Phytochem Rev (2013) 12:35–45
DOI 10.1007/s11101-012-9248-2

Analysis of betalains from fruits of Opuntia species

Sanjay P. Chauhan · N. R. Sheth · I. S. Rathod · B. N. Suhagia · Rajnikant B. Maradia

Received: 28 March 2012 / Accepted: 17 July 2012 / Published online: 29 July 2012

Abstract Betalains are of great taxonomic significance in higher plants and occur only in 10 families of the order Caryophyllales (Centrospermae). They are water-soluble nitrogenous pigments. They can be divided into two major structural groups, the red to red-violet betacyanins and the yellow betaxanthins. Betalains are widely used as natural red food colorant as well as antioxidant potentials. Several methods have been published for the determination of betalain in fruits of Opuntia species. The purpose of the current review is to provide a systematic survey of the analytical techniques for the determination of betalain from fruits of Opuntia species.

Keywords Betalain · Betacyanin · Cactaceae · Opuntia · Prickly pear

S. P. Chauhan (✉) · B. N. Suhagia · R. B. Maradia
Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, Gujarat, India
e-mail: sanjulmcp@rediffmail.com

N. R. Sheth
Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India

I. S. Rathod
L. M. College of Pharmacy, Ahmedabad, Gujarat, India

Introduction

Opuntia (subfamily: *Opuntioideae;* Family: Cactaceae) is a large genus of succulent shrubs, native of the new world, now widely grown in the warmer parts of the world, on account of their unique appearance and attractive flowers. They are commonly known as Prickly pears, because of their edible fruits. Recent data revealed the high content of some chemical constituents, which can give added value to this fruit on a nutritional and technological functionality basis. High levels of betalains, taurine, calcium, magnesium, and antioxidants are noteworthy. Another important compositional factor of prickly pear is the presence of pigments, which give particular attractiveness to fruit and products (Piga 2004). Betalains have a number of health properties. Infusions of betalains from the bracts of *Bougainvillaea* mixed with honey, for example, are used to treat coughs in some regions of Mexico. Some anticancer, antiviral and antioxidant activity has been attributed to betalains. The main focus of interest, however, has recently been on betalain pigments as natural antioxidants (Yahia and Castellanos-Santiago 2008). The purpose of the current review is to provide a systematic survey of the analytical techniques for the determination of betalain.

The most common connotation with pigmented flower petals and fruits is the attraction of animals both for pollination and seed dispersal. Anthocyanins mask the chlorophyll containing organelles and thereby protect chloroplasts against high light intensities to

prevent photoinhibition (Stintzing and Carle 2004). Chalker-Scott (1999) suggested three functions of anthocyanins in plants, namely as absorbers of harmful radiation, as transport vehicles for monosaccharides and as osmotic adjusters during periods of drought and low temperature. The anthocyanins are a subgroup within the flavonoids characterized by a C_6–C_3–C_6 skeleton.

Betalains are of great taxonomic significance in higher plants. The presence of betalains in members of the order Caryophyllales has been an important criterion for their classification. The presence of betalains and anthocyanins is mutually exclusive in the angiosperms. Betalains are water soluble nitrogenous chromoalkaloids and can be divided into two major structural groups, (1) The red to red-violet betacyanin (Latin *Beta*, beet and Greek *kyanos*, blue color) and (2) The yellow betaxanthins (Latin *Beta* and Greek *xanthos*, yellow). Betalains may function as osmolytes to uphold physiological processes, to stabilize subcellular structures, to reduce nitrogen toxicity and to be an excellent radical scavenger. Structurally, betacyanins are characterized by a cyclo-Dopa structure with additional substitutions through varying glycosylation and acylation patterns at C_5 or C_6 whereas the betaxanthins are condensation products of betalamic acid and various amino compounds. Betacyanins can be further classified by their chemical structures into four types: betanin-type, amaranthine-type, gomphrenin-type and bougainvillea-type (Stintzing and Carle 2004; Cai et al. 2005). Structures of betacyanins and betaxanthins found in the fruits of different *Opuntia* spices summarized in Fig. 1. The biosynthetic steps involved in betalain biosynthesis are summarized by (Strack et al. 2003).

Numerous analytical methods have been designed and developed for the qualitative and quantitative determination of betalains in fruits of *Opuntia* species and are reviewed as follows.

Qualitative analysis

Chemical tests

Harborne (2007) reported chemical tests for the identification of betacyanins. Red color of betacyanin vanishes upon heating with 2 M HCl for 5 min at 100 °C and color changes to yellow by adding 2 M NaOH dropwise indicate presence of betacyanins.

