Betalains`s structure, existence and biological importance

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ABSTRACT

Betalains are water-soluble pigments derived from tyrosine. They are found almost exclusively in the Caryophyllales family. They can be classified into two major groups; red-violet betacyanins and yellow-orange betaxanthins. Besides being used as natural colourants instead of artificial dyes, betalains gained a lot of attention as biologically active compounds. This review summarizes the therapeutic effects of betalains in human health, including the antioxidant, anti-inflammatory, anti-bacterial, anti-cancer, anti-diabetic, neuroprotective and cardiovascular-protective effects.

KEY WORDS: Betalains, Colourants, Antioxidant, Anti-inflammatory, Anti-bacterial, Anti-cancer, Anti-diabetic, Cardiovascular diseases, Neurodegenerative disorders.

1. INTRODUCTION

Betalains are water-soluble nitrogen-containing pigments derived from tyrosine and normally accumulate in the vacuoles of the epidermal and subepidermal tissues (Tanaka, 2008; Hussain, 2018; Slimen, 2017; Rodriguez-Amaya, 2019; Timoneda, 2019). They are considered one of the most common plant pigments in nature along with carotenoids, chlorophylls and anthocyanins. Unlike those pigments, which are ubiquitous in the plant kingdom, betalains are present in a much smaller group of plants (Choo, 2019). Their presence is restricted to the order Caryophyllales and some inedible mushrooms such as *Amanita muscaria*, *Hygrocybe* sp. and *Hygrosporus* sp. (Choo, 2019; Delgado-Vargas, 2000; Georgiev, 2008). Recently, some researchers reported the synthesis of betalains in the bacterium *Gluconacetobacter diazotrophicus* (Contreras-Llano, 2019).

In a plant, betalains can be present in seeds, leaves, flowers, roots, and even in fruits (Azeredo, 2009; Ge Li, 2019). The presence of these colourful pigments in flowers is an important characteristic known to attract pollinators, along with the fact of attracting fruit-eating animals for dispersal of the indigestible seeds, which are essential for facilitating plant propagation (Gandia-Herrero, 2005; Gandia-Herrero and Garcia-Carmona, 2013; Solymosi, 2015; Guerrero-Rubio, 2020).

The rare attention to betalains could be attributed to the fact that red beet represents the only edible betalains source for a long time. However, many plants provide betalains to our diet along with red beets such as swiss chard, *Amaranthus*, cactus pear (prickly pear), dragon fruit, and some tubers (Giuliani, 2016; Villano, 2016; Coy-Barrera, 2020).

Betalains Chemical Structure: For a long time, betalains were mistakenly classified as flavocyanins (betaxanthins) and nitrogenous anthocyanins (betacyanins). Only in 1968, these two dyes were first addressed as ‘‘betalains’’, according to beet (*Beta vulgaris*) which they were extracted from for the first time (Slimen, 2017; Calogero, 2015; Akhavan and Jafari, 2017).

It`s worth noting that betalains and anthocyanins are two different types of pigments and they have never been reported together in the same plant. This has not been explained well, but at the biochemical level, it has been illustrated that the anthocyanins biosynthesis enzymes are suppressed in betalain-producing plants (Choo, 2019; Gandia-Herrero and Garcia-Carmona, 2013).

To date, studies identified about 78 betalains (Slimen, 2017). They can be found in the majority of families of the Caryophyllales (Achatocarpaceae, Aizoaceae, Amaranthaceae, Basellaceae, Cactaceae, Chenopodiaceae, Didiereaceae, Halophytaceae, Hectorellaceae, Nyctaginaceae, Phytolaccaceae, Portulacaceae, and Stegnospermataceae), while anthocyanins are produced by only two families of the Caryophyllales (Caryophyllaceae and Molluginaceae) (Hussain, 2018; Choo, 2019; Gandia-Herrero and Garcia-Carmona, 2013; Giuliani, 2016; Strack, 2003; Stintzing and Carle, 2004; Rosa, 2007; Moreno, 2008).

The most common and well-known sources of betalains are those belonging to the Amaranthaceae (*Beta vulgaris* L. and *Amaranthus* sp.) and Cactaceae families (*Opuntia* sp. and *Hylocereus* sp.) (Choo, 2019).

Betalains include two classes of compounds, betaxanthins and betacyanins. Betaxanthins are derived from betalamic acid via conjugation with different amines and amino acids, while betacyanins are derived by condensation of betalamic acid with cyclodihydroxyphenylalanine (*cyclo*-DOPA) (Timoneda, 2019; Stafford, 1994).

