Betanin: A Bioeconomy Insight into a Valued Betacyanin

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ABSTRACT: Sourced so far mostly from beet root juice, betanin is a red-violet natural colorant increasingly used by the food, beverage, and nutraceutical industries. The production and purification methods, emerging sources, extraction technologies and applications are reviewed. Moreover, we provide an updated bioeconomy perspective on this valued betacyanin whose supply and applications, as we argue in this study, will rapidly expand.

KEYWORDS: Betanin, Natural red, Pigment, Betalain, Opuntia, Beet root

Late re-evaluated and reapproved as a food additive in the European Union (labeled E162), betanin (betaninid-5-O-β-glucoside) is a red-violet betalain widely used as a food colorant and, more recently, also as an antioxidant dietary supplement. Betalains are water-soluble natural pigments containing betalamic acid (the chromophore), which conjugates either with cyclo-3,4-dihydroxyphenylalanine (DOPA) to produce red-violet betacyanins or with different amino acids or amines to afford yellow-orange xanthins.2

Added to food and beverage products (for example, at 0.5% level in ice creams), betanin-based extracts impart a striking red color without altering the flavor, whereas the pigment stability between pH 3 and 7 makes its use particularly attractive for the food industry, transitioning from synthetic to natural colorants. In 2007, indeed, scholars in psychology and child health in the U.K. published the outcomes of randomized, double-blind, placebo-controlled trials on artificial food additives and hyperactive behavior in 300 children aged three or eight/nine.3 Findings revealed a significant increase in hyperactive behavior, including impulsive behavior and loss of concentration, in children consuming artificial additives in both age groups. This led the U.K. Food Standards Agency (which had funded the study) recommending to food and beverage manufacturers the voluntary removal of six artificial colorings, which are the six main food and beverage synthetic colorants (allura red, E129; ponceau 4R, E124; tartrazine, E102; sunset yellow, E110; quinoline yellow, E104; and carmoisine, E102).4

Following deliberation of the EU Parliament in 2008,5 in 2010, the European Commission required food and beverage manufacturers to insert the warning "may have an adverse effect on activity and attention in children" on the label of beverage and foodstuff products containing any of the six food color additives linked to hyperactivity in children.

As threatening as it may sound for European consumers, in a thorough recent account on betalain as natural colorant, Ferreira and co-workers emphasized how several food colorants that are allowed in the European Union by the Council regulation (EC) 1129/2011, including carmoisine (E122), amaranth (E123), and ponceau 4R (E124), are no longer permitted in the United States. This spurred the industry’s interest for natural colorants and, in particular, for those capable, likewise to lycopene, to synergistically provide color and health benefits.

Reviewing the impact of extraction and processing conditions on betalains and comparing their properties with anthocyanins, scholars in Canada lately reported how information about the effects of processing on betalains physicochemical properties and stability is scattered.6 This study provides a unified and updated bioeconomy perspective on betanin, aimed at bioeconomy scholars and practitioners. In parallel, the impact of processing on color and anthocyanin content of blueberry (Vaccinium spp.) fruits was analyzed,7 contributing to selecting the best manufacturing procedure to prepare blueberry-derived products keeping their healthy properties.

Production, Purification, and Emerging Applications. The betanin molecule has a high molar extinction coefficient ε