Spectrophotometric method

Harborne (2007) reported visible spectrum of betacyanin in methanol-HCl give maximum absorbance in the range of 532–554 nm. Viloria-Matos et al. (2001) reported visible spectra of fruits of *Opuntia boldinghii* Br. et R., maximum absorbance at 537 nm at pH 6.1 which is similar to the earlier reported value of betacyanin (Bilyk 1979, 1981; Delgado-Vargas et al. 2000). Fernández-López and Almela (2001)extracted pigments from the prickly pear fruits (Opuntia ficus-indica), of reddish purple and yellow color, by homogenization of fruit flesh in methanol, with a ratio mass fruit (g)/solvent (ml) of 1:5 and two main pigments were obtained, which were identified as indicaxanthin (λ_{max} 484 nm) and betanin (λ_{max} 535 nm). The spectrophotometric analysis suggests that the external color of prickly pear fruits depends on the relative concentration of betacyanins (red pigments with maximum absorbance at around 535 nm) and betaxanthins (yellow pigments with maximum absorbance at around 480 nm) (Schliemann et al. 1996, 2000, 2001; Wybraniec et al. 2001; Fernández-López and Almela 2001; Cai and Corke 1999; Stintzing et al. 2003, 2005).

Chromatographic method

Thin layer chromatographic (TLC)

Harborne (2007) reported chromatography in 1 % aqueous HCl and n-butanol:acetic acid:water (BAW; 4:1:5) give high and very low R_f value, respectively.

High performance thin layer chromatographic (HPTLC)

Viloria-Matos et al. (2001) isolated and identified betacyanin from fruits of *Opuntia boldinghii* Br. et R. by HPTLC using two solvent systems (System I: isopropanol:ethanol:water:acetic acid 55:20:20:5; System II: isopropanol:ethanol:water:acetic acid 30:35:30:5) in one dimension. Results showed a major red fraction with a maximum absorbance at 537 nm which is similar to the reported value for betacyanin.

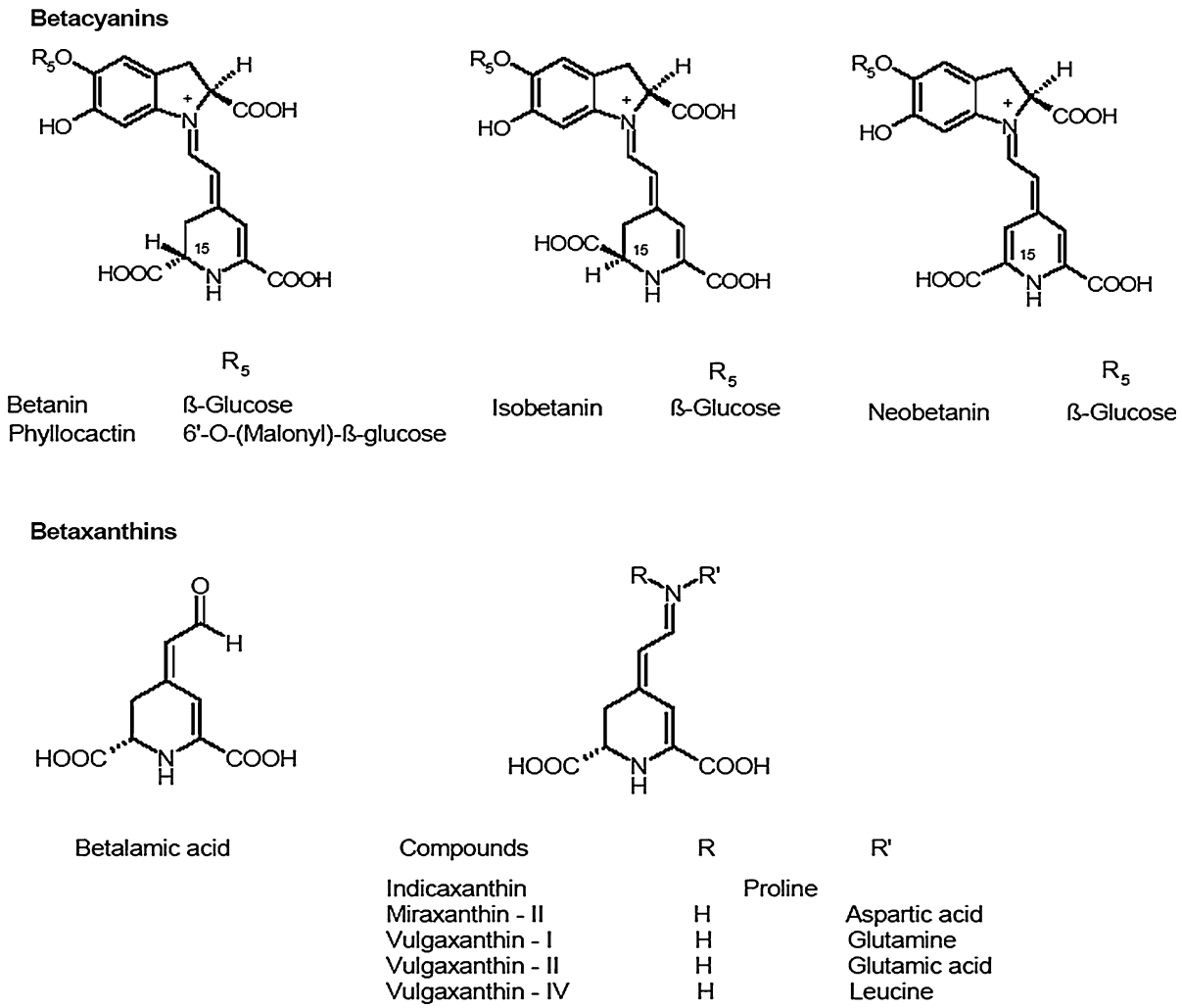

Fig. 1 Structures of betacyanins and betaxanthins found in prickly pear

High performance liquid chromatographic (HPLC)