Betalains Biosynthesis: Betalains biosynthesis starts from tyrosine (Figure 1), which is derived from the shikimate pathway in the chloroplast. Tyrosine is exported to the cytosol and converted to L-DOPA (dihydroxyphenylalanine) via tyrosine hydroxylase. Then L-DOPA undergoes a ring-opening oxidation reaction by the enzyme L-DOPA 4,5-dioxygenase (DODA) to produce the 4,5-*seco*- DOPA, which converts to betalamic acid via spontaneous intramolecular condensation. Alternatively, L-DOPA can be oxidized to dopaquinone which cyclizes to form *cyclo*- DOPA. Betalamic acid can spontaneously conjugate with the imino group of *cyclo*-DOPA, leading to the formation of betacyanins. On the other hand, betalamic acid may spontaneously condense with the imino or amino group of amino acids or other amines to give betaxanthins (Hussain, 2018; Timoneda, 2019; Martins, 2017, Polturak and Aharoni, 2018; Schenck and Maeda, 2018).

Different amino acids are reported to conjugate with betalamic acid to form betaxanthins; mainly tryptophan, serine, valine, phenylalanine, isoleucine, alanine, histidine, methionine, threonine, arginine and lysine (Khan and Giridhar, 2015). As regards amines, ethanolamine, putrescine and phenethylamine have been reported in betaxanthins (Khan and Giridhar, 2015).

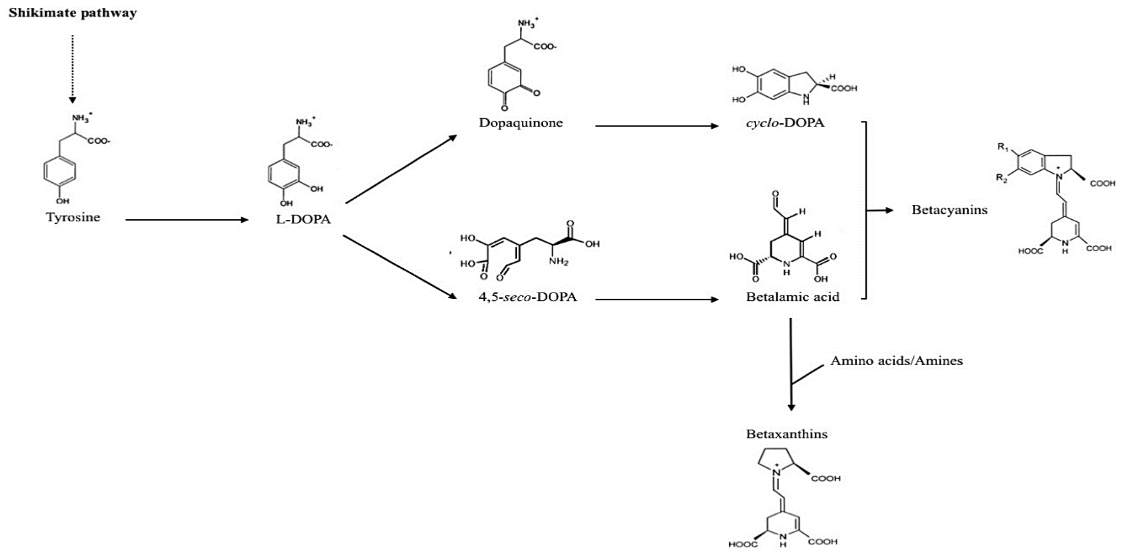

Figure.1. Plant betalains biosynthesis scheme (Martins, 2017)

Betacyanins structures differ in their sugar moieties and acyl groups (Delgado-Vargas, 2000; Coy-Barrera, 2020; Calogero, 2015; Moreno, 2008; Polturak and Aharoni, 2018; Gandia-Herrero, 2009; Sigurdson, 2017; Miguel, 2018), whereas betaxanthins have a wide range of amines and amino acids in their structures (Delgado-Vargas, 2000).

Betacyanins are usually 5-O-glucosylated (like betanin) and rarely 6-O-glucosylated (like gomphrenin II), but never linked to sugar moieties in both positions (Esatbeyoglu, 2015). On the other hand, betaxanthin glycosylation tends to be extremely rare or absent (Polturak and Aharoni, 2018).

