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review

Complex Biochemistry and Biotechnological Production of Betalains

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Summary

The demand for natural food colourants is increasing because of public awareness of their health benefits. Betalains are nitrogen-containing plant pigments whose colours range from red-violet betacyanins to yellow betaxanthins. They are used for colouring dairy products, meat and frozen desserts. Betalains have attracted additional interest because of their antioxidative, anti-inflammatory and anticarcinogenic properties. The main source of commercially produced betalains is red beet root, but alternative sources are found in plants from the Amaranthaceae and Cactaceae families. Another alternative source is plant cell culture in bioreactors, although optimization of pigment production seems necessary. In this paper we synthesize the results of recent studies on betalain biosynthesis, chemical properties, sources, biotechnology and applications.

Key words: antioxidants, betacyanins, betaxanthins, food colourants, pigments, reactors, red beet

Introduction

The addition of colourants to foods stretches back to ancient Egypt around 1500 BC, and their use expanded greatly after the Industrial Revolution (*1*). In modern times, health concerns have shifted public preferences towards stricter safety regulations, organic and natural food production and led to the ban of a large number of synthetic colourants previously in use. For example, the number of colourants widely used in the USA since 1970 has fallen from 700 to only 36, of which 7 are synthetic and 26 are natural (*2*). In 1994 the European Union authorized the use of just 43 colourants as food additives: 17 synthetic, 13 natural and 13 nature-identical (*2*). These compounds carry the E denomination to indicate their approved status. As a result of this shift in public attitudes and laws, plant pigments have been increasingly used to substitute for synthetic colourants in the food, pharmaceutical and cosmetic industries. The global market in colour additives was estimated to be $940 million in the 1990s, of which 42 % of additives were synthetic, 27 % natural, 20 % nature-identical and 11.4 % caramel colours. The annual growth of the natural colour market has been predicted to be 5–10 % in the future, twofold higher than that of synthetic colours (*1*).

Betalains are water-soluble, nitrogen-containing plant pigments whose colours range from red-violet betacyanins to yellow betaxanthins (*3*). Like anthocyanins, betalains serve as optical attractors for pollinators and for seed dispersion. Their production is induced by UV radiation in *Mesembryanthemum crystallinum* L. (ice plant) and by viral infection in *Beta vulgaris* L. (red beet), suggesting that betalains also have radioprotective and antiviral activity (*4*,*5*). The recent increase in the interest in betalains stems from their desirable chemical, medical and pharmacological properties: they are chemically stable over a broader pH range than anthocyanins, they are powerful antioxidants, and they have anti-inflammatory and anticarcinogenic activity (*6–8*). The most abundant of all betalains is betanin, found in *B. vulgaris* roots. In Europe, it is listed as E162 and is used in a variety of

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processed foods such as dairy products, frozen desserts and meat (*9,10*). Currently, the main sources of betalains are crop plants, but researchers are investigating how to obtain comparative yields from alternative sources like cell suspensions or hairy roots grown in bioreactors (*11*).

The aim of the present review is to cover several aspects of these important pigments. First, classification and biosynthesis of the main betalains will be discussed. Traditional and biotechnological sources of betalains, efforts to improve their yield, their antioxidative properties and their possible beneficial effects on human health will be described. The review will conclude by discussing broader applications of the pigments.

Classification and Biosynthesis

While anthocyanins are present in virtually all members of Angiospermae, betalains are restricted to the suborder Chenopodineae within the Caryophyllales (*12*) and to some genera of the fungi Basidiomycetes (*13*). However, there are two exceptions: the Caryophyllaceae and Molluginaceae plants, which accumulate anthocyanins instead of betalains. Interestingly, these two pigment families are mutually exclusive and cannot be found together in the same plant. This has given rise to a considerable taxonomic debate (*12*). Plants accumulating betalains express at least some of the flavonoid biosynthetic enzymes and can accumulate significant quantities of flavonols, flavonoids, and in some cases even proanthocyanidins (*14*). A possible explanation for the lack of anthocyanins in Caryophyllales is that the promoter regions of the genes for dihydroflavonol 4-reductase and anthocyanidin synthase differ from those of anthocyanin-producing species (*15*). These enzymes catalyze the first committed step in anthocyanin biosynthesis from dihydroflavonols, namely the NADPH-dependent reduction of dihydroflavonols into leukoanthocyanidins. Anthocyanidin synthase catalyzes the next step and is responsible for the formation of coloured anthocyanidins from colourless leukoanthocyanidins.

More than 100 betalains are classified in two main groups: red-violet betacyanins and yellow betaxanthins. According to Strack, the former group is further divided into four subgroups and the latter one into three subgroups (Fig. 1) (*16*). Betalains share the same basic structure, with a substituted dihydropyridine serving as the chromophore with a system of conjugated double bonds. Betalains arise as immonium conjugates of betalamic acid with *cyclo*-3-(3,4-dihydroxyphenyl)-L-alanine (*cyclo*-DOPA), amino acids, or amines (Fig. 2) (*16*,*17*). The biosynthetic pathways consist of several enzymatic and spontaneous reactions (Fig. 3). Betalains are synthesized in the cytoplasm and then stocked in vacuoles that are present mainly in flowers and fruits and occasionally in vegetative tissues of plants (Table 1; *17–37*).