HPLC is an excellent means in the analysis of betalains. The most common support is C_{18}-derivatized silica providing adequate efficiency and retention of betacyanins as well as their sufficient resolution on conventional stationary phases. Because betacyanins exist in aqueous solution in different ionisation forms at changed pH values, the use of typical acidic eluents with or without buffers is a useful factor governing their separation (Schliemann et al. 1996, 2000, 2001; Wybraniec et al. 2001, 2006). Fernández-López and Almela (2001) separated and identified betalain pigments from methanolic extract of two cultivars of prickly pear (*Opuntia ficus-indica*) fruits using reversed-phase high performance liquid chromatography and photodiode array detector. The chromatographic separation program consisted of a 30 min linear gradient elution from solvent A (1 % acetic acid in water) to 12 % solvent B (1 % acetic acid in acetonitrile) with a flow of 1 ml/min. The chromatographic pattern of the methanolic extract showed two major peaks with a retention time of 16.2 min at 484 nm and 17.4 min at 535 nm, identified as indicaxanthin and betanin, respectively. Fernández-López et al. (2002) also analyzed presence of betalains using method proposed by Fernández-López and Almela (2001) from the fruits of *Opuntia stricta, Opuntia undulata* and *Opuntia ficus-indica* and found HPLC patterns of betalains with retention time at 16.8 min

Table 1 Qualitative analysis of betalains by HPLC

Solvent system	Chromatographic separation tech.	R_t (min)	λ_{max} (nm)	Compound	Reference
A (1 % acetic acid in H_2O) B (1 % acetic acid in acetonitrile)	30 min linear gradient elution from solvent A to 12 % solvent B with a flow of 1 ml/min	16.2	484	Indicaxanthin	Fernández-López and Almela (2001)
		17.4	535	Betanin	
		16.8	484	Indicaxanthin	Fernández-López et al. (2002)
		19.6	537	Betanin	
		22.8	537	Isobetanin	
A (1 % v/v of formic acid in H_2O) B (Aq. MeCN, 80:20 MeCN/H_2O, v/v)	Isocratically with 100 % A for 2 min, a linear gradient was followed from 0 to 20 % B in 60 min and then from 20 to 100 % B in 5 min.	10.4	470	Histidine-betaxanthin	Stintzing et al. (2006b)
		16.3	470	Glutamine-betaxanthin	
		29.2	470	GABA-betaxanthin	
		29.9	470	Isoproline-betaxanthin	
		31.2	470	Proline-betaxanthin	
		36.8	538	Betanin	
		40.7	538	Isobetanin	

(λ_{max} 484 nm), 19.6 min, and 22.8 min (λ_{max} 537 nm) assigned to indicaxanthin, betanin and isobetanin, respectively. Stintzing et al. (2003) separated betalains from *Opuntia ficus-indica* cv. 'Rossa' and cv. 'Gialla' using aqueous 0.2 % trifluoroacetic acid and 10 % formic acid solutions at a ratio of 65/35 (v/v) as eluent A, and a mixture of 100 % acetonitrile and 10 % aqueous formic acid (80/20,v/v) as eluent B. After 15 min of isocratic elution with 100 % A, a linear gradient was followed from 0 % B to 20 % B in 60 min. Betaxanthins was monitored at 470 nm and betacyanins at 538 nm, respectively. Stintzing et al. (2006b) developed a process for the production of both juice concentrates and powders from *Opuntia ficus-indica* fruits of the cultivar 'Gialla' at laboratory and pilot plant-scale, respectively. Since betalains are regarded as thermolabile compounds, alternative processes for juice concentration and preservation, including cross-flow microfiltration and freeze drying, considered. HPLC–DAD peak separation was achieved using mobile phase A (1 % v/v formic acid in water) and B (Aqueous MeCN, 80:20 MeCN/H_2O, v/v). Starting isocratically with 100 % A for 2 min, a linear gradient was followed from 0 to 20 % B in 60 min and then from 20 to 100 % B in 5 min. Pigment retentions of the major betaxanthins and betacyanins were determined at 10.4 min (Histidine-betaxanthin), 16.3 min (Glutamine-betaxanthin), 29.2 min (GABA-betaxanthin), 29.9 min (Isoproline-betaxanthin), 31.2 min (Proline-betaxanthin) at 470 nm and 36.8 min (betanin) and 40.7 min (isobetanin) at 538 nm.