Betacyanins are sub-divided into four groups: betanin-type, amaranthin-type, gomphrenin-type and bougainvillein –type (Coy-Barrera, 2020; Polturak and Aharoni, 2018; Miguel, 2018; Esatbeyoglu, 2015; Pavokovi and Krsnik-Rasol, 2011).

Betacyanins have red to red-violet colour and show maximum absorption at 532–550 nm, with an absorbance spectrum centred at 536 nm. By contrast, betaxanthins are yellow-orange and their maximum absorption is around 457–485 nm (Rodriguez-Amaya, 2019; Azeredo, 2009; Gandia-Herrero and Garcia-Carmona, 2013; Coy-Barrera, 2020; Calogero, 2015; Khan, 2016).

Betaxanthins can be sub-divided into two groups: amine-derived and amino acid-derived conjugates (Coy-Barrera, 2020; Pavokovi and Krsnik-Rasol, 2011). Betaxanthins are fluorescent pigments. They absorb blue light and emit light between 509-512 nm within the green area of the electromagnetic spectrum (Hussain, 2018; Slimen, 2017; Gandia-Herrero, 2005; Miguel, 2018; Escribano, 2017). The strong fluorescence is attributed to the structural property of these pigments (betalamic acid moiety + an amine group) (Guerrero-Rubio, 2020).

The Importance of Betalains:

Betalains as natural colourants: Recently, there was a strong demand for replacing artificial food dyes with natural pigments, considering the potential health benefits of the latter. Yet, natural colourants are generally more costly, less stable and not easily used comparing to synthetic ones, together with their interaction with food ingredients, and narrow range of hues (Rodriguez-Amaya, 2019; Rosa, 2007; Miguel, 2018).

Like other natural pigments, colour stability is a major concern with betalains. It is increased by high betalain concentration, high degree of glycosylation and acylation, low temperature, pH 3 to 7, chelating agents (EDTA, citric acid) and antioxidants. On the contrary, stability is decreased by degrading enzymes (peroxidase, polyphenol oxidase, glucosidase), low degree of glycosylation and acylation, metal cations, pH<3 or>7 and high temperature (Hussain, 2018; Slimen, 2017; Rodriguez-Amaya, 2019; Azeredo, 2009; Sigurdson, 2017; Miguel, 2018; Esquivel, 2016).

Betalain containing plants such as cactus pears are commercially investigated as food colourants. They may expand the colour spectrum provided by red beet, and help the sustainability of semiarid and arid regions at the same time (Slimen, 2017).

Despite the increased attention to betalain sources, the majority of studies of betalains have been conducted using red beetroot as a source of these pigments. Consequently, red beet betanin is a well-known red food colourant. Betanin (EEC No. E 162) is approved as a red food colourant by the European Union and under Section 73.40 in chapter 21 of the Code of Federal Regulations (CFR) by the Food and Drug Administration (FDA) in the USA (Martins, 2017; Khan, 2016). Stable at a broad pH range from pH 3–7, betalains are mainly applied in low-acid foods such as yoghurt, soft drinks, powdered drink mixes, cake mixes, ice cream, candies, marshmallow candies, meat substitutes, gravy mixes, and gelatin deserts (Choo, 2019; Solymosi, 2015; Akhavan and Jafari, 2017; Khan, 2016).

Besides, since betalains are sensitive to heat, and that`s what makes their colours more suitable to frozen or chilled products (Cai, 2005; Attokaran, 2017).

Given that betalains containing betacyanin are more stable than anthocyanins, so they usually complement anthocyanins in food applications, principally in low-acid and neutral foods (Tanaka, 2008; Hussain, 2018; Rodriguez-Amaya, 2019; Giuliani, 2016; Strack, 2003; Sigurdson, 2017; Esatbeyoglu, 2015; Esquivel, 2016; Dias, 2020; Skalicky, 2020).

In addition to the use as food colourants, betanin can be used as a natural colourant for pharmaceutical products (Solymosi, 2015; Martins, 2017; Esatbeyoglu, 2015; Silva, 2019). It can be also added together with other betalains as a colourant for cosmetic products (Solymosi, 2015; Martins, 2017; Esatbeyoglu, 2015; Azwanida and Afandi, 2014).

Physiological effects of betalains: Apart from being perfect examples as prominent colourants, betalains show a wide variety of biological properties, mainly antioxidant, anti-inflammatory, antimicrobial, anticancer and neuroprotective besides their positive influence on the cardiovascular system and metabolic disorder (Coy-Barrera, 2020; Martins 2017).