The complete biosynthetic scheme of betalains has been described in detail in reviews by Strack *et al.* (*16*) and Han *et al.* (*38*). The starting point is an amino acid tyrosine (Tyr), but tyramine may also be a starting point in the synthesis of some betalains (*39*).

The Tyr pathway (Fig. 3) begins by hydroxylation of Tyr to L-DOPA by the action of tyrosinase (EC 1.14.18.1), which belongs to a group of copper-containing bifunctional enzymes involved in the hydroxylation of phenols to *o*-diphenols. Subsequently, DOPA can be converted in three ways: it can be oxidized by the same enzyme (EC 1.10.3.1) to dopaquinone, it can be cleaved and opened into 4,5-*seco*-DOPA in a reaction catalyzed by DOPA 4,5--dioxygenase (DOD), or it can undergo decarboxylation to form dopamine, in a reaction catalyzed by DOPA-decarboxylase. Dopaquinone spontaneously forms *cyclo*--DOPA, while spontaneous cyclization of 4,5-*seco*-DOPA yields betalamic acid, the central intermediate in the formation of all betalains.

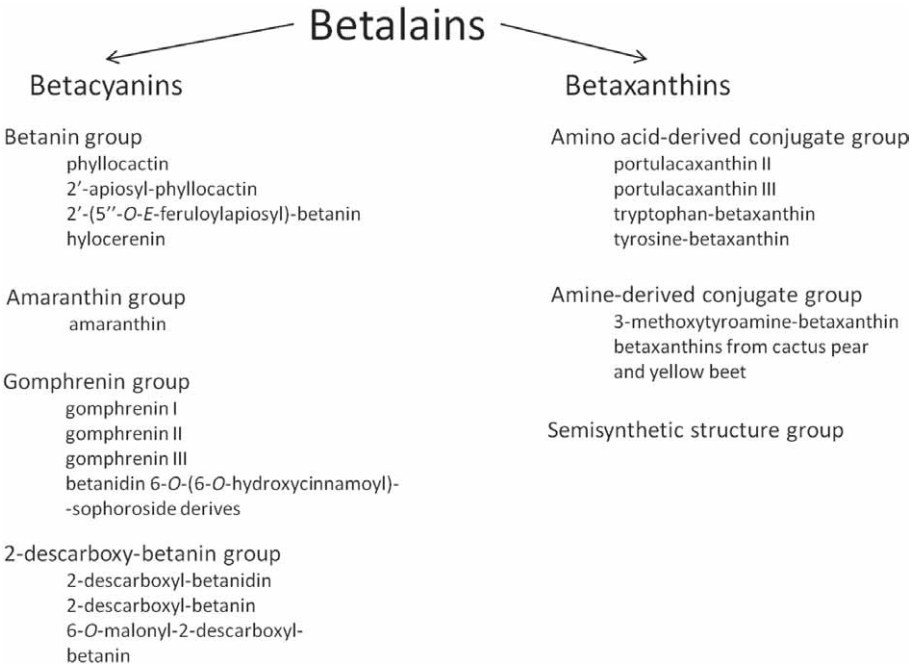

Fig. 1. General classification of betalains, according to Strack *et al.* (*16*)