Wybraniec (2006, 2008) reported the effect of tetraalkylammonium salts on retention of betacyanins and decarboxylated betacyanins in ion-pair reversed-phase HPLC and investigated chromatographic acyl migration in betacyanin and their decarboxylated derivatives. Identification of betalains from the fruits of 10 Mexican prickly pear cultivars by HPLC–DAD analysis was performed by Yahia and Castellanos-Santiago (2008) using water (eluent A) and methanol (eluent B) mixture at a flow rate of 1 ml/min. Betalains were separated starting isocratically with 100 % A in 10 min followed by a linear gradient from 0 % B to 30 % B in 30 min, and finally a linear gradient from 30 % B to 100 % B in 20 min, before re-equilibration to the starting conditions. Betaxanthins and betacyanins were monitored at 482 and 535 nm, respectively. Several solvent systems were used for betalain analysis; the best results were obtained in water/methanol system than other methods, acetic acid in water/acetic acid in acetonitrile or phosphoric acid solution buffer. Table 1 shows the qualitative data of betalains by HPLC.

Liquid chromatography–Mass spectroscopy (LC–MS)

The use of mass spectrometry (MS) coupled to HPLC complements the use of photodiode-array detectors (PAD) and permits immediate identification of components of a mixture and characterization of an extract in terms of its chemical composition. MS provides molecular weight and structural information of the

Table 2 Qualitative data of betalains in prickly pear (Opuntia spp.) fruit by HPLC–ESI–MS

Solvent system	Chromatographic separation tech.	R_t (min)	λ_{max} (nm)	$[M + H]^+$ m/z	Daughter ions	Compounds	Reference
A (88 mM acetic acid in H_2O) B (88 mM acetica acid in acetonitrile	Linear gradient from 100 % Solvent A to 12 % solvent B for 30 min	16.8	484	309	263, 217	Indicaxanthin	Fernández-López et al. (2002)
		19.6	537	551		Betanin	
		22.8	537	551		Isobetanin	
A (1 % Formic acid in Water) B (Methanol)	Start isocratically with 100 % A, followed by a linear gradient from 0 to 10 % B in 20 min, and then a linear gradient from 30 to 100 % B in 5 min	1.6	438	325	309	Portulacaxanthin i	Yahia and Castellanos-Santiago (2008)
		1.8	470	269	225	Portulacaxanthin iii	
		5.1	474	326	295, 149	Vulgaxanthin iii	
		5.2	478	349	215, 124	Muscaaurin	
		6.5	478	305	172, 149	Unknown	
		7.3	472	299	268, 136	Unknown	
		9.4	475	340	323	Vulgaxanthin i	
		14.5	474	341	325, 149	Vulgaxanthin ii	
		18.9	535	713	551, 389	Betanidin-5-O-β-sophoroside	
		20.1	470	297	253, 149	Unknown	
		21.0	483	309	263, 188	Unknown	
		22.0	483	309	263, 219	Indicaxanthin	
		27.2	478	329	295, 297	Unknown	
		27.3	538	551	389, 149	Betanin	
		27.3	540	389	345, 150	Betanidin	
		28.5	538	551	389, 149	Isobetanin	
		30.2	472	311	175, 137	Unknown	
		30.3	470	311	299, 137	Unknown	
		32.0	475	398	353, 311	Unknown	
		33.5	480	549	387	*Neo*-betanin	
		33.9	472	325	308, 219	Unknown	
		34.1	473	325	209	Vulgaxanthin iv	
		34.1	535	459	443, 413	Unknown	
		34.4	467	359	312, 225	Unknown	
		36.0	475	315	270	Unknown	

chromatographic bands so that fully-resolved peaks are not required, thus shortening chromatographic runs and reducing sample preparation while ensuring high sensitivity and selectivity. This technique is commonly used in investigations on betalain pigments (Schliemann et al. 1996, 2000, 2001; Wybraniec et al. 2001). Fernández-López et al. (2002) screened the presence of betalain pigments in fruits of *Opuntia stricta, Opuntia undulata* and *Opuntia ficus-indica,* also Yahia and Castellanos-Santiago (2008) identified betalains from the fruits of 10 Mexican prickly pear cultivars by HPLC and ESI–MS, qualitative data summarized in Table 2.

