Erum Akbar Hussain · Zubi Sadiq
Muhammad Zia-Ul-Haq

Betalains: Biomolecular Aspects

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Erum Akbar Hussain
Lahore College for Women University
Lahore, Pakistan

Zubi Sadiq
Lahore College for Women University
Lahore, Pakistan

Muhammad Zia-Ul-Haq
Lahore College for Women University
Lahore, Pakistan

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Preface

Besides the health-promoting effects of betalains and adding splashes of color to the world that we live in, betalains are still not considered "nutrients." Nonetheless, betalains are receiving increased attention as a group of phytochemicals important for optimal health. Betalains are a significant source of important antioxidants, and plant-based diets are consumed by populations most at risk for deficiency of antioxidants. Providing information about health-promoting and disease-lowering effects of betalains may decrease the prevalence of various diseases. The overarching goal of this book is to convince the reader that betalains can contribute to overall health and well-being in addition to their well-known color-imparting function.

The inspiration for *Betalains: Biomolecular Aspects* was to provide health-care and nutrition professionals and medical, graduate, and senior undergraduate students with a resource of up-to-date information on betalains. Betalains are ubiquitous pigments found mainly in plants and fungi and are considered as major contributors to the health benefits associated with diets rich in fruits and vegetables. The major objective of this comprehensive book is to review the growing evidence that betalains are bioactive molecules that can be of value to many aspects of health.

The different chapters of this book complement each other and provide distinct areas to be used for teaching. The first chapter, "Introduction," provides essential background for all readers on dietary sources of betalains and introductory material on various aspects of betalains that can be used in graduate-level instruction. The second chapter, "Sources of Betalains," may be used by practitioners and for senior undergraduate, graduate, and medical school-level courses on the importance of betalains in human health and development. Health-care and nutrition professionals will find this section most informative as they advise patients and clients.

The third chapter, "Chemistry of Betalains," is an up-to-date comprehensive review of the science behind the active molecules of betalains. It is of great importance for chemistry, pharmacy, and health graduates who have to rationalize chemical aspects with bioactivities of these colorful pigments. The fourth chapter, "Biosynthesis of Betalains," elaborates various pathways of synthesis of betalains in plants and describes research concerning the metabolizing enzymes and sites of their activities as well as the mechanisms of transport of betalains in plants.

The fifth chapter, “Role of Betalain in Human Health,” examines the role of betalains in human health and begins with the importance of adequate betalains intake to assure personal health and well-being. This chapter will serve the reader as the most authoritative resource in the field to date.

The sixth chapter details “Bioactivities of Betalains.” Special focus is given on antioxidant and singlet oxygen quenching reactions performed by betalains and their biological significance in promoting health. This chapter reviews the data from relevant clinical studies so that the current and past findings can be placed in the proper perspective, especially with regard to the potential for new data to suggest that certain betalains may have benefit as anticarcinogenic agents. Betalains, have the capacity to impart colors. The seventh chapter, “Betalains as Colorants and Pigments,” highlights the coloring characteristics of betalains.

The eighth chapter, “Analysis of Betalains,” reviews progress in analysis of betalains. All techniques starting from simple like paper chromatography till advanced like spectroscopy have been described including pros and cons of every technique and method.

The next chapter, “Bioavailability of Betalains,” summarizes the impact of other dietary components on the metabolism of betalains. In addition to dietary factors affecting betalains absorption and metabolism, host factors also can greatly affect betalains bioavailability. Host factors can influence the ability to absorb, convert, and metabolize dietary betalains. Factors such as gender, body fat, and genetic variation play an important role in this process. The last chapter, “Processing of Betalains,” examines the effects of various processing techniques on chemistry and biology of betalains. This chapter is useful to the nutrition community as well as for health professionals who have to answer client or patient questions about this area of clinical research.

It is hoped that this book will ignite scientists, practitioners, and students to evaluate their work and endeavors in the scheme of global public health. Although betalains are not currently considered essential nutrients, as we move from prevention of nutrient deficiency to supporting optimal human health and prevention of disease, evidence presented in this book should compel the reader to contemplate what truly defines a nutrient.