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= 6.56 × 10^4 \text{ L mol}^{-1} \text{ cm}^{-1} \text{ in the visible region, and the spectrophotometric method to quantify it in solution, via Beer’s law, taking into account light absorption at the maximum absorption wavelength (then considered 530 nm) was described as early as 1938 (today’s value for } \lambda_{\text{max}} \text{ is 536 nm).}^{10} \text{ Recently, scholars in Egypt introduced the use of betanin as a novel colorimetric sensor for the naked eye detection of copper ions in water samples, providing a fast and low-cost colorimetric determination of Cu}^{2+} \text{ ions, simply based on the coordination between the copper ion and betanin dye, which leads to a large change in absorbance intensity (Figure 1).}^{11} \text{ Betanin shows excellent antiradical activity because of both the good electron donor properties of its two main molecular moieties (betalamic acid and DOPA) and their strong electronic conjugation. Its high free radical-scavenging activity is associated with the ability to act as hydrogen and electron donors, which is particularly high at neutral and basic pH, namely, going from the cationic form to mono-, di-, and trideprotonated species present in increasingly basic solution (Figure 2).}^{12} \text{ Present in many plants, fruits, and flowers, betanin shows also distinctive biological activity, which includes scavenging of reactive oxygen species, gene-regulatory activity, and enzyme-inducing and antioxidant defense mechanisms, as well as the prevention of LDL oxidation and DNA damage.}^{13} \text{ At neutral pH and depending on concentration, betanin exhibits a stimulating or inhibitory effect on the chlorination activity of the heme enzyme myeloperoxidase (producing cytotoxic HOCl from } \text{H}_2\text{O}_2 \text{ and chloride), thereby acting as modulator in all inflammatory conditions mediated by HOCl.}^{14} \text{ Moreover, the hydrolytic degradation of betalains can be partially reversible, causing the regeneration of betanin. This process occurs by Schiff base condensation between the nucleophilic amine of cyclopropane-5-O-glycoside and the aldehyde group of betalamic acid.} \text{ In 1980, R. A. Harmer, an industrial chemist at a food company, published a first comprehensive study on the occurrence and chemistry of betanin in light of food coloring applications.}^{15} \text{ Betanin was reported to be stable (bluish-red color) between pH 3 and 7, a very broad pH range, turning to yellow-brown only at basic pH. Water was also found to promote degradation of the aldime bond between betalamic acid and DOPA, which is due to hydrolysis (favored at higher pH). The other molecular degradation mechanisms, favored at lower pH, include decarboxylation and dehydrogenation (Figure 3).}^{16} \text{ The hydrolysis degradation mechanism explains why red beet (and cactus pear) whole extracts, which contain high levels of pectin, are considerably more stable than purified beet pigments.}^{17} \text{ Pectin hydrocolloid indeed lowers water activity (a similar beneficial effect is exploited to stabilize anthocyanins).}^{18} \text{ Heating, air, and light also cause change of the red color to light brown. The temperature dependence of color degradation follows a first-order kinetics governed by the Arrhenius equation (Figure 4).}^{19} \text{ It is known since the early 1970s that betanin heat degradation can be significantly slowed by the addition of 1% citric acid, which acts also as a preserving of color stability, by chelating metal ions, among which iron is particularly prone to decompose the betacyanin. Eventually, in 2006, Stintzing and co-workers reported that the addition of a similar amount of ascorbic acid (vitamin C) to the betalain extract obtained from the purple pitaya fruit results in even better stabilization of the pigment against heat, eventually making it suitable for food coloring.}^{20} \text{ In industry, betanin is obtained via conventional solid–liquid extraction from beet juice or root macerates, using water as a solvent and water as an extracting agent. The process is carried out under mild conditions to prevent degradation of the pigment. Extraction is followed by concentration, purification, and, if necessary, stabilization with ascorbic acid. The final product is a concentrated solution of betanin, which is then used as a food coloring agent.}^{21} \text{ The use of betanin as a food coloring agent is regulated in many countries, and its consumption is considered safe according to the latest scientific evidence.}^{22} \text{ In conclusion, betanin is a versatile pigment with a wide range of applications in various food products. Its unique properties, including its color stability, antioxidant activity, and biological activity, make it a valuable addition to the food coloring arsenal.}^{23} \text{ Future research should focus on optimizing the extraction and purification processes, as well as on exploring new applications for betanin in the food industry and beyond.}^{24} \text{ DOI: 10.1021/acssuschemeng.7b04163} \text{ ACS Sustainable Chem. Eng. 2018, 6, 2860–2865}
solvent at room temperature. The beet roots are pressed, typically in the presence of citric acid, ultrafiltered to concentrate, and pasteurized.

