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Pigment pattern and expression of colour in fruits from different *Hylocereus* sp. genotypes

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Abstract

Pigment profiles, betalain contents, colour and pH-values were studied in fruits from five *Hylocereus* sp. genotypes originating from Costa Rica. Significant colour differences between fruit pulps from "Nacional" and "San Ignacio" were found for *C**-values and "Lisa" exhibited the most reddish tint *h*°. Whereas highest betalain contents were registered in "San Ignacio" and "Orejona", lowest values were found in "Nacional". Additionally, pigment patterns of the genotypes were found to differ: While "Lisa", "Nacional" and "Orejona" were characterised by a similar betalain pattern, "Rosa" and "San Ignacio" were significantly different offering a valuable tool for genotype comparison. Moreover, "Rosa" showed the highest betanin and isobetanin contents, whereas phyllocactin and hylocerenin were predominant in "San Ignacio". Apart from the newly reported betalains neobetanin and gomphrenin I, indicaxanthin was the first betaxanthin so far detected in pitaya fruits.

Keywords: Hylocereus; Dragonfruit; Genotypes; Betalains; Colour; Betanin; Phyllocactin; Hylocerenin; Neobetanin; Gomphrenin I; Indicaxanthin

Industrial relevance: Natural substitutes for synthetic colourants are increasingly gaining importance on the global market. In particular, the so-called colouring foodstuffs representing aqueous or oily plant extracts extend their market share with red-coloured preparations being particularly requested. In this regard, pitaya fruits from the genotype *Hylocereus* have been proposed as promising colour sources, recently. To secure the authenticity of the plant material, a reliable differentiation of pitayas from different origins is required. In addition, *Hylocereus* fruits may differ in their colour quality and pigment content, the knowledge of which is crucial for the selection of appropriate plants for an emerging pitaya market.

1. Introduction

There is a growing interest in the use of natural pigments for food colouring because natural products are associated with quality and health promotion whereas synthetic pigments are critically assessed by consumers (Downham & Collins, 2000).

Among the former, betalains have recently re-gained interest in food science (Delgado-Vargas, Jiménez, & Paredes-López, 2000; Stintzing & Carle, 2004). In contrast to anthocyanins, they maintain their colour over a wide pH range from 3 to 7. This property makes betalains ideal for colouring low-acid foodstuff such as dairy products (Stintzing & Carle, 2004). The most important betalain sources for natural red colouring are selected varieties of red beet, commercial preparations of which are mainly composed of the red-purple betanin and its C_{15}-isomer isobetanin. However, because of the unfavourable earth-like flavour characteristics caused by geosmin and pyrazine derivatives, as well as high nitrate concentrations associated with the formation of carcinogenic nitrosamines, there is a demand for alternative compounds (Castellar, Obón, & Fernández-López, 2006; Stintzing & Carle, 2004). Hence, fruits from the Cactaceae have been proposed as a promising betalain source (Stintzing, Schieber, & Carle, 2001, 2003), offering preparations with a broader colour spectrum and being devoid of the mentioned drawbacks (Stintzing et al., 2001). In addition to cactus pears (*Opuntia ficus-indica* [L.] Mill.), purple-fleshed pitayas (*Hylocereus polyrhizus* [Weber] Britton & Rose) have very recently been suggested as viable betalain sources (Stintzing, Schieber, & Carle, 2002; Wybraniec Platzner, Geresh, Gottlieb, Haimberg, Mogilnitzki, et al., 2001).

However, variability in the pigment composition within members of the genus *Hylocereus* has been scarcely addressed. So far, Wybraniec and Mizrahi (2002) reported the colour

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characteristics of several *Hylocereus* clones cultivated in Israel to range from purple to red.

Selected species in this genus have been developed as fruit crops. Among them, *H. undatus* [Haworth] Britton & Rose (red pericarp, white pulp) has been widely planted, while others such as *H. polyrhizus* [(F.A.C. Weber) Britton & Rose] (red pericarp, red-violet pulp) and *H. costaricensis* [(F.A.C. Weber) Britton & Rose] (red pericarp, red pulp) are grown at smaller scale (Mizrahi & Nerd, 1999). In spite of the recent commercial importance of this crop, there is still some uncertainty with respect to the taxonomic placement of some of the selected genotypes (Mizrahi, Nerd, & Nobel, 1997). In Central America, where pitayas are native to, particular names have been coined depending on individual morphological characteristics such as fruit shape, scale number and profile (Anonymous, 1994; Vaillant, Perez, Davila, Dornir, & Reynes, 2005). “Lisa”, “Orejona”, “Rosa” and “San Ignacio” are the most common genotypes commercialised in Nicaragua, the Central American country with the highest pitaya production of about 3000 t on 420 ha (Vaillant et al., 2005).