Nuclear magnetic resonance (NMR)

Unambiguous betalain structures can only be elucidated by nuclear magnetic resonance (NMR) measurements, requiring tedious isolation and solid experimental set up (Strack et al. 2003; Stintzing and Carle 2007). Stintzing et al. (2004) analyzed betacyanin pigments by LC–NMR and 2D NMR spectroscopy from red–purple pitaya (*Hylocereus polyrhizus* (Weber) Britton and Rose) at neutral pH (Table 3). Wybraniec et al. (2006) elucidated decarboxylated betanin, phyllocactin and hylocerenin of purple pitaya (*Hylocereus* polyrhizus) fruits by ^{1}H and ^{13}C NMR

Table 3 ^{1}H and ^{13}C NMR data of betacyanins; D_2O, ref $\delta = 4.7$ ppm; 500 MHz

Position	Betanin		Isobetanin		Phyllocactin		Hylocerenin	
	^{1}H NMR δ[ppm], mult, J[Hz]	^{13}C NMR δ[ppm]	^{1}H NMR δ[ppm], mult, J[Hz]	^{13}C NMR δ[ppm]	^{1}H NMR δ[ppm], mult, J[Hz]	^{13}C NMR δ[ppm]	^{1}H NMR δ[ppm], mult, J[Hz]	^{13}C NMR δ[ppm]
2	4.92, dd, 3.1; 10.3	65.0	4.97, dd, 2.7, 10.1	64.7	4.90, dd, 3.1, 10.5	64.9	4.85, dd, 3.1, 10.5	64.6
3a	3.53, dd, 11.5; 16.9	32.7	3.51, dd, 11.6, 16.7	33.5	3.56, dd, 11.6, 16.5	33.0	3.56, dd, 11.6, 16.5	32.9
3b	3.10, dd, 4.3; 16.8	nf	3.18, bd, 16.5	nf	3.11, dd, 4.3, 16.5	nf	3.11, dd, 4.3, 16.5	nf
4	7.06, s	113.9	6.93, s	113.9	7.00, s	113.9	7.00, s	113.9
5	nf	144.0	nf	144.1	nf	143.9	nf	143.9
6	nf	146.1	nf	146.6	nf	146.2	nf	146.7
7	nf	100.0	6.91, bs	100.3	6.94, bs	100.1	6.94, bs	99.9
8	nf	137.4	nf	137.9	nf	137.9	nf	138.0
9	nf	124.1	nf	124.3	nf	124.1	nf	124.3
10	nf	175.8	nf	176.1	nf	176.5	nf	176.5
11	8.19, bs (d, 12.6)	144.4	8.11, bs (d, 11.6)	144.6	8.14, bs (d, 11.8)	144.3	8.14, bs (d, 11.8)	143.7
12	5.84, bs (d, 12.6)	106.9	5.84, bs (d, 12.46)	nf	5.84, bs (d, 10.5)	106.4	5.80, bs (d, 12.6)	106.1
13	nf	117.7	nf	nf	nf	nf	nf	nf
14a	3.20, bm	26.5	3.13, bd, 17.6	26.4	3.24, bm	26.7	3.14, bm	26.4
14b	3.12, bm	nf	2.94, bdd, 7.0, 17.0	nf	3.16, bm	nf	nf	nf
15	4.40, bt, 7.1	53.1	4.31, bs	53.0	4.37, bt, 6.5	53.1	4.33, bt, 7.2	53.3
18	6.22, bs	105.2	6.18, bs	nf	6.22, bs	nf	6.20, bs	nf
1′	4.98, d, 7.4	101.4	4.95, d, 7.1	101.0	4.98, d, 7.1	101.4	4.99, d, 7.2	101.1
2′	3.55 (overlap)	75.7	3.55 (overlap)	73.4	3.56 (overlap)	75.2	3.56 (overlap)	75.2
3′	3.55 (overlap)	73.9	3.55 (overlap)	75.8	3.57 (overlap)	72.8	3.56 (overlap)	72.5
4′	3.41 (overlap)	69.3	3.45, pt, 8.8	69.7	3.50, t, 9.2	69.4	3.47, pt, 9.51	69.4
5′	3.52 (overlap)	76.2	3.51 (overlap)	76.6	3.76, ddd, 2.3, 5.6, 9.5	73.8	3.73, ddd, 1.9, 6.3, 9.4	73.5
6′a	3.85, dd, 1.6, 12.3	60.6	3.86, dd, 1.4, 12.4	60.8	4.46, dd, 2.2, 12.3	63.7	4.45, dd, 1.6, 12.1	63.02
6′b	3.70, dd, 5.3, 12.3	nf	3.71, dd, 5.3, 12.4	nf	4.33, dd, 5.6, 12.3	nf	4.23, dd, 6.4, 12.2	nf
1″	nf	nf	nf	nf	nf	170.1	nf	172.9
2″a	nf	nf	nf	nf	3.34, s	43.3	2.68, d, 14.4	45.0
2″b	nf	nf	nf	nf	nf	nf	2.64, d, 14.4	nf
3″	nf	nf	nf	nf	nf	172.9	nf	69.9
4″	nf	nf	nf	nf	nf	nf	1.24, s	26.8
5″a	nf	nf	nf	nf	nf	nf	2.56, d, 15.0	44.4
5″b	nf	nf	nf	nf	nf	nf	2.52, d, 15.0	nf
6″	nf	nf	nf	nf	nf	nf	nf	175.6

nf not found

Table 4 ^{1}H (600 MHz, D_2O) and ^{13}C (150 MHz, D_2O) NMR data of decarboxylated betanin