Lahore, Pakistan

Erum Akbar Hussain
Zubi Sadiq
Muhammad Zia-Ul-Haq

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Chapter 1
Introduction

1.1 Historical Aspects

Man is always fascinated with colors, especially when edibles are under consideration; we are all captivated with colorful foods. Betalain is one of the important natural pigments of the food industry and safe from the health point of view. Due to inextensive research in chemistry, biosynthesis and ecophysiological factors affecting betalain accumulation and evocation in situ/ex situ for its improved production were for the first time calculated by its annual production potential estimation, and relevant future study was attempted [1]. Betalains are named as chromo-alkaloids that are polar, hydrophilic nitrogenous pigments which mainly exist in most plants of Caryophyllales order [2, 3]. It is derived from *Beta vulgaris* from which its extraction was done for the first time and well recognized as a chief natural source. Beetroot is the main part of plant which has enormous quantity of betalain than any other part. The presence of carboxylic acid is responsible for the acidic nature of this important bioactive molecule, which is why it is not included in alkaloids [3]. The earliest chemically identified betalains were thought to be anthocyanins till 1957 or nitrogenous anthocyanins more incisively [1] because the biological functions of anthocyanins were replaced by these nitrogenous compounds in plants [4]. This term incorrectly suggested structural resemblance between the two pigment classes: both betaxanthine and anthocyanin [5].

The structure elucidation was made possible in the recent years due to the advancement in spectroscopic techniques particularly the nuclear magnetic resonance technique. Within the last era, ample characterization of some presumed betalains as well as their biosynthetic pathways was described that was helpful in better understanding of betalain structure. Biogenetic and structural evidences showed that the term "betalains" was introduced by Mabry and Dreiding. By spectroscopic techniques, betaxanthins with some other amines and amino acids in addition to several betanidin conjugates (glycosides and acylglycosides) were recognized afterward [6–8].

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Betalains are nitrogenous pigments which are subdivided into betacyanins (red-violet) and betaxanthins (yellow-orange) (Fig. 1.1). Phenylalanine-based, red/violet secondary metabolic anthocyanin pigments are produced by all existing flowering plants except for a few families of the order Caryophyllales in which dissimilar violet/red/yellow beet pigments or betalain is produced.

Betalain and anthocyanin pigments are equally exclusive; no taxa are known to produce both pigments [1]. Betalains replace the anthocyanin physically and functionally, in all biological contexts. They are free oxygen radical scavengers which are nutritionally beneficial in ways similar to the phenylpropanoids such as the anthocyanins [4]. All the major crop families produce anthocyanins. The betalain-producing families include such crops that are widely grown and associated with various important agricultural economic systems, such as beets, Swiss chard, spinach, *Amaranthus*, *Chenopodium quinoa*, and prickly pear. Evidences by crystallization of betanin, hydrolysis of betanin to betanidin, and subsequent report on indicaxanthin isolation show that they are a different set of pigments having a 1,7-diazaheptamethin system which is responsible for their chroma.

Unique biosynthetic pathway toward betanin and indicaxanthin through DOPA incorporation further reinforces this concept. Caryophyllales-specific occurrence of betalains shows chemotaxonomic pertinence to secondary metabolites of plants. Betalains are still a secret due to evolution in plant and net gain/loss of anthocyanins. Clarification of evolutionary mechanisms leading to the communal segregation of betalain and anthocyanin pathways in flowering plants requires more extensive molecular studies [6]. In case of leaf margin coloration, the pigment responsible for reddening/purpling is presumed to be anthocyanin, and there are no reports of leaf margin reddening by carotenoids or betalains [9].

1.2 General Characteristics

Betalains show a large diversity in structures due to acylation and glycosylation. In plants, conjugates of the chromophore betalamic kingdom, betanin (betanidin-5-O-β-glucoside) is the most common betacyanin acid [7], derived by an oxidative 4,5-extradiol ring-opening mechanism from 3-(3,4 dihydroxyphenyl)alanine (DOPA) [10].