The extracts thereby obtained contain 0.5–1% of betanin along with a small quantity of yellow vulgaxanthin plus significant amounts of sugars and other compounds. The enzymes present in the extract, such as polyphenoloxidases (PPOs), also play an important role. This is shown, for example, by freeze-drying the beet root juice immediately after its extraction (initial pH 6), which results in browning due to the increase in the concentration of PPOs catalyzing the oxidation of o-hydroquinones to o-quinones (which polymerize producing brown and red pigments related to fruit browning). 21

In industry, the beet root juice is also spray-dried onto a carrier like maltodextrin, yielding a final betanin percentage of about 0.3–0.5%. 22 Eventually, along with fresh beet root juice, commercial lyophilized (freeze-dried) beet root and commercial betanin diluted with dextrin are the main betalainic commercial sources. 23 Another important factor responsible for enhanced stability of betalains is the concentration of the pigment molecule itself, as observed in the case of betacyanins extracted from Opuntia fruits. 24

Attempts to concentrate aqueous extracts containing betalains in low amount, via chromatography on a preparative scale, go back to the early 1980s: using macroreticular, cross-linked, nonionic resin adsorbent, the betanin pigment concentrates on the adsorbent, being removed by elution. 25

Important progress was reported in 2008, introducing hydrophobic adsorbent in which betalains will associate with the organically modified resin via “pi-stacking”, while allowing undesirable compounds to pass. 26 Remarkably, the nutritional profile of such a pigment-enriched (41% betalain) beet root extract is significantly richer than a conventional extract obtained via simple extraction in water, as it contains also a large number of nutritionally important compounds such as vitamin C, nonprotein nitrogen, tryptophan, and numerous minerals. 27

Indeed, the latter beet root betalain-enriched extract (trade named Racerunner) supplemented to 13 competitive male runners in two double-blind, crossover, randomized trials (BRC and control) led to significantly improved exercise performance (3% lower heart rate, 15% lower rate of perceived exertion, and a 14% lower blood lactate concentration compared to the control). 28 In 2010, a group of researchers from Poland found that individuals consuming betalain-rich extract (35–100 mg capsules, twice daily for 10 days) experienced a clear reduction of discomfort (joint pain) associated with osteoarthritis. 29 Six years later, new evidence showed that consuming beet root juice before strenuous sporting enhances performance and shortens the recovery time. 30

Comparing seven different methods to purify betanin in fresh beet root juice, food-grade beet root powder, and betanin standard diluted in dextrin, in 2012, scholars in Brazil demonstrated that food-grade beet root powder had the largest amount of violet-red impurities, likely formed during processing. 31 In further detail, the team proved that even though ion-exchange chromatography is the best method to separate betanin from its epimer isobetanin, both reverse-phase HPLC and column chromatography provide excellent separation at much higher speed.

Today, improvements in processing and extraction techniques, as well as in storage conditions, enable the production of betalain pigments of enhanced stability, resulting in commercial betanin extracts having a shelf life of 2 years, stored in a tightly closed container kept away from heat and sunlight. The degradation induced by light is, indeed, enhanced in the presence of oxygen, 32 making it possible to reduce light-induced degradation by addition of ascorbic acid at levels of 0.1–1.0%. 33

Based on these advances and on further innovation in separation technology in mid-2016, a food colorant company in the U.S. launched the first heat-stable betanin-based food colorant capable of retaining its vibrant red color even at the high cooking temperature of baking goods. 34 The new purification technology leads to a purified betanin vegetable juice (trade named SupraRed) which avoids the unwanted Maillard reaction causing browning and affecting color. Available in both liquid and powder forms, the product contains beet juice, maltodextrin, citric acid, and ascorbic acid only. 35

Emerging Sources and Extraction Technologies. Conventional betanin extraction is based on maceration carried out by stirring the plant betalain source immersed in water or in a water–ethanol mixture kept in a closed vessel. The process is not efficient, but chemical degradation is minimized being conducted at room temperature. 22 Recent research efforts applying innovative techniques, such as microwave-assisted extraction (MAE), led to dramatically enhanced extraction yields. For example, a two-step MAE process affords extraction percentages higher than 50% of the maximum extractable

| Table 1. Color Properties of Produced Opuntia spp. Fruit Extract and Commercial Red Beet Concentrate<sup>a,b</sup> |
|---|---|---|---|---|---|---|---|---|
| **Color parameters** | **Color strength** | **L*<sup>a</sup>** | **a**<sup>b</sup> | **b**<sup>c</sup> | **C**<sup>d</sup> | **h**<sup>e</sup> | **ΔE**<sup>f</sup> | **RGB color** |
| **Opuntia spp. fruit extract** | 5.0 | 28.7 | 62.4 | 24.6 | 67.1 | 0.38 | 0.0 | Red |
| **Red beet concentrate** | 5.2 | 69.5 | 57.9 | -1.0 | 57.9 | 359 | 8.4 | |  

<sup>a</sup>Chroma = saturation of color; hue angle = chromatic perceived color determined by the wavelength.  
<sup>b</sup>Reproduced from ref 37, with kind permission.