Position	2-Decarboxybetanin		17-Decarboxybetanin		2,17-Bidecarboxybetanin	
	^{1}H NMR δ[ppm], mult, J[Hz]	^{13}C NMR δ[ppm]	^{1}H NMR δ[ppm], mult, J[Hz]	^{13}C NMR δ[ppm]	^{1}H NMR δ[ppm], mult, J[Hz]	^{13}C NMR δ[ppm]
2	4.11, bt, 5.6	50.1	4.72, dd, 3.4, 10.5	64.8	4.06, bt, 7.5	49.7
3a	3.16, bt	26.6	3.50, dd, 10.5, 16.6	33.0	3.15, bt	26.7
3b	3.16, bt	nf	3.11, dd, 3.4, 16.6	nf	3.15, bt	nf
4	7.08, s	113.7	7.00, s	113.8	7.08, s	114.0
5	nf	143.3	nf	143.0	nf	143.6
6	nf	145.8	nf	146.1	nf	145.9
7	6.98, s	99.7	6.88, s	99.2	6.94, s	99.4
8	nf	136.9	nf	137.9	nf	137.5
9	nf	124.1	nf	123.5	nf	125.8
10	nf	nf	nf	176.7	nf	nf
11	8.17, bd, 11.0	144.0	8.03, bs	142.8	8.10, bs	138.5
12	6.02, bd, 12.4	106.3	5.57, bs	103.8	5.84, bs	109.1
13	nf	117.4	nf	nf	nf	nf
14a	3.23, bm	27.0	3.15, bm	27.0	3.20, bm	27.1
14b	3.09, bm	nf	3.15, bm	nf	3.20, bm	nf
15	4.23, bt, 7.4	53.8	4.18, t, 7.9	53.7	4.20, t, 8.3	53.8
17	nf	nf	7.52, d, 5.7	155.3	7.52, d, 5.5	154.4
18	6.17, bs	104.1	5.76, bs	105.3	5.78, bs	104.4
19	nf	nf	nf	176.0	nf	nf
1′	4.98, d, 6.7	101.3	4.90, d, 7.0	101.5	4.97, d, 7.9	101.4
2′	3.55 (overlap)	75.2	3.49 (overlap)	75.9	3.55 (overlap)	76.0
3′	3.53 (overlap)	72.8	3.46 (overlap)	72.7	3.53 (overlap)	75.6
4′	3.41 (overlap)	69.3	3.40 (overlap)	69.3	3.41 (overlap)	69.2
5′	3.53 (overlap)	76.0	3.49 (overlap)	76.0	3.52 (overlap)	72.8
6′a	3.87, bd, 12.3	60.4	3.79, dd, 1.6, 12.4	60.3	3.83, dd, 2.2, 12.5	60.4
6′b	3.71, bdd, 5.2, 12.3	nf	3.64, dd, 5.3, 12.4	nf	3.71, dd, 5.2, 12.5	nf

nf not found

spectroscopy summarized in Tables 4, 5 and 6, respectively. Betaxanthin structure was elucideted based on ^{1}H NMR data (Trezzini and Zr 1991) and Stintzing et al. (2006b) has reported ^{13}C NMR data of two betaxanthins (indicaxanthin and miraxanthin) by applying only slightly acidic conditions.

Quantitative analysis

Spectrophotometric method

The most convenient way to quantify betalains is spectrophotometric method. First, Nilsson (1970) established a method to quantify pigments in beetroot. The total contents of betacyanins and betaxanthins were determined using the formula reported by Cai et al. (2005); Nilsson (1970); Chethana et al. (2007); Fernández-López and Almela (2001); Fernández-López et al. (2002). Their molar absorptivity (ε) values were 5.66×10^4 (amaranthin, $E^{1\%}_{1cm}$ 536 nm = 779), 6.16×10^4 (betanin, $E^{1\%}_{1cm}$ 536 nm = 1120), and $5.06 \times 10_4$ (gomphrenin I, $E^{1\%}_{1cm}$ 540 nm = 920). The mean molar absorptivity (ε) value for betaxanthins is 4.80×10^4. Another formula for determination of betalain content was described by (Cai and Corke 1999; Stintzing et al. 2003, 2005): [BLC [mg/l] =

Table 5 ^{1}H (600 MHz, D_2O) and ^{13}C (150 MHz, D_2O) NMR data of decarboxylated phyllocactin