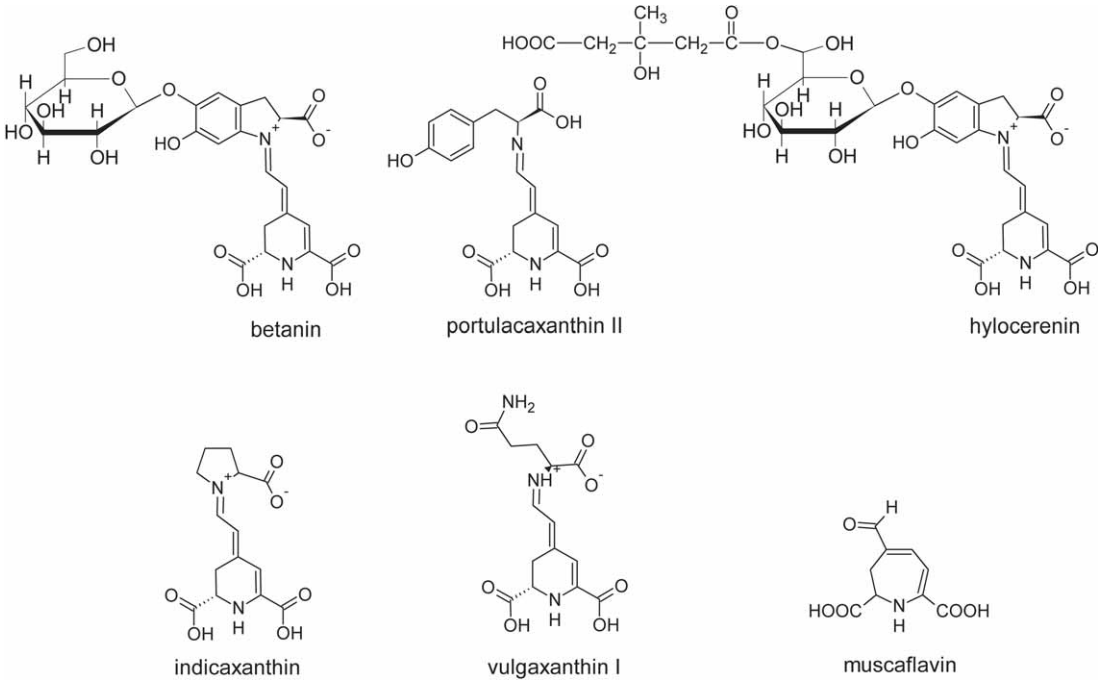

Fig. 2. Chemical structure of common betalains

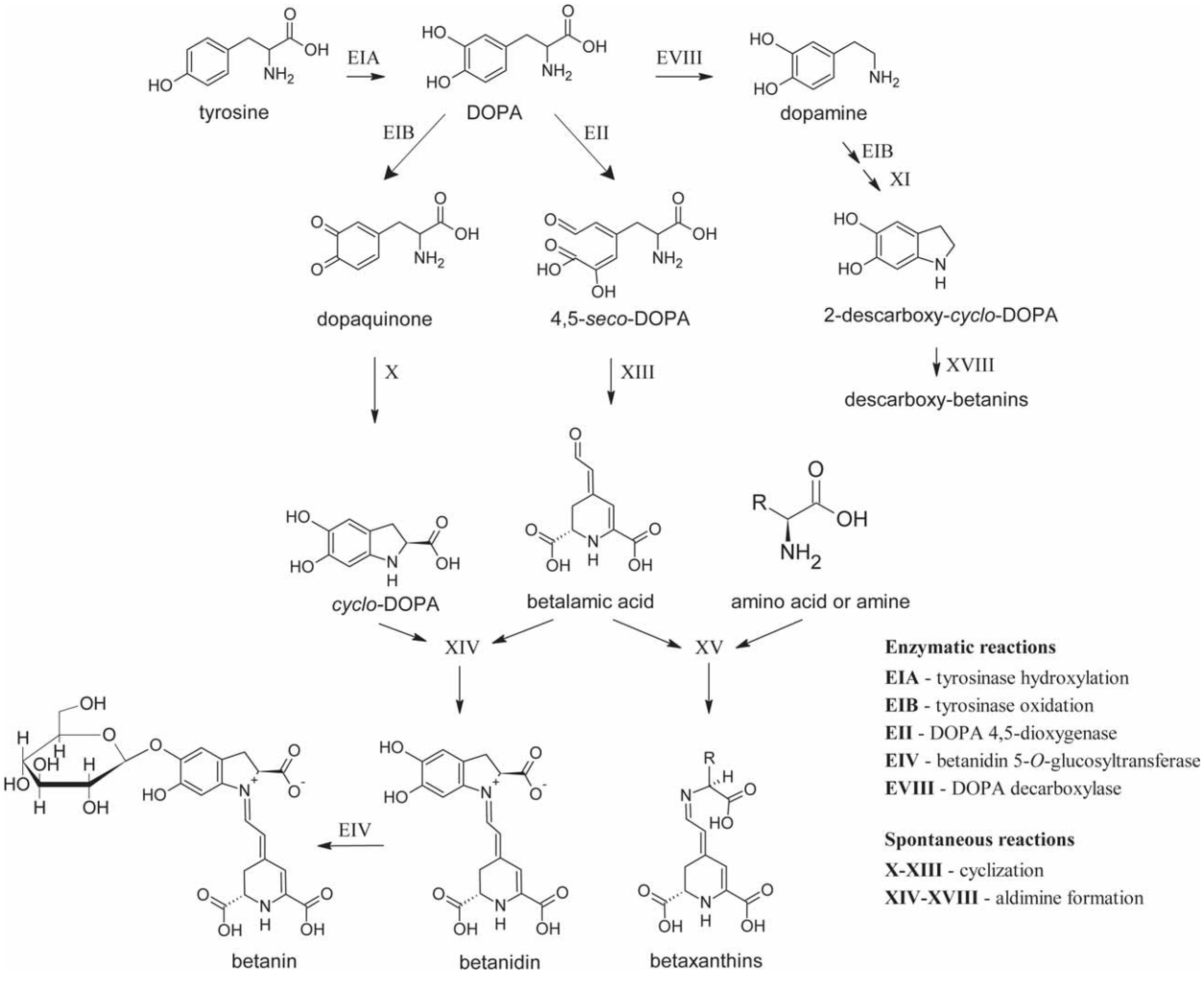

Fig. 3. Schematic representation of the biosynthetic pathway of some betalains

Table 1. The major betalain-producing plant species, modified after Moreno *et al.* (*18*)