Due to antioxidative, anti-inflammatory, and anticarcinogenic properties, betalains are of additional interest. They are used for coloring dairy products, meat, and frozen desserts. Like anthocyanins, betalains act as optical attractors for pollinators and for distribution of seeds. Their synthesis induced in *Mesembryanthemum*

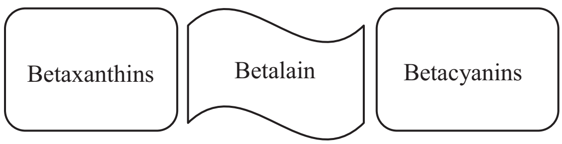

Fig. 1.1 Classification of betalain

crystallinum L. (ice plant) by UV radiation and in *Beta vulgaris* L. (red beet) by viral infection reveals that betalains also have radioprotective and antiviral activity showing its recent increase in interest due to their desirable chemical, medical, and pharmacological properties; i.e., they are chemically stable over a wide range of pH as compared to anthocyanins, possessing anticarcinogenic and anti-inflammatory activities, and they serve as strong antioxidants [11].

The natural pigments that are safe for human health are quiet scarce to be used in food. Pigments are under strict regulations, because the US Food and Drug Administration (FDA) considers the pigments as additives so the endorsement of new sources is challenging in terms of its consumption for human. The better use of pigments will be improved by an adequate empathy of the actual sources and preferred if proved safe and derived from natural source [3]. Betalains have many sources as mentioned in Fig. 1.2. In the order Caryophyllales, plants Molluginaceae and Caryophyllaceae are exceptions which produce anthocyanin, while betalains are produced in roots as well as flowers in *Basidiomycetes* (i.e., *Hygrocybe conica, A. muscaria*) [7].