The preliminary findings on the pigment potential and the scant knowledge about *Hylocereus* colour characteristics prompted more thorough investigations. To gain a more complete insight into pigment-colour relationships, the present work aimed at the determination of colour properties, pigment contents and betalain patterns of *Hylocereus* sp. fruits belonging to different genotypes cultivated in Costa Rica.

2. Materials and methods

2.1. Plant material and sample preparation

Three-year-old plants of the genotypes “Lisa”, “Rosa”, “San Ignacio”, “Orejona” and “Nacional”, cultivated organically in Barranca, Puntarenas, Costa Rica (N 9°57.566′ W 084°43.217′) were used for this work. The plants were brought from Nicaragua plantations several years ago, except for those of the genotype “Nacional”, which were formerly collected from the surroundings of the pitaya plantation and grown there for several years. Samples of each of the analysed genotypes were deposited at the Herbarium at the Universidad de Costa Rica (USJ), where they received the following accession numbers: Rosa 88680, San Ignacio 88681, Nacional 88682, Lisa 88683 and Orejona 88684. Six fruits of each genotype were harvested from July to August 2004, when their peel colour started to turn from green to purple. Fruits were stored at room temperature until the skin colour change was completed. At that moment, peel was manually removed and processed in an Oster extractor (Model 5720-08, Mexico City, Mexico). Clarified juice was obtained by passing the extracted pulp through a filter paper (Whatman No. 4, Whatman Inc., NJ, USA).

2.2. Betacyanin content, colour analysis and pH value determination

The colour of the processed pulp (20 ml) was determined with a Colourflex (Hunterlab, VA, USA) instrument, using illuminant D_{65} and 10° observer angle. Colour was expressed as L^*, a^*, b^*, chroma $[C^*=(a^{*2}+b^{*2})^{½}]$ and hue angle $[h°=\arctan b^*/a^*]$ as described by Stintzing et al. (2003). Colour differences (ΔE^*) were determined according to Gonnet (1998) with $\Delta E^*=[(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{½}$. After pH determination of the juice (Corning pH meter, Model 430, Corning, NY, USA), photometric quantification of total betalains was carried out following the method by Stintzing et al. (2003) on a Shimadzu (Model UV-1203, Tokyo, Japan) UV/Vis spectrophotometer. An aliquot of the samples was membrane filtered (0.45 µm) for HPLC measurements.

2.3. HPLC–UV

The pigment patterns of the juices were determined using an HPLC system (Merck, Darmstadt, Germany) equipped with an analytical Sunfire C_{18}-column (250×4.6 mm i.d., Waters, Wexford, Ireland) with a particle size of 5 µm. Separation was achieved at 30 °C using a flow rate of 1 ml min^{-1}. The mobile phase A consisted of 0.2% (v/v) formic acid in water, while MeCN:H_2O (80:20, v/v) was used as the mobile phase B. The first 5 min were performed isocratically with 100% A. Then, a

Table 1
Betalain concentration, pH and chromatic parameters of the extracted fruit pulp obtained from different *Hylocereus* genotypes

Genotype	Betalains (mg l^{-1})	L^*	a^*	b^*	C^*	$h°$	pH
“Lisa”	554.0±17.1ab	6.2±0.6b	32.4±1.6ab	2.0±1.1a	32.6±1.6ab	3.6±1.8a	4.70±0.03a
“Nacional”	474.1±54.8b	7.6±0.5ab	31.7±1.3b	0.5±1.3ab	31.8±1.3 b	0.6±1.6ab	4.89±0.05a
“Orejona”	707.6±93.2a	6.0±0.5b	32.6±1.6ab	0.3±1.0ab	32.7±1.6ab	0.3±1.8ab	4.71±0.06a
“Rosa”	629.2±43.1ab	6.5±0.6ab	33.7±1.4ab	−1.0±1.0b	33.8±1.4ab	358.4±1.8b	4.84±0.08a
“San Ignacio”	717.1±50.1a	8.1±0.6a	35.9±1.1a	−1.57±0.3b	35.9±1.1a	357.5±1.6b	4.30±0.07 b

$\triangle E^*$

	“Lisa”	“Nacional”	“Orejona”	“Rosa”	“San Ignacio”
“Lisa”	–	–	–	–	–
“Nacional”	2.2	–	–	–	–
“Orejona”	1.7	1.9	–	–	–
“Rosa”	3.4	2.8	1.8	–	–
“San Ignacio”	5.4	4.7	4.2	2.7	–

Significant differences within values in the same column are indicated by different letters ($P<0.05$).