use. Not only do these studies show that the encapsulation process helps to increase the shelf-life of betalains, but betalains in this encapsulated form are highly water soluble and are easily incorporated into food systems.

Encapsulation seems like the ideal form for stabilizing betalains due to its practicality, proven success, and pre-established and well-known method of spray drying. However, food systems are very diverse and different forms of stabilizing betalains may be ideal in different
systems. It is important that research be conducted to see how different forms of stabilized betalains react in general food categories where betalains would be used. Suggested food categories to be further researched include thermally processed foods, dairy, baked items, and juices, as they show the most potential for the application of betalains. Surely each different food system, and even each different food product, presents unique characteristics that alter the way stabilized betalains behave. Because of the great variation of betalain behavior in food systems, consumer demand, profit as a result of this demand, and the health benefits betalains provide when incorporated into food products, it is important that methods for stabilizing betalains for use in food systems be researched and applied.

**Conclusion**

After this discussion concerning the different betalain stabilization techniques being researched and applied, it is clear that a strong potential exists for consumers to start reading “natural betalain color” as an ingredient in their favorite strawberry milk or raspberry muffin mix, among a plethora of other products. Though all artificial colors currently used in the food industry are safe and approved by the FDA, many consumers trust food products more when the ingredients used in their food are natural, rather than artificial. Not only would the addition of betalains in our food products help consumers feel more confident in the American food supply, consumers would gain additional health benefits like increased antioxidant, antimitagenic, and antimicrobial activities by consuming betalainic food products. All of these are highly marketable benefits that will appeal to many consumers. If the trend towards “natural” continues, and these health benefits continue to be sought after, betalains will become a practical and high demand food ingredient that is sure to provide great financial opportunity for individual food companies and the industry as a whole. As the food industry is supplied with monetary
resources, further research is made possible and food scientists can continue making advances with food ingredients and products to better our food supply.

Nevertheless, just because betalains naturally exist, produce bright colors, and provide health benefit does not mean they are the perfect solution to replacing artificial colors in our food supply. Betalains are generally not sufficiently stable on their own to be used in most food systems, as they are highly sensitive to light, oxygen, pH, water activity, enzymes, metallic ions, and abusive storage conditions. Because of their instability, food scientists researchers are conducting studies to establish methods to stabilize betalains for food use. Some of the most promising methods, as discussed in this review, include complex formation, copigmentation, and encapsulation. Complex formation, especially in cyclodextrin complexes, provides excellent protection for the betalains against degradation. However, the artificial status of cyclodextrins as a food ingredient presents issues in preserving the “natural” qualification of betalains stabilized in this way. Copigmentation using other natural and valuable ingredients to stabilize betalains provides many benefits as a commercial stabilization option and has already been applied in many food systems in research. However, standard methods are not as ready to be applied in food production facilities as copigmentation techniques are new and not as well established. On the other hand, spray drying is already common practice in the industry for other food applications, and the encapsulation method via spray drying for stabilizing betalains is the most promising of all the stabilization methods discussed in this review.

Though much research has been conducted concerning the stabilization of betalains, it is important that food scientists continue research involving the stabilization of betalains. The importance is made clear when considering the great demand consumers present for naturally sourced food ingredients like betalains, the great financial opportunity this consumer demand
presents to the food industry, and the benefits consumers obtain as they intake the vibrant and valuable betalain pigment.
References