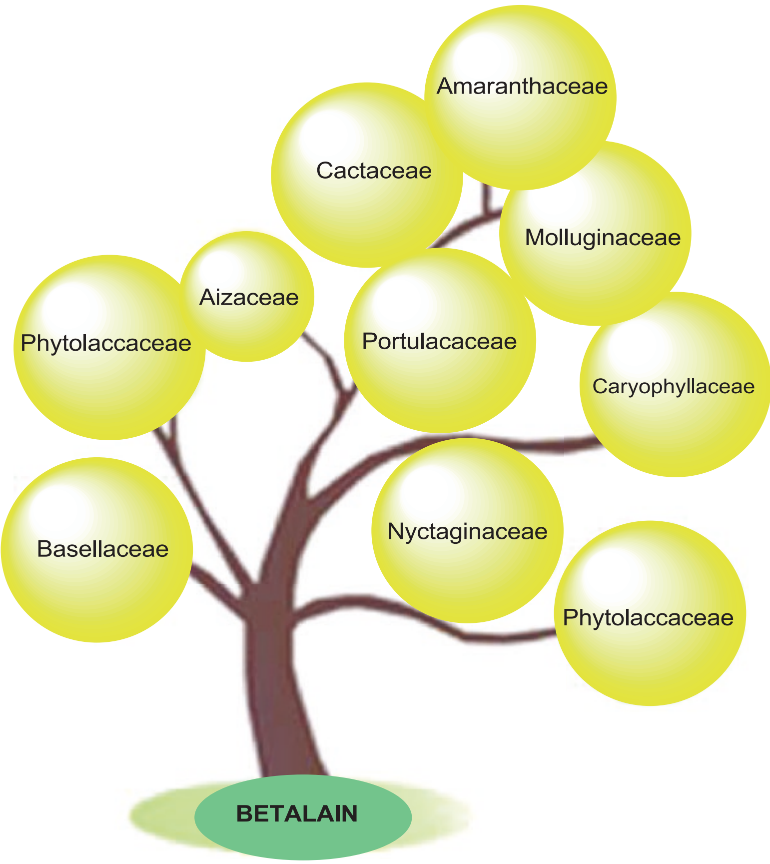

Fig. 1.2 Sources of betalain

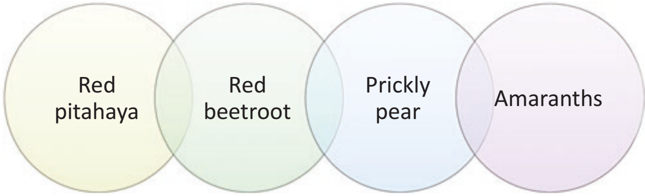

Fig. 1.3 Chief sources of betalains

The existing sources of betalains such as amaranths, prickly pear, and red pitaya other than red beetroot should be grown in appropriate quantities to make sure they are manufactured at a large scale to be used in the food industry. Their growth can be enhanced via breeding and high-yielding biotechnological tools, such as plant tissue cultures and genetic engineering [14] (Fig. 1.3).

Betalain occurrence is restricted in plant families belonging to the Caryophyllales and higher fungi, where seeds, fruits, flowers, leaves, stems, and/or roots are the specific parts of plants which contain betalain pigment. The distribution of betalains in plant parts, their colors, and sources are mentioned in Table 1.1. In cacti, betalain synthesis is restricted to flowers and fruits (reproductive organ) from a wide range of natural environment or in both vegetative and reproductive structures of ice plant such as leaves and flowers. Betalains and anthocyanins share similar histological locations in dermal, ground, and vascular tissues of vegetative organs and are stored as glycosides in the cell vacuole [5].

1.3 Betalains in Vegetables and Fruits

Toadstool "fly agaric" (*A. muscaria*) is a mushroom; its study demonstrated that the most significant findings of betalain biosynthesis have been acquired, which reveal that it gathers in the cap and their biosynthesis is subjected to evolving guidelines [15].

The vegetable "*Beta vulgaris*" is the most common source of red beet juice extracted from its roots which is cultivated in North and Central America and Britain [14]. North American red beets are grown in the Midwest region of the United States and harvested in the third quarter of the year (August to October). Betanin is the main coloring compound present in red beetroot juice color. Historically, it has imparted additional color to wines and is responsible for the color of red hue of red beet juice and comprised of red and yellow pigments known as betacyanins and betaxanthins, the magenta pigment and the yellow pigment, respectively. Red beetroot hues vary depending on betalains' source of extract. The dissemination of extracted pigments differs owing to aspects such as beetroot cultivar and extraction method.

A prevalent extraction technique includes a sequence of size reduction processes followed by hydraulic filtration and condensation. Extraction parameters are controlled in such a way that color is protected from heat, light, pH, and enzymatic degradation during the process.

Table 1.1 Distribution of betalains in plants

Structure of plant	Leaves and stems Bracts Flowers Roots Seeds Fruits
Color produced	A long range of colors Color ranges Yellow, orange, red, and pink Red-purple Red and yellow, among others Purple, red, and yellow
Sources	Teloxys spp. *Bougainvillea* spp. *Portulacaceae* plants and *Aizoaceae* Red beetroot *Amaranthus* spp. Prickly pear, *Opuntia stricta*

The contents of beetroot products depend upon the red beets' cultivar. The major yellow components are xanthin I and vulgaxanthin II and red components are betanidin and betanin (as well as their isomers). These are a unique source of phytonutrients. Vulgaxanthin and betanin are well-explored betalains obtained from red beets. Anti-inflammatory, antioxidant, and detoxification maintenance are associated with both of these. The detoxification maintenance by betalains comprises sustenance of certain important Stage 2 detox steps comprising glutathione. Although these pigments can be used in diet (such as chard or rhubarb stems), the betalain concentration in the bark and flesh of beets gives an unpredictably pronounced chance for health fitness.