Antioxidant effects: Biomolecules (DNA, lipids and proteins) are the main target of radicals if they exceed the endogenous antioxidant capacity. This fact leads to biomembranes destruction, genetic material disruption, and enzyme dysfunction. To prevent/correct that, endo or exogenous antioxidants have a crucial role in scavenging on those radicals (Coy-Barrera, 2020).

Earlier studies strongly demonstrated the high radical scavenging activity of betalains. This antioxidant capacity depends on the chemical structure of these pigments (Slimen, 2017; Esatbeyoglu, 2015; Escribano, 2017; Cai, 2003; Wootton-Beard and Ryan, 2011; Koubaier, 2014; Asgary, 2016; Rahimi, 2019).

Structurally, these dietary cationized antioxidants "betalains" represent ammonium derivatives of betalamic acid, which contain an aromatic amino compound that can stabilize radicals. This stabilization ability is closely connected to the electron donation ability of betalains (Slimen, 2017).

Antioxidant potential increases with the increasing number of hydroxyl and amino residues in betaxanthins (Stintzing and Carle, 2004; Cai, 2005), while in betacyanins, acylation generally raises the antioxidant capacity glycosylation ease it (Coy-Barrera, 2020; Stintzing and Carle, 2004; Fu, 2020).

According to many reports, the antiradical activity of betalains is much higher than that of the water-soluble derivate of vitamin E, ascorbic acid, rutin, catechin, ß-carotene and α-tocopherol (Slimen, 2017; Cai, 2005; Skalicky, 2020). Generally, betacyanins are stronger free radicals scavengers compared to betaxanthins (Esatbeyoglu, 2015; Fu, 2020).

Anti-inflammatory properties: Inflammation is a symptom related to many disorders. This symptom is treated with anti-inflammatory drugs (steroidal and non-steroidal), which may cause serious side effects after the usage for a long time. Natural products represent an alternative source for compounds with anti-inflammatory activity, such as betalains. Some betacyanins and betaxanthins inhibit inflammatory mediators-converting enzymes, such as cyclooxygenase (COX-1 and COX-2) and lipoxygenase (LOX). They notably reduce carrageenan-induced superoxide anion, tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-1β levels, reverse the pro-inflammatory cytokines (interleukin-8 (IL-8), and interleukin-6 (IL-6)) induction and improve IL-10 levels (Coy-Barrera, 2020; Asgary, 2016; Fu, 2020; Martinez, 2015; Ahmadi, 2020; Hadipour, 2020; Dia, 2021).

Anti-microbial activity: Betalains inhibit a wide spectrum of microorganisms. They showed antimicrobial activity against different types of microbes such as malaria parasites, gram-positive bacteria (e.g. *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Listeria monocytogenes*) and gram-negative bacteria (e.g. *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhymurium*, *Yersinia enterocolitica*, *Citrobacter freundii*, *Citrobacter youngae*, *Enterobacter cloacae*, *Enterobacter aerogenes* and *Klebsiella pneumonia*) (Coy-Barrera, 2020; Canadanovic-Brunet, 2011; Madadi, 2020). Betalains-rich extracts also inhibit yeasts (like *Candida albicans*, *Rhizoctonia solani*) and moulds (such as *Fusarium oxysporum*, *Cladosporium herbarum*, *Botrytis cinerea*, *Aspergillus flavus*) (Coy-Barrera, 2020; Miguel, 2018).

The antimicrobial activity of betalains is thought to be a result of their negative impacts on the microbial cell membrane because they alter its function and in some cases its structure and increase its permeability leading to cell death (Canadanovic-Brunet, 2011; Madadi, 2020).

Anti-cancer properties: Betalains exhibit antiproliferative action on different cancer cells probably via their free-radical scavenging ability, but without affecting normal cell lines (Coy-Barrera, 2020). Besides, recent studies with different cancer cell lines indicated the high chemopreventive potential of betalain containing extracts (Kapadia, 2011; Gandia-Herrero, 2016; Lechner and Ston, 2019). Thus, betalain-rich extracts significantly inhibit the growth of human ovarian, immortalized cervical epithelium cervical, prostate, melanoma and chronic human myeloid leukaemia cancer cells (Coy-Barrera, 2020; Madadi, 2020; Kapadia, 2011; Gandia-Herrero, 2016).