Figure 4. First-order plot of color (Hunter “a/b”) degradation in beet root puree at 50, 90, and 110 °C. [Reproduced from ref 19, with kind permission].
pigments, whereas the amount of pigments extracted with ethanol:water (1:1) at 23 °C or at 80 °C were considerably lower (10–20%).36

More recently, an even higher extraction yield (83% of maximum extractable pigment) was obtained from cactus pear using high pressure CO₂-assisted extraction.37 Likewise to commercial betanin extract from beet root (red beet concentrate), the latter Opuntia spp. extract presented high color strength, with a total color difference ΔE of 8.4. They are, thus, easily distinguished by human eye, with the Opuntia spp. fruit extract being the reddest colorant.

The team went further and used the color parameters recommended by the Commission International de L’Eclairage to determine the color coordinates L* (luminosity, from black to white), a* (coordinate from red to green), and b* (coordinate from yellow to blue). Due to their red color, both Opuntia spp. and Beta vulgaris L. extracts had positive a* values, whereas the b* parameter showed greater dispersion in the Opuntia extract reflecting more yellowness color than blueness (Table 1). Dutarte and co-workers concluded that Opuntia spp. fruit extract is a promising natural betalain food colorant.

In 2005, Stintzing and co-workers in Germany were the first to demonstrate the feasibility of betalain extraction from the fruits of Opuntia ficus-indica (OFI) for food coloring purposes as a viable alternative to red beet.38 Obtained directly on a pilot plant scale starting from 20 kg of fruit, neither the red and yellow extracts obtained from red and yellow OFI fruits grown in Sicily exhibited negative sensorial impact nor high nitrate levels, but they did offer a broad color range. In closer detail, after squeezing the fruits, the preclarified juice at pH 6 was adjusted to pH 4 with citric acid followed by pectin hydrolysis with pectinase enzyme. Filtration gave the clarified juice that underwent thermal preservation (pasteurization).

No browning was observed during juice production, including in the pasteurization step, due both to acidification and to the specific amino acid composition of prickly pear. In place of beet root, often suffering from geosmine (earth smell), the Opuntia red extract has several advantages, such as neutral smell and absence of nitrate. Similar good results were lately reported by scholars in Colombia studying the stability of Opuntia dillenii extracts added to foodstuffs at different pH and temperatures.39

Stintzing’s team concluded that the process developed was suitable for scaling up to industrial-scale production, especially because the cactus pear juice could be processed into concentrates and fruit powders, which are easier to handle during transportation and storage. Indeed, the process has been adopted by industry, even though the juice extracted, because of its exceptional nutritional value, is not used for coloring purposes but rather sold as a nutraceutical product by a German company which offers the product to sports practitioners and for those who suffer from physical exhaustion.40 From a bioeconomy viewpoint, it is relevant to notice that the packaged juice was sold online by the end of 2017 at €3.98/100 mL, showing the entity of the value addition process upgrading the OFI fruit (currently traded in Italy at €1.60/kg).

Bioeconomy Aspects. Driven by a profound change in consumers’ demand across the world, the food industry is redefining its supply aiming to promote healthfulness through its renewed products. It is enough to review the outcomes of a recent survey to learn that half of the U.S. consumers have “changed their purchasing or engaged friends, family or coworkers as a result of reading or seeing something that examined the food system and/or commonly held beliefs about it”; and while taste and price consistently have been the top two factors that impact consumers’ food and beverage purchases (84% and 71%, respectively), healthfulness in 2016 almost entirely closed the gap with price, rising from 48% of consumers in 2006 to 64% ten years later, almost a 20 percentage-point increase.41

In China, the health food market was recently estimated to grow at an average annual rate of 10–15%, with consumers’ mind-set about health food gradually shifting from being seen as luxury goods to ordinary consumer products. The Outline of the Programme for Food and Nutrition Development in China (2014–2020) mentions that the government will make the development of health food and nutrient fortified food one of its priorities.42 In Russia, in 2017, sales of organic food reached unprecedented levels (hitting $250 million), with the government envisioning a future for the country becoming a leading supplier of healthy and high-quality food products in the world market in which their demand is steadily growing.

