Betalains-natural pigments for healthy food

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ABSTRACT

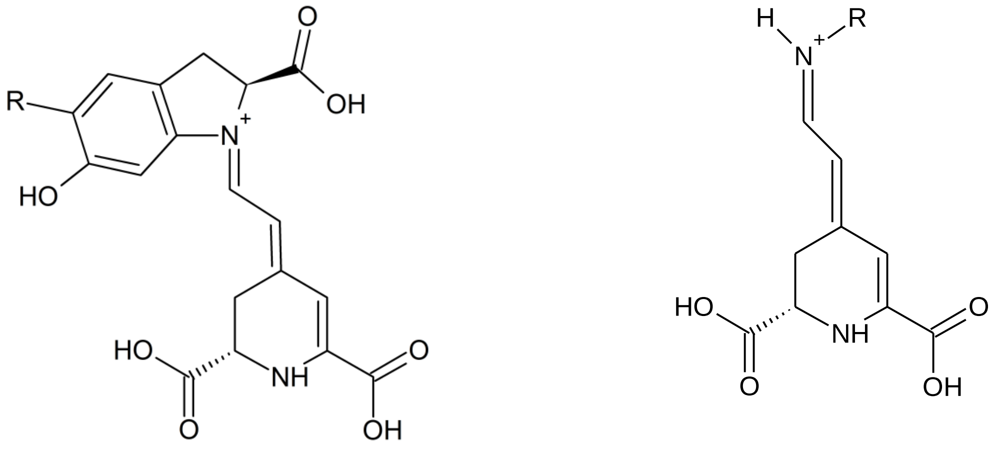
The application of artificial colors in practice has shown many negative effects on food safety and human health. Allergic reactions to artificial colors are very common, especially in children. Some research suggests that prolonged use of artificial colors can be the cause of cancer. Betaine is one of the most widespread groups of plant pigments, which is increasingly being researched in recent times. Therefore, the use of cheap plant pigments such as betaine is important. They are safe for use in food and have good biological and pharmacological properties. Some of them have an antioxidant, antimicrobial, antilipidemic, anticancer role. They can be used in food instead of anthocyanins. Some properties are beneficial to our health due to which they can be used to enhance the flavor and color of ice-cream, jellies, jams, desserts, sauces, sweets, tomato paste, breakfast cereals.

Keywords: betalains, isolation, identification, stability, application, health benefit

Introduction

The development of synthetic chemistry has led to the production of numerous artificial colors, which were widely used in the food industry after the Second World War. The prolonged use of artificial colors has caused numerous adverse effects on human health. Allergic reactions in children to artificial colors were particularly pronounced. Some colors have been found to have a carcinogenic effect. All this has directed scientists to research natural colors, which provide bright coloration to fruits, flowers, and roots of plants, and do not have negative effects on health; on the contrary, they have many beneficial effects.

So far, betalains have been much less studied than other natural plant pigments: chlorophylls, carotenoids and anthocyanins. Betalains are water-soluble and nitrogen-containing pigments, divided into betacyanins and betaxanthins. Red and violet tonalities result from different substitution patterns in betacyanins, while different amino acid or amine side chains determine the color of betaxanthins (Figure 1).



(a) (b)

Figure 1. Structures of (a) betacyanins and (b) betaxanthins.

Glycosylation and acylation result in a diversity of betacyanin structures. The betanidin aglycone is usually linked with glucose, occasionally with glucuronic acid, sophorose, rhamnose, and apiose, at the C-5 or C-6 position. Further modification occurs by aliphatic or aromatic acid esterificationof the sugar moiety. Malonic, 3-hydroxy-3-methyl-glutaric, caffeic, *p*-coumaric, cinnamic, and ferulic acids are typical acid substituents (Strack et al., 2003).