Family	Species	Common name	Type of produced betalains	Refs.
Chenopodiaceae	*B. vulgaris*	red beet root	vulgaxanthin (I, II), indicaxanthin, betanin, prebetanin, isobetanin, neobetanin	(*19–22*)
	B. vulgaris L. ssp. *cicla* [L.] Alef.	Swiss chard	betaxanthins (20 different ones) betacyanins (9 different ones)	(*23*)
	Chenopodium rubrum	red goosefoot	vulgaxanthin I and II, amaranthin, celosianin, betanin	(*24,25*)
Cactaceae	*Hylocereus polyrhizus*	red-purple pitaya	betanin, isobetanin, phyllocactin, isophyllocactin, hylocerenin, bougainvillein-r-I	(*26–28*)
	Opuntia ficus-indica	cactus pear	hylocerenin and isohylocerenin, minor apiofuranosyl betacyanins	(*29,30*)
Amaranthaceae	*Amaranthus* sp.	amaranth	amaranthine, isoamaranthine	(*31,32*)
Portulacaceae	*Portulaca grandiflora*	moss rose	dopaxanthin, vulgaxanthin I, portulacaxanthin II, miraxanthin V, indicaxanthin	(*33,34*)
Aizoaceae	*Mesembryanthemum crystallinum*	ice plant	betacyanins, mesembryanthin	(*4*)
Nyctaginaceae	*Bougainvillea* sp.		gomphrenin I, derivatives of bougainvillein V, various betaxanthins	(*35–37*)

The critical step in the production of betalamic acid is catalyzed by DOD, one of the most important and well-characterized enzymes in betalain biosynthesis. The enzyme was first purified and its gene characterized in the mushroom *Amanita muscaria*, where it was shown to be composed of 4–7 identical 22-kDa subunits (*40,41*). The enzyme cleaves double 2,3- and 4,5-extradiol aromatic rings. The 2,3-extradiol-cleaving activity generates the fungal-specific betalain muscaflavin (*42*). In higher plants, DOD was first purified and characterized in *Portulaca grandiflora* and afterwards in *Opuntia ficus-indica*, *Mirabilis jalapa* and *Phytolacca americana* (*43–46*). Interestingly, the enzyme from higher plants shares no homology with the enzyme from *A. muscaria* and it has only 4,5-extradiol-cleaving activity (*43*). In all plants analyzed so far, DOD is encoded by a single-copy gene, while *Ph. americana* contains two copies (*46*). The promoter region of the gene contains MYB, bHLH and environmental stress-responsive transcription factor binding sites, suggesting complex regulation of the genes by abiotic and biotic stress (*46*).

From betalamic acid, several reaction pathways are possible. One possibility is a spontaneous condensation of *cyclo*-DOPA with betalamic acid to form betanidin, the aglycone form of most betacyanins. Betanidin is glycosylated on the DOPA side with the aid of betanidin-5--glucosyltransferase, to form betanin (*16*). As is the case with many other plant natural products, betalains are stored in the vacuole as glycosides. Alternatively, *cyclo*--DOPA can be glycosylated by *cyclo*-DOPA-5-glucosyltransferase to form *cyclo*-DOPA glucoside. The product of this reaction can condense with betalamic acid to form betanin directly without passing through the betanidin intermediate (*47,48*).

Subsequently, spontaneous condensation of betalamic acid with an amino acid or amine, involving the formation of an aldimine bond, generates betaxanthins like indicaxanthin or vulgaxanthin (*49*). A combination of betalamic acid with 2-descarboxy-*cyclo*-DOPA will result in the formation of 2-descarboxy-betanidin, a minor betacyanin found in the yellow beet (*50*). If betalamic acid is back-condensed with tyrosine, the starting molecule of the pathway, tyrosine-betaxanthin (portulacaxanthin II) is generated. This compound is found in *Portulaca* sp. and other species (*39,51*).

Several *Amanita* species from Basidiomycota fungi contain the betaxanthins muscaaurins I–VII, responsible for the characteristic red-orange colour of the caps. The first two are biosynthesized from nonprotein amino acids, ibotenic acid and stizolobic acid, while the other muscaaurins are derivatives of common proteinogenic amino acids (*52*).

The tyramine-derived betacyanin biosynthetic pathway was proposed by Kobayashi *et al.* (*50*). Betacyanins and betaxanthins originating from this pathway were identified in plants such as *B. vulgaris* (yellow beet root), *Celosia* sp. inflorescences or *Mirabilis jalapa* (*50,53,54*). The starting molecule, tyramine, is directly hydroxylated to dopamine and then oxidized to dopamine-quinone. Dopamine-quinone is converted to 2-descarboxy-*cyclo*-DOPA, which further condenses with betalamic acid to form 2-descarboxy-betanidin. The existence of another branch of the same pathway has been proposed by Gandia-Herrero *et al.* (*39,51*). In this branch, tyramine can be attached immediately to betalamic acid or it can first be hydroxylated to dopamine in a reaction catalyzed by hydroxylase-type tyrosinase, and then attached to betalamic acid. The dopamine-betaxanthin may be oxidized further by a tyrosinase with phenoloxidase activity to yield dopamine-betaxanthin-quinone, which may ultimately be converted to 2-descarboxy-betanidin *via* intramolecular cyclization. However, definitive proof of such a pathway is still missing.