These are just three selected examples of a widespread and rapidly increasing societal demand for natural ingredients replacing synthetic chemicals in food, beverage, cosmetic, and nutraceutical products. As a result, the global market of natural food colorants, valued $1.31 billion in 2015, is expected to grow at over 5% annual rate between 2016 and 2021.43

Growth is further compounded by the rising demand for processed and packaged food, driven by the growing urban population in huge economies and by chemical technology advancements, which are eventually making possible the incorporation of natural colors into all sort of food products (see above the case of the SupraRed colorant) without color degradation, even during cooking or heat processing.

Indeed, processed foods such as beverages, confectionary, bakery, meat, dairy, and frozen products intrinsically drive demand for colorants. Consumers like colors even more than taste,44 and since during processing the original color of food products turns out to lighten or to brown, colorants are added to restore the color and boost consumer’s preference. Accordingly, the EU legislation on food additive colors defines them as “substances which add or restore color in a food, and include natural sources which are normally not consumed as a foodstuff as such and not normally used as a characteristic ingredient in food”.45

In general, betanin-rich extracts (so far commercially obtained from red beet, but in the future, also from fruit byproducts rich in betalin, similarly to grape’s anthocyanins, which are extracted from grape residues) are sold on the basis of betanin content. For example, by the end of 2017, powdered betalin ready for use as natural food colorant was sold online by an Italian food colorant manufacturer at €130/kg.46 Globally, revenue from the beet root powder market (90,000 tons by 2016) was valued more than $ 15 billion in 2016, forecasted to expand at a compound annual growth rate of 5% over the 2017–2027,47 to reach more than 1.1 million tons by 2027.

Outlook and Conclusions. Because of rapid recent progress in extraction, purification, and stabilization technologies, betain extracts from botanical sources can now be effectively used as red colorant in a broad variety of food and beverage products, including baked goods (and even at low usage rate, thereby reducing the overall cost of use).
Furthermore, numerous new nutraceutical products will comprise among the active ingredients these powerful antioxidant, anti-inflammatory, antifatigue, and anticarcinogenic agents.

To meet the increasing demand of betanin-based extracts of high quality, the old extraction processes, based on solid–liquid extraction with acidic water, will be replaced by advanced and entirely clean technologies, such as supercritical fluid37 and solvent-free microwave-assisted extraction.36

One way to achieve the required cost reduction through enhanced supply is to expand betanin botanical sources from beet root to more abundant and faster growing plant and fruit sources, as would happen sourcing the betalain from Opuntia fruit and, even better, from the fruit peel. Though containing a lesser betanin amount than beet root,39 sourcing this important colorant from Opuntia in addition to the current industrial practice to extract the pigment from Beta vulgaris L. would provide significant advantages. Contrary to beet root, mostly grown in the U.S., Russia, Germany, Poland, and France, which slowly affords its high betanin content after two years of cultivation, the Opuntia plant is ubiquitous in semiarid countries, giving its numerous fruits twice a year between mid-June and the late November.

Remarkably, when extracting betanin from the peel of both red and white OFI fruits, the mild extraction process applied using microwaves, and not even adding water, affords an integral extract, whose other key components such as pectin and polyphenols are efficiently extracted and kept intact.36 This provides one with unprecedented stability in the new extracts, bottled in semitransparent plastic bottles without even excluding air, whose deep red color lasts unvaried for several months, thereby also addressing in a natural and straightforward way the key stability issue of betalain extracts for future applications.36

In conclusion, as the market for natural food colors continues to grow at quick rate, numerous new companies extracting said valued colorants from multiple botanical sources with the above-mentioned green chemistry technologies will emerge. This study will hopefully assist bioeconomy practitioners engaged in the transition from synthetic to natural red colorants beyond anthocyanins.

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**Notes**

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