Betalains are found in numerous sources (flowers, fruits, roots, leaves, stalks, seeds, grains) in the plant kingdom. In food, however, their occurrence is limited, the red beet is being regarded for a long time as practically the sole source of betacyanin. Betalaines are found in various plant organs and accumulate in cellular vacuoles, mainly in the epidermal and subepidermal tissues. However, they are sometimes accumulated in plant stems, such as subterranean parts, seeds, flowers and leaves (Delgado-Vargaset al., 2000).

About 75 betalains have been structurally identified from plants of about 17 families out of 34 families under the order Caryophyllales, such as Aizoaceae, Amaranthaceae, Basellaceae, Cactaceae, Chenopodiaceae, Didiereaceae, Holophytaceae, Nyctaginaceae, Phytolaccaceae, and Portulacaceae families, to name a few. Betalains have also been found in mushrooms in the genera Amanita, Hygrocybe, and Hygrophorus. Only a few fruits and vegetables contain betalains and the best known is beet roots (*Beta vulgaris*) that produces betanin ($C_{24}H_{27}N_2O_{13}$), an important natural food colorant (Slimen et al., 2017).

The other edible sources known to contain betalains are fruits of *Opuntia* spp. (*e.g.*, *O. ficus-indica*, commonly known as prickly pear), Swiss chard (*Beta vulgaris* subsp. *vulgaris*, Cicla-Group and Flavescens-Group), grains or leaves of amaranth (*Amaranthus* spp.), pitahaya (*Hylocereus* spp.), also known as dragonfruit, ulluco (*Ullucus tuberosus*), djulis (*Chenopodium fromosanum*, a native cereal in Taiwan), and bilberry cactus (*Myrtillocactus geometrizans)*, an endemic plant locally consumed in Mexico, which contains 233 mg/kg of total betalains in its fruit pulp (Delgado-Vargas et al., 2000).

Betanin was discovered around 1920, while the crystalline form of betanin was produced in the 1960s (Nottingham, 2004).

At the root of beetroot, betanin is normally found in a much higher amount than other betacyanin pigments. Like all betacyanins, betanin is metabolically derived from a molecule known as 3,4-dihydroxyphenylalanine (L-DOPA) (Gandía-Herrero and García-Carmona, 2012).

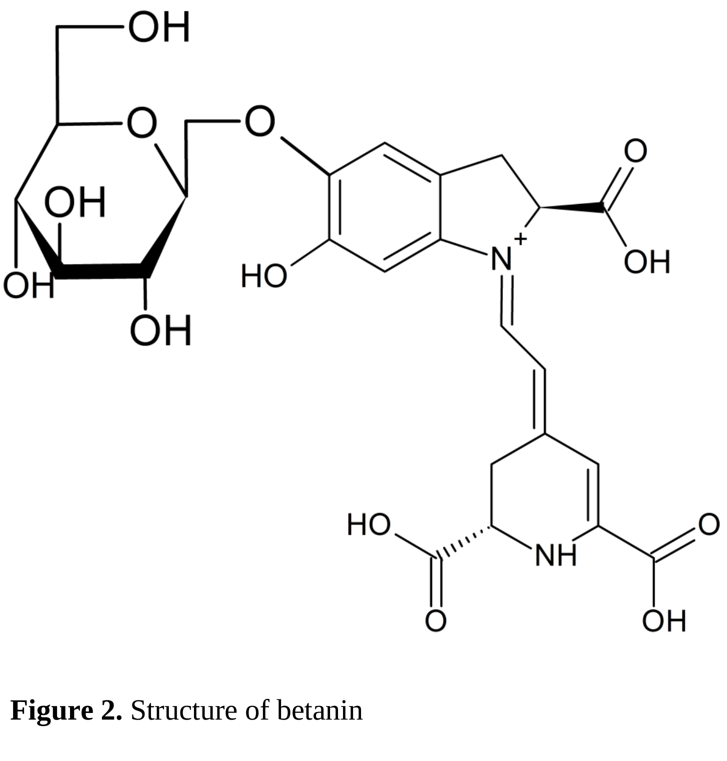


Figure 2. Structure of betanin

The biosynthesis of betalains is discussed in detail in a review article (Khan and Giridhar, 2015). Betanin is formed from two L-DOPA molecules. One molecule undergoes a change to the form of betalamic acid. Another molecule of L-DOPA is changed to cyclo-DOPA glucoside (CDG), which condenses with betalamic acid and builds betanin (Hussain et al., 2018b).