Commercial Sources of Betalains

There are several sources of betalain pigments (Table 1) and the most important one is *B. vulgaris* root, which produces mainly betanin. Crop yield is 50–70 t/ha, with betanin content ranging from 0.4 to 20 mg/g of root.

Due to the high yield of betanin from this crop, it has been the only one commercially exploited for betalain production (*10*). Attempts to increase the betanin content of this crop involved the addition of nitrates to the soil in the form of ammonium nitrate or sulphate, as well as leaf application of Fe and B (*55*). Fertilization of table beet plants by ammonium nitrate in combination with spraying of Fe, K, B, Fe+K, and Fe+B increased the leaf anatomical dimensions, while spraying with Fe+B or B alone in combination with fertilization with ammonium sulphate or ammonium nitrate increased the contents of betanin and root anatomical traits. Despite its high betanin content, *B. vulgaris* root has several drawbacks (*10*). It has a limited pigment spectrum and adverse flavour due to geosmin (4,8a-dimethyldecalin-4a-ol) and various pyrazines. It contains a high nitrate level, which may form carcinogenic nitrosamines in the human body when ingested. Moreover, there is a risk of carry-over of soil microbes from the raw material during betanin production. These shortcomings prompted a search for more convenient sources of the pigments. In the plant family Amaranthaceae a total of 16 kinds of betacyanins and 3 kinds of betaxanthins have been identified and elucidated (*32, 56,57*). The genotypes of the *Amaranthus* and *Celosia* genera were found to encode superior pigments, better agronomic traits and higher biomass yield. However, *Amaranthus* sp. contains saponin (*58*) and *Celosia* sp. contains high concentrations of dopamine (*53*). The presence of these chemicals may restrict their global acceptance (*56*). In semiarid regions, important sources of betalains can be found in the Cactaceae family, specifically in the genera *Opuntia* and *Hylocereus* and in some *Mamillaria* species. *Opuntia stricta* Haw. fruits showed a betalain level of 0.8 mg/g of fresh fruit, which is higher than in some commercially used *B. vulgaris* varieties. *Opuntia* fruit shows adequate nutritional properties, including high levels of calcium, magnesium and vitamin C, and it contains interesting functional compounds like quercetin (*59–61*), which argues for its use over the *B. vulgaris* root (*62*).

Sources of yellow betaxanthins may be found in Swiss chard and in yellow beet, albeit at a lower level than in *B. vulgaris* root. However, the intensely coloured juices from these plants can be mixed with red juice from *B. vulgaris* to produce a wider colour palette (*10*).

Not all plant species that produce betalains can be exploited: *Phytolacca americana* L. (the deeply red pokeberry) is another source of betacyanins, but it has been banned as a food colourant because of toxic saponins and lectins (*63*).

Biotechnology to Produce Betalains

Biotechnology offers an opportunity to exploit cells, tissues, organs or entire organisms by manipulating them genetically to produce desired compounds and growing them *in vitro* (*64*). Such production offers several advantages over field cultivation: it is independent of geographical and seasonal variations, environmental factors, and political interference; in addition, it allows optimal and stable growth conditions, voluntary modulation of growth parameters, and constant quality control (*18,64*). Moreover, colourants produced in this way are classified as 'natural' rather than 'nature-identical', which increases their desirability to customers (*65*). Several plants have been introduced in *in vitro* culture with the purpose of studying the biosynthesis and eventual commercial production of betalains, such as *Myrtillocactus geometrizans* (Mart.) Console stem tissues, *Portulaca grandiflora* Hook. and *B. vulgaris* cell lines, and tissues of *Chenopodium rubrum* L., *Ph. americana*, and others (*24,66–69*). The betalain yield of several such production systems is presented in Table 2 (*11,24,66,70–77*).

Chemical synthesis of betalains does not seem feasible for commercial production of pigments due to the numerous steps involved in betalamic acid synthesis and the low yield of the final products (*78*).

Optimization of betalain yield

Since the yield of betalains from *in vitro* systems is lower than from crops, it has been necessary to optimize the pigment production either by selection of highly productive cell lines or by modification of growth conditions. Careful selection of *B. vulgaris* cells during subsequent subcultures allowed Akita *et al.* (*71*) to increase betalain yield nearly twofold.