Some evidence referred to the ability of betalains to modify a particular gene expression associated with apoptosis and cell growth and stop mitochondrial transmembrane potential, which works as a proapoptotic factor (Coy-Barrera, 2020; Madadi, 2020).

Anti-diabetes effects: Diabetes, which resulted from chronic hyperglycemia, is considered a silent killer with a rising number of diagnosis year after year. Recent studies referred to the ability of betalains to counteract diabetic complications like chronic hyperglycemia.

Many investigations have been made on the hypoglycemic effect of betalain-rich preparations or purified betalains. Those pigments can maintain glucose homeostasis (Hadipour, 2020; Murthy and Manchali, 2013; Kaur, 2018). In a previous study, high neobetanin containing red beet juice caused a significant reduction in insulin and postprandial glucose responses of healthy adults in the first 30 minutes of intake (Wootton-Beard, 2014). Insulin response was also recorded suggesting reduced insulin requirement. Besides, it was evident that a betalain-rich diet markedly reduced glucose absorption (Khan, 2016).

It can be implied that betalain extracts can effectively minimize the impact of insulin resistance similar to the drugs (Mirmiran, 2020).

Effects on the cardiovascular system (CVD): Unhealthy diet is one of the most serious risk factors for cardiovascular diseases (CVD), which are the major cause of death worldwide (Madadi, 2020). Hyperlipidemia could cause atherosclerosis among other CVD (Fu, 2020).

Betalains rich-extracts increase high-density lipoprotein (HDL) (Asgary, 2016; Rahimi, 2019; Singh, 2015) and glutathione (GSH) which reduce their risk of CVD development (Madadi, 2020), while decreasing both systolic blood pressure (Asgary, 2016; Rahimi, 2019; Fu, 2020; Hadipour, 2020; Mirmiran, 2020; Jajja, 2014; Rahimi, 2019), diastolic blood pressure (Fu, 2020; Mirmiran, 2020; Rahimi, 2019; Coles and Clifton, 2012), lipid peroxidation (Fu, 2020; Kaur, 2018), low-density lipoprotein (LDL) (Madadi, 2020; Murthy and Manchali, 2013; Singh, 2015; Coles and Clifton, 2012), and total cholesterol (Hussain, 2018; Asgary, 2016; Rahimi, 2019; Madadi, 2020; Rahimi, 2019).

Effects on neurodegenerative disorders: Neurodegenerative disorders are characterized by the gradual degeneration of the structure and function of the central or peripheral nervous system. They include Alzheimer's disease and Parkinson's disease.

Alzheimer's disease (AD) is an irreversible, progressive neurologic disorder that slowly impairs memory and cognition skills and, ultimately, the ability to do the simplest tasks (Dia, 2021; Madadi, 2020). Beta-amyloid is an adhesive fragment of a protein or peptide that increases in the brain of AD patients and obstructs the connection between nerve synapses. Betalains extracts (betanin) can inhibit the progression of AD by reducing oxidation and slowing down the accumulation of beta-amyloid protein (Hadipour, 2020; Madadi, 2020).

Parkinson's disease (PD) is a neurologic disorder that causes shaking, stiffness and trouble with walking, balance, and movement coordination. In Parkinson’s disease, up to 70% of dopaminergic neurons are broken down in substantia nigra pars compacta (Mosley, 2004). L-dopa is an intermediate compound in betalain biosynthesis and can be converted to dopamine, thereby alleviate AD development (Hadipour, 2020; Madadi, 2020). Betalain-rich extracts reduce the intensity of muscular rigidity, tremors number, vacuous chewing movements, and increase motor activity and grip strength (Nade, 2015).

The Side Effects of Betalains: To date, there are no reported studies about the toxicity of betalains to human, so they are considered safe for consumption.

As a consequence of the red colour of some betalains (betanidin and betanin), they may colour both urine and faeces with a bright red colour. This condition is called beeturia or betaninuria (Delgado-Vargas, 2000; Kanner, 2001), and is not harmful, but it could be an indicator of problems in iron metabolism (Clementa and Ashford, 2011).

2. CONCLUSION

Betalains are nitrogenous containing pigments. They are colourful compounds that existed in different plant organs (seeds, leaves, flowers, roots, and fruits). Numerous studies have revealed the health benefits of betalains arising from their high antioxidant potential. Therefore, this review points out the therapeutic impacts of betalains on human chronic disorders.

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