The betanin concentration is decreasing in the following order:

Peel > crown > flesh

Red beet contains about 300–600 mg/kg betanin. Various factors affecting its content in betanin are cultivar, farming conditions, temperature during the growing season, soil fertility, soil moisture, storage temperature, etc. In the food industry red beet extracts containing betalain are allowed in the USA as natural food colorants [7] (Fig. 1.4).

Swiss chard scientifically known as *Beta vulgaris* is a large green leafy vegetable having a bushy and crispy stem that exists in red, white, or yellow fanlike leaves. Chard, spinach, and beets belong to the same family and have the familiar strong bitter and somewhat salty taste. It exhibits exceptional health-improving properties. It grows faster in June and August, but it is widely available for the whole year.

The term "greens" is widely referred to leafy vegetables like Swiss chard, mustard, cabbage, and collard greens possessing curly or smooth leaves, liable upon the diversity, and light-colored spine feature distributed end to end. It exhibits orange, yellow, red, or white variety in colors to the stem that is nearly two feet long. In the

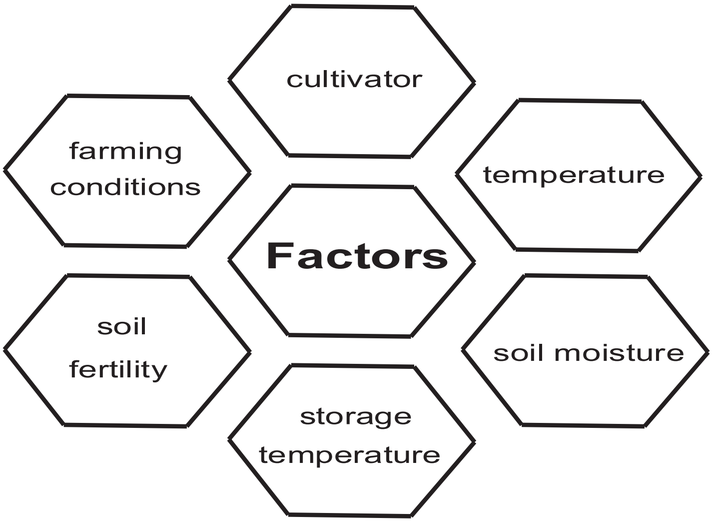

Fig. 1.4 Various factors affecting red beet content in betanin Swiss chard

market, occasionally, clusters of various colors are huddled and branded as "rainbow" chard. It is a regular biennial plant which is seeded in June, July, August, September, or October in Northern areas. Its garnering is a nonstop method due to the production of three or more harvested crops by a number of chard classes. It contains betalains, phyto-nutritional pigments such as betaxanthins (yellow) and betacyanins (red). Its royal purple-colored veins and stem exhibit different betacyanins such as isobetanidin, betanidin, isobetanin, and betanins. In stems and veins possessing yellow color, 19 different betaxanthin pigments are present such as triamine-betaxanthin, alanine-betaxanthin, histamine-betaxanthin, as well as 3-methoxytyramine-betaxanthin. Its biological profile includes anti-inflammatory, antioxidant, and detoxification support. Phytonutrients present in betalains can rapidly detoxify the gut due to its anti-inflammation and antioxidant properties. The nerve that is responsible for vision signaling is well protected by betalains.

Betalains are present in the fruits of prickly pear or cactus plant scientifically known as *Opuntia ficus-indica* L. mainly in purple variety as betacyanins and in orange variety as betaxanthins. The origin of cactus pear is from Mexico, but it also comes from Africa, Middle East, and Europe later on revealing extraordinary alteration to semiarid and arid type of weather in subtropical and tropical areas of the universe. It is self-generated in Italy and is cultured in Southern areas such as Calabria, Apulia, Sardinia, and Sicily.

Due to flavorsome properties, fruits of this plant are commercially significant. Usually, the fruit is taken fresh in July to October that is the seasoning period, but its shelf life has also been increased by various procedures developed by food technicians due to growing arcade request for health-encouraging food. Due to its nutritive and health-encouraging features, it has gained a considerable prominence. It is also associated with bioactive ingredients such as antioxidants like polyphenols, ascorbic acid, and betalains. Apart from this, antiproliferative, hepatoprotective, neuroprotective, anticancer, and antiulcer organic activities are also associated with it. It is also an excellent source of yellow and red dyes for food.