Position	2-Decarboxyphyllocactin		17-Decarboxyphyllocactin		2,17-Bidecarboxyphyllocactin	
	^{1}H NMR δ[ppm], mult, J[Hz]	^{13}C NMR δ[ppm]	^{1}H NMR δ[ppm], mult, J[Hz]	^{13}C NMR δ[ppm]	^{1}H NMR δ[ppm], mult, J[Hz]	^{13}C NMR δ[ppm]
2	4.07, bt, 5.6	50.0	4.78, dd, 3.5, 10.3	64.9	4.02, bt, 7.1	49.7
3a	3.13, bt	26.9	3.58, dd, 10.4, 16.2	33.2	3.14, bt	26.8
3b	3.13, bt	nf	3.08, dd, 3.5, 16.2	nf	3.14, bt	nf
4	7.03, s	113.3	7.05, s	114.5	7.03, s	113.4
5	nf	143.8	nf	142.6	nf	143.1
6	nf	145.6	nf	146.1	nf	145.7
7	6.93, s	99.6	6.95, s	99.4	6.89, s	99.3
8	nf	137.1	nf	138.3	nf	137.4
9	nf	126.4	nf	123.3	nf	125.6
11	8.10, bd, 10.9	143.7	8.10, bs	142.9	8.05, bs	143.3
12	5.98, bd, 11.0	104.1	5.63, bs	104.0	5.80, bs	104.2
13	nf	118.1	nf	nf	nf	nf
14a	3.21, bm	27.0	3.21, bd, 7.8	27.2	3.16, bm	27.1
14b	3.10, bm	nf	3.21, bd, 7.8	nf	3.16, bm	nf
15	4.24, bt, 7.2	53.9	4.24, t, 8.2	53.9	4.19, t, 8.3	53.9
17	nf	nf	7.59, d, 5.6	155.3	7.51, bs	154.2
18	6.17, s	106.7	5.84, bs	105.5	5.76, bs	104.2
1′	4.97, d, 6.6	101.0	4.96, d, 7.2	101.9	4.94, d, 5.8	101.1
2′	3.55 (overlap)	75.3	3.73, m	74.9	3.57 (overlap)	74.6
3′	3.56 (overlap)	72.8	3.56 (overlap)	73.1	3.55 (overlap)	73.0
4′	3.53 (overlap)	69.3	3.56 (overlap)	68.9	3.52 (overlap)	69.2
5′	3.75, m	73.7	3.57 (overlap)	73.7	3.77, m	73.7
6′a	4.40, dd, 12.4	63.5	4.40, dd, 12.4	63.2	4.46, dd, 12.1	63.4
6′ b	4.75, dd, 4.0, 12.4		4.35, dd, 4.0, 12.4		4.32, dd, 5.2, 12.3	nf
1″	nf	170.9	nf	nf	nf	nf
2″a	3.25, d	44.1	3.22, d	44.0	3.29, d	44.6
2″b	3.25, d	nf	3.22, d	nf	3.29, d	nf
3″	nf	172.6	nf	nf	nf	nf

nf not found

(A × DF × MW × 1000)/(e × 1)], where A is the absorption value at the absorption maximum, DF the dilution factor and 1 the pathlength (1 cm) of the cuvette. For quantification of betacyanins and betaxanthins, the molecular weights (MW) and molar extinction coefficients (ε) of betanin (MW = 550 g/mol; ε = 60,000 l/mol cm in H_2O; λ = 538 nm) and indicaxanthin (MW = 308 g/mol; ε = 48,000 l/mol cm in H_2O; λ = 480 nm) were applied, respectively. Stintzing et al. (2005, 2006a) developed a process for production of cactus pear juice and fruit powders. Quantitative and qualitative color changes during processing were monitored by analysing juice samples after each processing step in terms of CIE$L^*C^*h^\circ$ and betalain contents. Table 7 summarizes spectrophotometric quantification of betalains.

Yahia and Castellanos-Santiago (2008) extracted the pigments using two solvents, McIlvaine buffer (pH 6.5, citrate–phosphate) and water from the fruits of 10 Mexican prickly pear Cultivars. The betalain content (BC) was calculated according to literature with a

Table 6 ^{1}H (600 MHz, D_2O) and ^{13}C (150 MHz, D_2O) NMR data of decarboxylated hylocerenin