A small change in structure translates betanidin into betanin. Other small biochemical modifications yield other betacyanin pigment. The most important factors affecting the synthesis of beetroot pigments are temperature, soil fertility, soil moisture, irrigation and harvesting time (Jackman and Smith, 1996).

After betanin, yellow betaxanthins, vulgaxanthin-I and vulgaxanthin-II are the most significant beetroot pigments. These pigments were first described by Mario Piattelli with collaborators in the 1960s in Naples (Piattelli and Minale, 1964).

In the cultures under study (Piatta d'Egitto), they found at least six betaxanthins, all of which were in small quantities. In total, they listed sixteen different betalains, including indicaxanthin, isobetanin, neobetanin, and prebetanin. The characteristic color of the beetroot is due to variations in the betalain pigments, especially the relative concentrations of betanin and yellow betaxanthin. Species with a dark purple-red color have a high ratio of betanin to betaxanthin, while yellow and gold species such as Burpee's Golden have relatively large amounts of betaxanthin and have very little or no betanin at all. Species with white carrots, including Albina Verduna and Blankoma, have extremely low amounts of both betacyanin and betaxanthin (Nottingham, 2004).

Extremely dark and light rings are usually visible when the beetroot is crossed. This is due to the different amounts of pigments in the vascular system and the storage tissue of the root. The vascular system is seen as dark rings due to the large amount of pigments, while the storage tissue looks like brighter rings. In some dark red species such as Boltardy and Red Ace, these color differences are barely noticeable. The color difference is most evident in the Chioggia species, with concentric patterns (as targets) pinkish-red (vascular system) and white (storage tissue).

The content of betacyanin in beets is 75-95% and yellow betaxanthin vulgaxanthin I is the most abundant. In the other parts of the beet (shell), the highest concentration of betanin is present. Betacyanins are more stable than betaxanthin at 25ºC (Kujala et al., 2002).

Beetroot contains about 130 mg of betanin/100g of raw material. New selections can contain up to 450-500 mg betanin/100g. Betanin is the most prevalent among the betacyanins and the most significant. In addition to betanin, there are isobetanin, betanidin and isobetanidin (Kujala et al., 2002).

The betalains are generally arranged according to the outer parts of the beet root. However, they are not limited to the root, but give a red color that can be observed on leaves, stems and flowers. Of the yellow betaxanthins, vulgaxanthin I is the most abundant.

Isolation and identification of betalains

Betalains are generally extracted from ground plant material by maceration with water, at differents temperatures or Soxhlet extraction. For a complete extraction, it is preferable to use methanol or ethanol solutions (20–50%, v/v). Before extraction, a short heat treatment (70 °C, 20 min) can be made for inactivating degradative enzymes, despite the possible destruction of some pigments. A slight acidification with HCl or citric acid or acidified ethanol can be used for the precipitation of betacyanins. The addition of aqueous ethanol (95%) will give betaxanthins (Celli and Brooks, 2017). However, other techniques have been assayed to extract betalains, particularly from red beet: diffusion extraction, ultrafiltration and reverse osmosis and loose reverse osmosis (LRO), cryogenic freezing, aqueous two-phase extraction, pulsed electric fields and gamma irradiation, microwave(Bastos and Gonçalves, 2017) and ultrasound-assisted extractions (Cardoso-Ugarte et al., 2014).