As stability range of betalains ranges from 4 to 7 pH, it is most favorable for non-acidic food dyeing. Furthermore, the existence of both betaxanthins and betacyanins at the same time produces a broad range of color.

Almost 50% of the total cactus pear is produced in Mexico that is the main worldwide producer. After that, Italy comes next that produces 78,000 tons and 7400 Ha. In Italy, 90% production is that of Sicily, whereas Apulia documented in 2013 the creation of 2650 tons from 320 Ha, chiefly developed in North Apulia (Foggia province) with particular crops that are spineless. In Italy, exhaustive plantations principally propagate the spineless yellow variety. There grows an equal sharing of the two colored fruits in Apulia, predominantly in the South Apulia (Salento peninsula), from bristly genotypes, habitually rising privately or in wild country parks, by means of an unlike equilibrium of betacyanin (purple-red) present in purple variety and betaxanthin (yellow-orange) present in the orange variety.

Opuntia stricta is a cactus species commonly named as erect prickly pear from South to North America and the Caribbean. It is a sprawling or erect shrub up to two meters in height, bearing light yellow flowers from February to June and producing purplish-red fruits. *Opuntia stricta* contains five times higher concentration of betalain than *Opuntia ficus-indica* and partly higher as in red beet. *Opuntia stricta* occurs naturally in the Southern region of the United States as well as the Bahamas, Bermuda, the Caribbean, Central America, northern Venezuela, and Ecuador. Many regions of the world are beautified with this medicinally important plant; these regions include Africa, Southern Europe, and Asia particularly in Sicily. *O. stricta* is believed as a persistent species in South Africa. In Australia, it has been the subject of one of the first effective biological control exercises against the moth *Cactoblastis cactorum*. Literature has highlighted it as "Weeds of National Significance," though it must be used under controlled conditions.

New Zealand has ice plant, one of its inhabitant plants, which is regarded as a key source in augmenting crop capacity to salinity. This plant has red pigmentation that is believed to be one of the responsible factors for its saline resistance whose variable amount depends on its remoteness from the coastline. Betalain, the red pigment, in the ice plant, was studied, while the incorporation of betalains in green-leaved ice plant makes the plant tolerant from saline conditions which functions as a defense for plant tissues contrary to free radical destruction triggered by salt and too much sunlight. This result specifies the potential of betalain to be used in emerging and breeding crops to enrich forbearance under saline state.

Amaranthus, a member of family *Amaranthaceae*, is widely cultivated in some areas of Asia and Africa and contains complex mixtures of betalain. Several species of *Amaranthus* are often considered as weeds. Yet, in many countries, it is also used as vegetables, cereals, as well as medicinal and ornamental plants. Interestingly, the cultivated species contain more betacyanin and higher biomass than wild ones. Based on its physical/chemical properties, its pigments show possible potential as food colorant and also strong antioxidant activity, so it is recommended as an alternative source of betalain. In China, a natural pigment of *Amaranthus tricolor* has been legally accepted as food ingredient (Hygienic Standards for Food Additives, GD2760-89). The cultivars and the growth stage affect the pigment concentration which shows that the cultivated genotypes have a potential to be commercially developed as natural pigment sources. More than 60 species of short-lived herbs have a cosmopolitan genus that is known as *Amaranthus.* Cultivation of some amaranths is done as ornamental plants, leafy vegetables, or grain, whereas other amaranths are cultivated in the form of weeds. *Amaranthus tricolor* contains less betacyanins in their tissues than *Amaranthus hybridus*, *Amaranthus cruentus*, and *Amaranthus caudatus* (grain amaranths) [4]. Three kinds of betaxanthins and 16 kinds of betacyanins have been identified and elucidated in this plant family [11].