Several studies have sought to increase betalain yield by modifying growth conditions. The type and quantity of betalains is affected, respectively, by growth regulators auxins (*e.g.* 2,4-dichlorophenoxyacetic acid, 2,4-D) and cytokinins (*e.g.* 6-benzylaminopurine, BAP) (*79*). In *B. vulgaris* calli, applications of these chemicals in different ratios provoked colour changes: from white/green calli to a variety of colours, ranging from yellow, orange and red to deep violet (Table 2) (*73,80*). Auxins increase polysomaty, and recently published results on ploidy levels in *Beta vulgaris* organs, *in vitro* hairy roots and calli derived from hairy roots suggest that the level of endopolyploidy may affect the type of betalains expressed and their yield due to the presence of multiple copies of genes involved in the biosynthetic pathways of these pigments (*81*). In this way, betacyanin production was increased using moderate auxin concentrations, such as 10^{-7} M for 2,4-D, $3{\cdot}10^{-5}$ M for indole-3-butyric acid and 10^{-5} M for indole-3-acetic acid. However, the fact that 2,4-D is a herbicide makes it inappropriate for use in culture medium to produce food colourant (*80*). This problem can be alleviated by transformation of *B. vulgaris* roots with agrobacteria. Insertion of genes from T-DNA into the host genome allows cellular growth and division without exogenously applied growth regulators (*82*). In *Ph. americana*, the addition of cytokinins actually decreased the accumulation of betalains. Feeding experiments revealed that BAP reduced the incorporation of tyrosine into betacyanins up to 50 % (*69*).

The best carbohydrate source is sucrose, and concentrations of up to 5 % offered the highest betalain yield in cell suspensions of *B. vulgaris* (*71*). Bhagyalakshmi *et al.* (*83*) observed that in hairy roots of *B. vulgaris*, the use of 3 % fructose, lactose, xylose, galactose or glycerol totally suppressed both growth and betalain synthesis. When *B. vulgaris* var. *altissima* (sugar beet) was transformed with *Agrobacterium tumefaciens* and fed with glucose or fructose, the growth was markedly reduced, but cellular content of betanin was increased (*74*).

Table 2. Selected *in vitro* plant systems for producing betalain pigments, adapted from Georgiev *et al.* (*11*)

Species	Type of culture	Pigments produced	Pigment content	Refs.
B. vulgaris var. Boltardy	hairy root	betacyanins	0.7 mg/g fm	(*70*)
		betaxanthins	1.3 mg/g fm	
B. vulgaris L. cv. Detroit Dark Red	cell suspension	betacyanins	22 mg/g dm	(*71*)
B. vulgaris L. var. Ruby Queen	hairy root	betacyanins	202 mg/L	(*72*)
B. vulgaris var. bikores monogerm	callus cultures:			(*73*)
	yellow phenotype	betaxanthins	4.3 μmol/g dm	
	orange phenotype	betaxanthins	12.2 μmol/g dm	
	red phenotype	betacyanins	11.2 μmol/g dm	
	violet phenotype	betacyanins	28.0 μmol/g dm	
B. vulgaris L. var. altissima	transformed callus	betacyanins	13.8 mg/g dm	(*74*)
Myrtillocactus geometrizans (Mart.) Console	stem tissues	betalains	–	(*66*)
Chenopodium rubrum L.	cell suspension	betacyanins	3–4 mg/g dm	(*24*)
Portulaca grandiflora Hook	cell suspension	betaxanthins	–	(*75*)
Portulaca sp.	cell suspension	betacyanins	5.3 mg/g fm	(*76*)
Mammillaria candida	callus culture	modified betaxanthins	–	(*77*)

dm=dry mass, fm=fresh mass

Macro- and micronutrients in the medium also affect betalain production. A reduction in inorganic nitrogen concentration to 30 mM, modification of the ratio of ammonium to nitrate to 1:14, reduction of the Zn ion concentration to 0.3 μM and removal of Co and Cu from Linsmaier-Skoog medium caused an increase in the betacyanin yield from 400 to up to 550 mg/L on day 14 (*71*). In B5 medium, in contrast, a decrease in nitrate concentration from 24.72 to 12.36 mM, changes in the nitrate/ammonium ratios, and the removal of ammonium did not affect betalain production or cell growth in *B. vulgaris* cultures (*84*). Cell culture using modified B5 medium instead of original one was less expensive both on the laboratory scale and in bioreactors. Interestingly, in 2001, Trejo-Tapia *et al.* (*85*) discovered that adding small quantities of Co (up to 5 μM) to B5 medium increased betalain production by 60 % in cell suspensions, but only if it was added at the beginning of the culture.

Elicitation

Elicitors are traditionally used to stimulate cellular response, which leads to the production of valuable secondary metabolites. In suspension cultures of *Portulaca* cells, betacyanin levels increased 1.3- and 1.5-fold over controls in the presence of two abiotic elicitors (20 μM of $CuSO_4$ and 100 μM of Fe-EDTA), and they increased 1.8- and 1.6-fold in the presence of two biotic elicitors (0.5 mg/L of β-glucan and 0.5 mg/L of chitosan) (*76*). More extensive analysis of elicitor-induced betacyanin production was performed in hairy roots of *B. vulgaris* (*72*). The best elicitors were found to be calcium, purified pullulan (a polysaccharide consisting of maltotriose units) and *Penicillium notatum* powder, all of which increased betalain yield up to threefold compared to the control. Pullulan, for example, gave yields up to 202 mg/L on day 10. However, elicitors suppress biomass accumulation, prompting the development of a new strategy of adding them at the late exponential growth phase instead of the early one, which proved useful for scaling up experiments in bubble-column bioreactors (*72*).