Purification and isolation of betalains is needed before their qualitative and quantitative analysis for eliminating possible interfering compounds. Chromatographic and electrophoretic procedures have been used to separate and/or purify betalains. For the separation of these pigments, ion chromatography on cation exchange resins followed by adsorption chromatography on polyamide column or a sequential chromatography on a series of Sephadex ion exchangers can be used (Piattelli et al., 1964).

There are several chemical tests for the characterisation of betalains and distinguishing (recognizing) anthocyanins from betalains. In order to characterize betalains, some other important determinations can be made. Betalains, as pigments, have a maximum absorbance in the visible part of the spectrum, which characterizes them; it is clear that structural modifications can be monitored using UV-VIS spectroscopy.

Red betacyanins absorb at λ_{max} = 540 nm, and yellow betaxanthins at λ_{max} = 480 nm. Acetylated betalains show a second (secondary) absorbance maximum in the UV spectrum of 260 to 320 nm and the ratio of the maximum in the visible spectrum to the maximum in the UV spectrum is used as a measure of the number of acyl groups in the structure (Hussain et al., 2018a).

Separation, identification and quantification of betalains have been carried through high performance liquid chromatography (HPLC) coupled to ultraviolet-visible (UV-Vis) (Faridah et al., 2015), photodiode array (PDA), mass spectrometry (MS) and nuclear magnetic resonance detection (NMR) by using reversed phase columns and binary gradient dilutions (acidified water with formic acid 2–5%, v/v) and acetonitrile or methanol (Stintzing and Carle, 2004; Stintzing et al., 2004). As betaxanthins are fluorescent pigments, the detection of these pigments may be done using fluorescence methods (Gandía-Herrero et al., 2005).

By refining the methodological approach, the HPLC method can be coupled to DAD, MS and NMR (Castellanos-Santiago and Yahia, 2008). In this way, separation and identification can be performed simultaneously. In addition, several chemical tests have been introduced to characterize betalains, and many of them are based on the changes in pH (Delgado-Vargas, 2002).

Stability of betalains

Betalains are an alternative to synthetic colorants, having stability over a wide pH range and a high molar extinction coefficient and being neither toxic nor allergens. Degradation after processing or storage includes reactions such as isomerization, deglycosylation, hydrolysis, decarboxylation, and dehydrogenation, involving color alterations and absorption changes. Factors affecting the stability of betalains include chemical structure, pigment content, matrix, additives, enzymes, pH, thermal treatment, water activity, exposure to oxygen and light during storage, and storage temperatures (Herbach et al, 2006).

In order to retain the coloring strength and chromatic characteristics of betalains, reduced thermal exposure is recommended during processing, together with the inactivation of deleterious enzymes, avoidance of light and oxygen, and the addition of antioxidants and/or chelating additives.

In the food industry, betalaines represent an important natural color. The basic source of betalains is beetroot. During commercial extraction, the beetroot is first ground, the colored juice is collected and concentrated. Betalaine is sold to the food industry as a juice concentrate or powder. The juice is concentrated in vacuum until 60-65% of the total dry matter is reached. In the manufacture of powder (which usually contains 0.3-1.0% of pigments), lyophilization is applied (Chikara et al., 2019).

In juices and powdered products, a high concentration of pigments is achieved by fermentation. Beet juice soluble fermenters can be removed in the biofermenter, using yeasts such as *Candida utilis* and *Saccharomyces cerevisiae*, to remain a more concentrated (relative to pigments) product. Powders obtained after fermentation of beet juice contain 5-7 times the amount of betacyanin than powders obtained from unfermented juice (Hadipour et al., 2020).

Betalaine extracts can have a wide variety of colors, depending on the relative ratio of betacyanin to betaxanthin present. Dye products are usually odorless and tasteless, but can affect the aroma and flavor of foods. The most important coloring agent is pure betanin, E-162 (beet red), which is used to color a wide range of processed foods (Khan, 2016).