Pitaya (*Hylocereus undatus*) is the member of *Cacteacae* family, instinctive of Mexico and Central and South America. It comprises of numerous species though *Hylocereus undatus* is superlative to be cultivated. Pitaya peel is reflected as a residue from utilization and treatment of fruit, and it is usually discarded. However, this remainder can be consumed as feedstock for the extraction of pigments, due to the presence of betalains which display substantial and attractive color. Beet (*Beta vulgaris*) is the chief source of commercial extraction of betalains that comprises of geosmin and *pyrazines* which are accountable for the loss of original flavor due to deterioration. The betalains can be used in foods without flavor, which are extracted from pitaya, contrasting the red beet, and it covers an extensive spectrum of color from yellow-orange of *Opuntia* to red-violet of *Hylocereus*.

Strawberry blite belongs to the edible group of yearly grown plant. Moreover, *Blitum capitatum* syn. *Chenopodium capitatum* is also known as blite goosefoot, strawberry spinach, and strawberry goosefoot. It can be found in North America but may also be found in Canada, New Zealand, and the United States. Strawberry blite also occurs in damp mountain valleys. Its fruits are bright red and small but pulpy and edible just like strawberries. Natives use its fruit extract as red dye. The fruits have small, lens-shaped seeds that are black and 1.2–0.7 mm long. Its edible portion must be used in moderation due to oxalates. Seeds are toxic when used excessively (Table 1.2).

1.4 Betalain: A Versatile Color Source

Betalains obtained from red beet are responsible for brilliant colors in flowers and fruits belonging to species of the order Caryophyllales, excluding Caryophyllaceae and Molluginaceae used as natural colorant. The mutual clannishness of Caryophyllales has raised appreciable taxonomic debate. Lack of understanding of betalain biosynthesis in terms of molecular biology and biochemistry has prohibited researchers from interpreting this taxonomic puzzle. Betalain color is beneficial because this is independent of pH and is more stable than the color obtained from anthocyanins [12]. They are not only responsible for the bright coloration of fruits and flowers but also of leaves and roots of plants in Caryophyllales order [13].

Table 1.2 Major betalain-containing plant sources [11]

Common name	Species	Betalains produced
Beetroot	*B. Vulgaris*	Indicaxanthin, vulgaxanthin (I, II), betanin, isobetanin, prebetanin, neobetanin
Swiss card	*B. vulgaris* (L.)	20 different betaxanthins 9 different betacyanins
Prickly pear	*Opuntia ficus-indica* (L.)	Minor api of uranosyl betacyanins, hylocerenin, and isohylocerenin
Erect prickly pear	*Opuntia stricta*	Betalanin, betacyanin
Ice plant	*Mesembryanthemum crystallinum*	Mesembryanthin, betacyanins
Amaranth	*Amaranthus* spp.	Amaranthine, isoamaranthine
Pitaya peel	*Hylocereus undatus*	Isobetanin, betanin, isophylo cactin, phylocactin, hylocerenin, isohylocerenin
Strawberry blite	*Blitum capitatum*	Betanin
Red goosefoot	*Chenopodium rubrum*	Amaranthin, vulgaxanthin (I, II), betanin, celosianin
Moss rose	*Portulaca grandiflora*	Dopaxanthin, portulacaxanthin II, vulgaxanthin I, indicaxanthin, miraxanthin V

Its color shows stability in pH range 3–7. This range is useful for *Mesembryanthemum crystallinum* and *Portulaca oleracea* which are betalainic plants, and their carbon assimilation mode is switched from C4 and C3, respectively, to crassulacean acid metabolism (CAM) when it experiences water stress. A shift from a relatively stable vacuolar pH to large periodic fluctuations is observed in these plants. In the plants that occur in drought conditions, a stable red color reveals irrespective of vacuolar pH and maintenance of photo protection.

In betalain synthesis, nitrogen requires an additional cost; two atoms of nitrogen per betalain are required. So, there's a need to balance this economy, in spite of keeping equality between both pigment classes; anthocyanins are more successful than betalains as defensive molecules [5].