Recycling the cells

Techniques that are suitable for continuous production of secondary metabolites from plant cells without reducing their viabilities and biosynthetic capacities are of significant commercial value (*86*). Betalains are vacuole-stored pigments, but they can be forced out of the vacuole and into the extracellular medium by subjecting the culture to transient stress, which changes the permeability of cell membranes. Possible stress treatments include O_2 starvation for 16 h, which allowed an average extracellular production rate of pigment of 11.3 mg/(m·h) (*87*). Heat treatment at 42 °C for 45 min achieved a release of up to 15 % (by mass) of the product while having a minimal effect on cell viability (*88*). Higher temperatures were deleterious. A similar release of up to 15 % was obtained at ambient temperature (25 °C) by the addition of up to 20 mM $(NH_4)_2SO_4$ in the presence of 1 mM EDTA. Transient acidification of the nutrient medium to pH=2.0 for 10 min resulted in pigment release of 0.59 mg/g of dry mass (dm) during 24 h, corresponding to 36.8 % of the total pigment content (*89*). Permeabilization of the cell membranes using 0.7 mM Triton X-100® detergent during 10 min extracted 36 % of the pigments, while cell viability remained at 60–70 % (*90*). Thus, pH-mediated release of pigments seems to be a promising approach if factors that improve the viability of mature cells are taken into account. Combining two or more of the abovementioned stressors seems to be too damaging to the cells (*90*).

Bioreactors

Cell suspension cultures and hairy roots of *B. vulgaris* have been grown in shake flasks (*71,84*) and in several types of reactors. Until recently, the shake flask method

of cultivation offered higher betalain yield than cultivation of cells in bioreactors (*91*). Since then, higher yields have been obtained in a circulatory fed-batch bioreactor. This type of reactor was designed in order to sequester simultaneously betalains and peroxidase enzymes produced by *B. vulgaris* hairy roots, therefore improving usefulness of cultivating cells in bioreactors (*91*). In fluidized bed bioreactors, a betalain yield similar to that in shake flasks was achieved: 14–17 mg/g per day *vs.* 13–24 mg/g per day, respectively (*92*).

B. vulgaris cells in bioreactors, for example in a stirred tank type, are under much higher hydrodynamic stress than in shake flasks. This causes a high accumulation of extracellular proteins and polysaccharides, which changes the properties of the medium from Newtonian to pseudoplastic. To overcome this problem, air lift bioreactors with lower hydrodynamic stress than stirred tanks have been used (*93*). In studies with these reactors, the yield of betalains ranged widely from 4.2 to 35.0 mg/g dm, probably due to the mixing and aeration pattern present in each bioreactor. *B. vulgaris* hairy roots were also grown in a tubular-membrane bioreactor, where electropermeabilization of cell membranes and electrophoresis and diffusion of ionic products take place simultaneously (*86*). Higher betalain yield and cell viability were obtained by applying an oscillatory electrical field than by applying a continuous one.

A bubble-column type of reactor was used to test reverse sequestration and elicitation of betalain biosynthesis (*72,89*). In studies using this reactor, treatment with pullulan in the late exponential growth phase produced 280 mg/L of betalains from hairy roots. In the RITA® temporary immersion system with 15-minute flooding and 60-minute stand-by periods, hairy root cultures of *B. vulgaris* cv. Detroit Dark Red produced betalain at levels up to 18.8 mg/g dm (*94*).

Health and Food Industry Applications

One of the most interesting properties of betalains is their stability 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, where colouring with anthocyanins usually fails (*8*). Their exploitation started in the 1970s, when *B. vulgaris* was proposed for use in low-acid foods such as meat and dairy products (*9,95*). Undesirable properties of *B. vulgaris* juice require processing of the material, which now includes heat inactivation and oxygen removal to inhibit fast browning through polyphenol oxidase activity, as well as fermentation with bacteria to remove high nitrate concentrations (*96*). Geosmin and pyrazines are removed during the juice concentration, while soil-bound microbes are neutralized through pasteurization (*10*). 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 (*97*). It is mainly used in dairy products, yogurt, puddings, ice creams, frozen fruit desserts, gelatins, beverages, confectioneries, candies and baked foods (*62*).

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 (Hygienic Standards for Food Additives in China, GB2760-89) (*98*). 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 (*99,100*).

Health benefits

Reports that betalains and betalain-containing plant extracts have high antioxidant activity have certainly attracted interest (*101*). The chemical structure of betalains contains a cyclic amine, similar to the structure of the antioxidant ethoxyquine (*102*). This amine is considered to be the reactive group that gives these molecules reducing properties. Using the modified 1,1-diphenyl-2-picrylhydrazyl method of measuring antioxidant capacity, some betacyanins (average concentration of 3.7 μM) and betaxanthins (average concentration of 4.2 μM) demonstrated 3- to 4-fold greater antioxidant activity than ascorbic acid (13.9 μM); their activity was also greater than that of polyphenols rutin (6.1 μM) and catechin (7.2 μM) (*103*). In another study, radical scavenging activity of betanin in the Trolox equivalent antioxidant capacity assay was determined. At pH>4, betanin was about 1.5- to 2.0--fold more active than some anthocyanins that are considered very good free radical scavengers based on this assay (*6*).