Position	2-Decarboxyhylocerenin		2,17-Bidecarboxyhylocerenin	
	^{1}H NMR δ[ppm], mult, J[Hz]	^{13}C NMR δ[ppm]	^{1}H NMR δ[ppm], mult, J[Hz]	^{13}C NMR δ[ppm]
2	4.11, bt, 7.2	50.0	4.06, bt, 6.9	49.9
3a	3.16, bt	26.8	3.15, bt, 7.6	26.6
3b	3.16, bt	nf	3.15, bt, 7.6	nf
4	7.05, s	113.3	7.05, s	114.1
5	nf	143.8	nf	143.0
6	nf	146.7	nf	146.3
7	6.97, s	99.6	6.94, s	99.5
8	nf	137.4	nf	137.9
9	nf	126.2	nf	125.8
11	8.16, bd, 8.8	143.7	8.11, bs	139.2
12	6.02, bd, 8.8	106.5	5.83, bs	104.3
14a	3.26, bm	27.1	3.21, bm	27.1
14b	3.12, bm	nf	3.14, bm	nf
15	4.25, bt, 7.1	53.9	4.20, t, 8.5	53.8
17	nf	nf	7.51, bd, 5.6	154.4
18	6.18, s	104.5	5.78, bs	104.7
1′	5.10, d, 6.6	101.0	5.00, d, 7.4	101.3
2′	3.57 (overlap)	75.0	3.57 (overlap)	75.1
3′	3.56 (overlap)	72.9	3.55 (overlap)	72.5
4′	3.48 (overlap)	69.5	3.49 (overlap)	69.9
5′	3.74, m	73.6	3.74, m	73.8
6′a	4.26, dd, 11.5	63.3	4.46, dd, 12.0	63.2
6′ b	4.24, dd, 11.5	nf	4.26, dd, 6.0	nf
1″	nf	177.1	nf	177.1
2″a	2.60, d, 14.5	45.5	2.61, d, 14.5	45.7
2″b	2.64, d, 14.5	nf	2.63, d, 14.6	nf
3″	1.22, s	69.9	1.19, s	69.7
4″	nf	26.3	nf	26.2
5″a	2.41, d, 15.1	45.8	2.42, d, 15.1	47.3
5″b	2.46, d, 15.1	nf	245, d, 15.0	nf
6″	nf	172.6	nf	172.8

nf not found

slight modification; BC [mg/g]) [(A(DF)(MW)Vd/εLWd)], where A is the absorption value at the absorption maximum of 535 and 483 nm for betacyanins and betaxanthins, respectively, DF is the dilution factor, Vd is the dried pulp solution volume (ml), Wd is the dried pulp weight (g), and L is the path-length (1 cm) of the cuvette. In all cases, water extracted the highest level of pigments. Spectrophotometric quantification of betalains summarized in Table 8.

Conclusion

From the present review, analysis of betalains from the prickly pear carried out easily using visible spectrophotometer and HPLC. Presently, analysis of phytochemical constituents mostly carried out using HPTLC, but in case of betalains still such method is not available. Estimation of betalains carried out by spectrophotometer which is more reliable and economic. Though various analytical methods are

Table 7 Spectrophotometric quantification of betalains in prickly pear

Opuntia spp.	Betaxanthin	Betacyanin	Reference
Opuntia ficus-indica (reddish purple)	30 mg/100 g	19 mg/100 g	Fernández-López and Almela (2001)
Opuntia ficus-indica (yellow)	Not detected	25 mg/100 g	
Opuntia ficus-indica (L.) Mill.	Not detected	14.5 mg/100 g	Fernández-López et al. (2002)
Opuntia stricta Haw	Not detected	70 mg/100 g	
Opuntia undulata Griff	Not detected	18.5 mg/100 g	
Opuntia ficus-indica (L.) Mill. cv. 'Rossa' (red)	4.8–49.6 mg/l	66.5–80.4 mg/l	Stintzing et al. 2003 (betalains quantified at different pH and using different methods)
Opuntia ficus-indica (L.) Mill. cv. 'Gialla' (orange-yellow)	10.5–53.7 mg/l	5.4–19.6	

Table 8 Spectrophotometric quantification of betalains in the fruits of 10 Mexican prickly pear cultivars

Cultivar	Betacyanin content (mg/g dry pulp)		Betaxanthins content (mg/g dry pulp)		Total betalains (mg/g dry pulp)	
	Water	Buffer	Water	Buffer	Water	Buffer
Camuesa	5.29 ± 0.35	5.01 ± 0.60	2.86 ± 0.24	2.56 ± 0.42	8.15	7.57
Roja Pelota	2.06 ± 0.06	1.86 ± 0.28	0.99 ± 0.03	0.84 ± 0.12	3.04	2.71
Cardona	2.04 ± 0.20	1.83 ± 0.00	1.04 ± 0.09	0.80 ± 0.00	3.08	2.63
2142	0.71 ± 0.04	0.66 ± 0.01	0.44 ± 0.03	0.38 ± 0.01	1.16	1.04
Liria	0.39 ± 0.03	0.34 ± 0.02	0.14 ± 0.01	0.11 ± 0.00	0.53	0.45
Roja Lisa	0.27 ± 0.01	0.22 ± 0.02	0.23 ± 0.02	0.18 ± 0.00	0.50	0.40
Naranjona	0.065 ± 0.01	0.04 ± 0.01	0.16 ± 0.02	0.12 ± 0.00	0.23	0.16
2651	0.072 ± 0.00	0.04 ± 0.01	0.14 ± 0.02	0.09 ± 0.01	0.21	0.13
21441	0.071 ± 0.00	0.05 ± 0.01	0.41 ± 0.02	0.35 ± 0.04	0.48	0.40
Reyna	0.05 ± 0.02	0.03 ± 0.03	0.12 ± 0.01	0.23 ± 0.20	0.17	0.26
Red beet	5.41 ± 0.02	4.98 ± 0.00	3.21 ± 0.01	3.12 ± 0.00	8.60	8.10

reported, but still more focus is required towards HPLC and HPTLC with marker's evidence.

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