The biggest problem with the use of betalains in foods is stability, which limits their use. Betalains extracts must be carefully manipulated as they are sensitive to environmental influences, especially pH, heat, light, moisture and oxygen. Environmental influences have an interactive effect and pigments can quickly discolor under adverse conditions. Red pigment betanin degrades to a light brown color when exposed to air, bright light, and high temperatures. This discoloration is partly reversible, if the adverse conditions are only temporary (Azeredo, 2009).

Betalains remain unchanged in the pH range of 3.5 to 7.0. Beetroot extracts in most foods will therefore not discolour as a direct consequence of pH. The optimal pH for the color of betacyanin and betaxanthin is in a poorly acidic medium, in the range of 5.0-6.0. The color of beetroot extract changes from red to blue with a pH change over 7.0. The beet tissue exposed to high or alkaline pH (7.5-8.5) becomes discolored. Chopped beetroot retains its purple-red color well in acidic solutions such as acetic acid (Azeredo, 2009).

Betalain heating can cause discoloration. Red pigment betanin, for example, can turn light brown if gradually heated, especially if elevated temperatures are associated with an alkaline pH (Pedreño and Escribano, 2001).

According to the current legisalation in Republic of Serbia (Official Gazette SCG 56/2003 as amended 4/2004, 5/2004, 16/2005), the use of betanin, as the main pigment of beetroot, as a natural food color E162, is allowed. The FAO and WHO expert team recommends the application of beetroot products in dairy products, fresh fruits and vegetables, pastas, fresh meat, fresh eggs, baby food, fruit juices, nectars and wines, *etc*. They are best suited for use in food products that undergo minimal heat treatment, short durability, packaged in dried form with reduced oxygen, light and moisture.

As a result, red beet is well accepted as red food colourant, specially betanin, denoted as E-162 in the European Union (Downham and Collins, 2000) and 73.40 in the chapter 21 of the Code of Federal Regulations (CFR) section of the Food and Drug Administration (FDA) in the USA.

The stability of betalain is the greatest in low-moisture foods. High humidity increases the rate of degradation of pigments. Over time, oxygen exposure accelerates the darkening or discoloration of pigments in food products. Betalains react with air oxygen, but discoloration is partially reversible if oxygen levels decrease immediately (Martins et al., 2017).

Despite the relative lack of stability when compared to synthetic food colors, betalains are widely used in the food industry (Peschel et al., 2006).

Exposure of a solution of betanin to light at 25ºC significantly affects its stability, while degradation due to heat is so pronounced at 55ºC. Also, the degradation of betanin in solution is a partially reversible process (Pedreño and Escribano, 2001).

In general, high betalains content, high degree of glucosylation and acylation, antioxidant, chelation agents, low T, elimination of light, N_2 increases stability of betalains. The presence of metal ions, enzymes, light, high T and O_2 decreases the stability of betalains (Esatbeyoglu et al., 2015).

The betalain pigment is usually extracted from fruit pulp or pieces of other raw material (*e.g.*, red beet) with a solvent (water, ethanol, or methanol), with or without heat treatment or acidification, to improve the pigment yield. The stability of betanin can be affected by pH, water activity, exposure to light, oxygen, enzymatic activities, metal, and temperature. Temperature is the most important factor in pigment degradation during the separation and concentration processes (Vergara et al., 2015). Despite their coloring capacity and superior antiradical activity, betalains have not been considered by the food industry as potential additives. This is in part due to their instability, which prevents long-term storage (Gokhale and Lele, 2014). The susceptibility of betalains restricts their use as food colorants (Ravichandran et al., 2014). Thus, the stability of betalains could be improved by using microencapsulation technologies such as spray drying (Janiszewska, 2014).