Interestingly, it was reported that betalain extracts obtained from hairy root cultures of *B. vulgaris* cv. Detroit Dark Red had higher antioxidant activity than extracts obtained from mature beetroots (*104*). The high antioxidant activity of the hairy root extracts seems to be associated with increased concentrations of total phenolic compounds, which may have synergistic effects with betalains. Such properties of betalains could be beneficial against a number of health issues associated with an increase in oxidative stress, including obesity, cancer and atherosclerosis (*7,105,106*). *In vitro* experiments showed that betalains from *B. vulgaris* root juice and chips inhibited neutrophil oxidative metabolism, which is responsible for the activation of innate immune response in obese individuals, and that betalains had pro-apoptotic effects on stimulated neutrophils (*107*). Another study showed antiproliferative effects of the pigments on the human chronic myeloid leukemia cell line (K562) and induction of apoptosis (*108*).

Atherosclerosis is strongly linked to oxidative modification of LDL ('bad') cholesterol, and extracts from *Opuntia dillenii* Haw. fruit delayed oxidation of the lipoprotein particles, which may affect the onset of the disease (*109,110*). Betalains, especially betanin, are also powerful inducers of phase II detoxification enzymes, suggesting that they may help eliminate xenobiotic-induced oxidative stress (*111–113*).

An important determinant of whether a molecule with beneficial human health properties will be effective is its bioavailability, defined as the fraction or quantity of active substance absorbed after oral administration. Betalain bioavailability has been researched mainly using extracts from *O. ficus-indica* fruit and *B. vulgaris* root (*102, 114,115*). This work has shown that food source and pro-

cessing, food matrix and the type of betalain are key factors that affect bioavailability. Ingestion of 500 g of *O. ficus-indica* fruit pulp provided 28 mg of indicaxanthin and 16 mg of betanin, which was detectable in human plasma after 1 h. Both pigments had peak concentration in plasma at 3 h and were eliminated 12 h after ingestion. However, indicaxanthin concentration in plasma (7 μmol/L) was more than 20 times higher than that of betanin (0.2 μmol/L). *In vitro*-simulated digestion of betalains from *B. vulgaris* and *O. ficus-indica* fruit showed that indicaxanthin is more resistant than betanin to the physiological environment and enzymes in the gastrointestinal tract (*102,115*). Curiously, vulgaxanthin I from *B. vulgaris* was degraded faster than indicaxanthin. Within 12 h after ingestion, 76 % of indicaxanthin and 3.7 % of betanin were excreted in urine but some authors report that as little as 0.28 % of ingested betanin is excreted in urine in the 24-hour period (*102,114*). These results show that indicaxanthin is much more bioavailable than betanin and that digestive stability of the molecule may play an important role in the *in vivo* absorption (*115*). Betalains may exert health effects soon after their bioabsorption. They are able to traverse biomembranes of red blood cells and accumulate inside, and can be incorporated inside LDL particles, thereby inhibiting externally provoked hemolysis and lipid peroxidation, respectively (*102,116*).

Other Uses of Betalain Pigments and Future Perspectives

Betalains are versatile molecules that can be used in other fields, besides serving as food colourants. In electronics, they are being tested in dye-sensitized solar cells (DSSCs). These are devices for the conversion of visible light into electricity based on the photosensitization of wide band gap metal oxide semiconductors. The most efficient DSSCs to date are based on ruthenium-containing metallo-organic dyes adsorbed on nanocrystalline TiO_2, which show energy conversion efficiencies of 10–11 % (*117*). Natural pigments containing anthocyanins and carotenoids show overall efficiencies below 1 %, while betalains offer the best conversion efficiency of 1.7 % (*118*). In optics, microlenses can be produced using betanin-sensitized gelatin, and these lenses show good optical properties (*119*). In the chemical industry, acid solutions are used to remove the scales from metallic surfaces and inhibitors are commonly added to protect the metal from acids. Many synthetic compounds show good anticorrosive properties, but are very toxic for humans and the environment. When used in a corrosive environment of 1 M HCl solution, betanin was proven to be a good 'green' inhibitor of metal corrosion (*120*).

Considerable effort has led to the characterization of most of the betalain biosynthetic pathway. Still, not all enzymes have been identified and it remains unclear whether some chemical reactions are enzymatic or spontaneous (*38*). The main obstacle seems to be the lack of sequenced genomes of betalain-producing species. Highly productive *in vitro* plant systems have been reported, including cell suspensions and hairy root cultures, which may prove feasible when coupled with low-cost bioreactors such as the RITA® system, plastic-lined reactors or Wave bioreactors (*11*). Given that numerous factors can affect betalain biosynthesis in different ways depending on the production system and plant species, rigorous multivariate analyses should be performed to establish optimal nutrient media and conditions.

Due to increases in the human population and in the range of promising applications for betalains, these compounds are likely to have a colourful future.

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