1.5 Classification

In the class *Basidiomycetes* of fungi and within the Caryophyllales's suborder chenopodiaceae, betalains are restricted [11]. All betalains have the same basic structure in which R1 and R2 may be substituted with hydrogen and aromatic substituent. The colors of betalains are the result of their resonating double bonds [15]. A substituted dihydropyridine serving as the chromophore with a system of conjugated double bonds is the basic structure of betalains (Fig. 1.5). Betalamic acid forms immonium conjugate with amines or cyclo-3-(3,4-dihydroxyphenyl)-L-alanine (cyclo-DOPA) to form betalains (Fig. 1.6) [11].

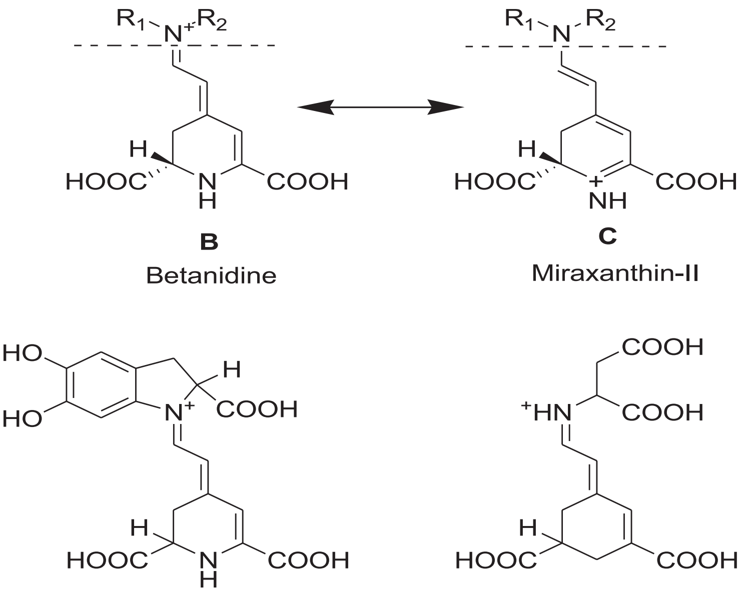

Fig. 1.5 Basic structure of betalain

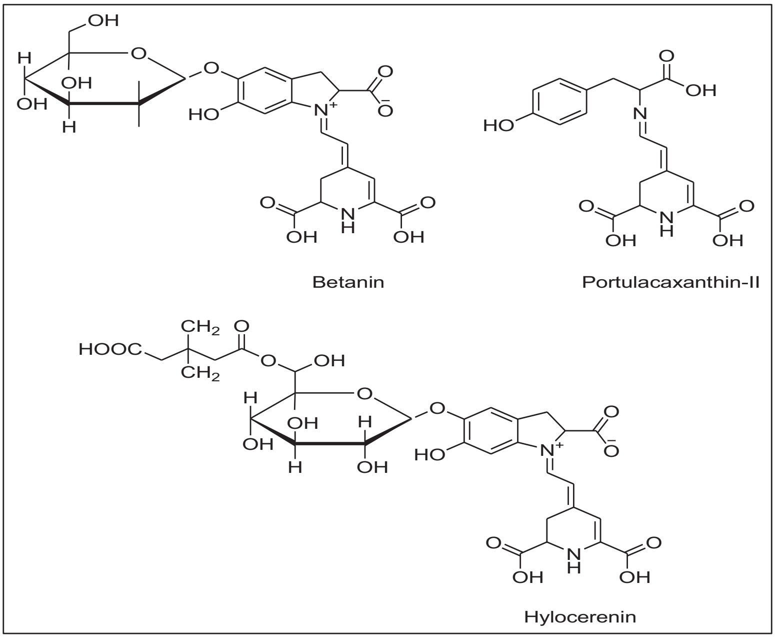

Fig. 1.6 Chemical structures of betalain