Potential health benefit of betalains

Beetroot pigments exhibit antioxidant properties. In various model systems, betacyanins have been shown to be potent antioxidants. Their positive electrostatic charge increases their affinity for biological membranes, which are major targets of oxidation. The antioxidant activity of betalains can be verified through different chemical and biological methods; anti-cancer, antiviral, and anti-oxidant activity has been attributed to betalain pigments. Due to their powerful anti-oxidant ability and their capacity to absorb free radicals, betalains can be used in the treatment of inflammatory and cardiovascular diseases, cancer, asthma, arthritis, oxidative stress, intestinal inflammation, diabetes, and other diseases associated with aging (Khan, 2016). The anti-oxidant properties of betalains can be related to structural features. In betaxanthins, an increasing number of hydroxy and imino residues improves free radical scavenging. In betacyanins, glycosylation reduces activity, while acylation generally raises the anti-oxidant potential (Stintzing and Carle, 2004).

- Free radical scavenging of reactive oxygen species (Escribano et al., 1998; Esatbeyoglu et al., 2014; Kanner, et al., 2001; Livrea and Tesoriere, 2004; Tesoriere et al., 2004;);
- Protection of LDL against oxidation (Neelwarne, 2012; Reddy et al., 2005; Siriwardhana et al., 2006; Tesoriere et al., 2003; Tesoriere et al., 2004);
- Prevention of DNA-damage (Zielińska-Przyjemska et al., 2012);
- Induction of antioxidant: paraoxonase 1 (Schrader et al., 2011**)**, heme oxygenase 1 (Kikuchi et al., 2005) and phase II detoxifying enzymes (Krajka-Kuźniak et al., 2013; Saw and Kong, 2011);
- Gene regulatory activity (*e.g.* Nrf2-dependent signal transduction pathway) (Na and Surh, 2014; Schrader and Rimbach, 2011);
- Anti-inflammatory activity (*e.g.* inhibition of cyclooxygenase-2) (Reddy et al., 2005; Ruiz et al., 2013; Zielińska-Przyjemska et al., 2016; ;
- Anticancer activity (Govind et al., 2011; Kapadia et al., 1996).

Application of betalains as a natural pigment in food

Stability of betalains goes over a broad pH range from 3 to 7, which makes them particularly suitable for use in a broad array of low-acid and neutral foods. Their exploitation started in the 1970s, when *B. vulgaris* was proposed for use in low-acid foods such as meat and dairy products (Elbe and Maing, 1973).

The colourant is commercialized as a liquid concentrate and spray-dried powder with a colour strength or tinctorial power (at 535 nm/1 % solution) ranging between 2 and 5. It is mainly used in dairy products, yogurt, puddings, ice creams, frozen fruit desserts, gelatins, beverages, confectioneries, candies and baked foods (Obón et al., 2009).

In different geographical regions, other plants may be used to provide colourants, such as Amaranthus pigments, which are added to beverages, bread, and other foods in the southwestern United States, Mexico, Bolivia, Ecuador, Argentina and China (Cai and Corke, 1999). Recently, a successful industrial process for juice production from *Opuntia ficus-indica* (L.) Mill. has been established, opening up the possibility of commercial use of this plant species from semi-arid areas (Mosshammer et al., 2006; Mosshammer et al., 2007).

Conclusion

The color of food is a significant factor in industry. However, to ensure the food safety, it is important to add up safe and healthy ingredients into the food products. The artificial colors may cause some allergic reactions; their long-term use is considered to be a cause of carcinogenesis. Therefore, the possibility of using inexpensive, natural plant pigments of betalains is significant. Betalain stability is affected by long term exposure to oxygen/air, light in the presence of oxygen, high temperature and water activity (wa) but it is highly stable in the presence of low moisture. Its pigments show more stability towards pH and temperature and are suitable for those foods in which anthocyanin cannot be used as a coloring agent. Betalains can enhance the flavor and color of icecream, jellies, jams, desserts, sauces, sweets, tomato paste and breakfast cereals. They have some properties which are beneficial to our health. Betalains exibit antioxidant, antimicrobial, antilipidemic and anticancer activity.

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Conflict-of-Interest Statement:

Authors declare no conflict of interest.

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