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By-product recovery of *Opuntia spp*. peels: Betalainic and phenolic profiles and bioactive properties

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ABSTRACT

Opuntia spp. are a tropical and subtropical plant that provides both edible green steams and fruits; however, the processing of this fruits results in the accumulation of enormous amount of by-products that can be a source of bioactive and pigmented compounds. Herein, three cactus pear from the species *Opuntia ficus-indica* var. sanguigna (OS) and gialla (OG) and *Opuntia engelmannii* (OE) were fully characterized regarding their phenolic and betalain composition and correlated with their antioxidant and antimicrobial activities. The hydroethanolic extracts of OE gave the highest amount of phenolic compounds isorhamentin-*O*-(deoxyhexosyl-hexoside) and betacianins (betanin); however, no betaxanthins were identified in this sample. This sample also revealed the lowest EC_{50} values in all the antioxidant activity assays. Regarding antimicrobial activity, the hydroethanolic extracts of all species revealed to be more active than ampicillin. The pivotal objective of this work was to focus on exploring by-product biocompounds and possible outputs, thus, we could suggest the use of these natural colorants with intrinsic antioxidant and antimicrobial activity, which would grant industries to produce cleaner label products with functional benefits.

1. Introduction

Opuntia spp., including their several varieties, belong to the dicotyledonous angiosperm and are the largest genus within *Cactaceae* family. *Opuntia* species are tropical and subtropical plants, able to grow in arid and semi-arid environments, with easy geographic adaptation due to their crassulacean acid metabolism (CAM). CAM enables the plant to survive to extreme heat, low temperatures (−40 °C) and drought because of their highly efficiency in the use of water (Nobel, 1998; Sudzuki et al., 1993). This plant provides both edible green steams and fruits. Cactus fruit is also known as prickly pear, cactus fig, Indian fig, cactus pear, Barbary fig, and is available across the 5 continents from early summer until late autumn. Although *Opuntia* species are native of Mexico, where the steams and fruits are consumed fresh in the local diet, fruits, are also processed and commercialized in numerous countries as jams, sweats, ice cream and alcoholic and not alcoholic beverages (Martins et al., 2016).

Opuntia spp. crops can be found in the five continents, being Mexico the majoritarian producer of commercial prickly pear with a total cultivated area of 72,500 ha. Italy, the second largest, harvest around 3000 ha with a 70,000 t yield per year. The processing of the fruits results in the accumulation of several quantities of by-products, namely prickly pear peels, which in the case of *Opuntia ficus indica*, accounts for around 30% of the fruit weight. In addition extensive but unknown areas of wild plants are exploited for food, feeds and materials (FAO, 2013). Proper utilization of this by-product could reduce waste disposal problems and serve as a potential new source of bioactive compounds and pigments. According to several authors (Belwal et al., 2016; Garcia-Castello et al., 2015; Pinela et al., 2016; Rao, 2010), one of the best ways to use this kind of by-products could be the application of an appropriate green solid-liquid extraction technology in order to obtain bioactive compounds with different properties and health benefits. Several studies marked fruits and fruits by-products as a rich source of natural molecules such as polyphenols (i.e. flavonoids), vitamins, colorants like betalains and carotenoids (Albuquerque et al., 2016; Alzate et al., 2016; Ayala-Zavala et al., 2011; Fernandez-Rojas et al., 2010; Silva et al., 2016), which could have high potential interest in human health, medicine and production of new added-value products.

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Table 1
Retention time (Rt), wavelengths of maximum absorption in visible region (λ_{max}), mass spectral data, tentative identification and quantification of phenolic compounds in Opuntia peels.

Peak	Tentative identification	Rt (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	OG	OS	OE	*t*-Student *p*-value
1	Piscidic acid[1]	4.65	222,276	255	193(34),179(6),165(100),149(3)	1.061 ± 0.04	nd	nd	–
2	Eucomic acid[1]	6.71	223,276	239	179(100),149(62)	1.40 ± 0.01	2.2 ± 0.2	nd	< 0.001
3	Isorhamnetin-*O*-(di-deoxyhexosyl-hexoside)[2]	17.09	354	769	315(100)	0.22 ± 0.01	0.56 ± 0.06	nd	< 0.001
4	Isorhamnetin-*O*-(di-deoxyhexosyl-hexoside) [2]	17.4	353	769	315(100)	tr	0.21 ± 0.03	nd	–
5	Isorhamentin-*O*-(deoxyhexosyl-pentosyl-hexoside) [2]	17.7	354	755	315(100)	0.77 ± 0.02	1.3 ± 0.1	nd	< 0.001
6	Quercetin-3-*O*-rutinoside[2]	17.98	354	609	301(100)	nd	nd	tr	–
7	Isorhamentin-*O*-(deoxyhexosyl-pentosyl-hexoside)[2]	18.14	354	755	315(100)	tr	tr	nd	–
8	Isorhamentin-*O*-(pentosyl-hexoside)[2]	19.58	354	609	315(100)	0.100 ± 0.001	0.67 ± 0.06	nd	< 0.001
9	Kaempferol-3-*O*-rutinoside[2]	21.31	348	593	285(100)	nd	nd	tr	–
10	Isorhamentin-*O*-(deoxyhexosyl-hexoside)[2]	21.67	354	623	315(100)	tr	tr	0.48 ± 0.01	–
11	Isorhamentin-*O*-(deoxyhexosyl-hexoside)[2]	22.25	354	623	315(100)	0.77 ± 0.01[c]	1.7 ± 0.1[b]	5.99 ± 0.04[a]	–
12	Isorhametin-3-*O*-glucoside[2]	23.08	354	447	315(100)	nd	nd	tr	–
	Total phenolic compounds					3.26 ± 0.04[c]	3.7 ± 0.3[b]	6.5 ± 0.1[a]	–

Phenolic compounds were expressed in mg/g extract; nd- not detected; Calibration standards: 1- *p*-hydroxibenzoic acid ($y = 208604x + 173056$, $R^2 = 0.9995$); 2-quercetin-3-*O*-glucoside ($y = 34843x - 160173$, $R^2 = 0.9998$). OG, *Opuntia ficus-indica* var gialla; OS, *Opuntia ficus-indica* var sanguigna; OE, *Opuntia engelmannii*; nd, not detected; tr, traces.
In each row different letters mean significant differences ($p < 0.05$). *Statistical differences (< 0.001) were observed when t-student test was applied.

Scientists have lately reported multiple properties regarding to phenolic compounds as antioxidants endowed with anticancer, anti-inflammatory, antimicrobial and antidiabetic activities (Dias et al., 2016; Kaur Kala et al., 2016). Among the most cited properties attributed to polyphenols it is the protective effect against damage of the human body caused by reactive oxygen, nitrogen and sulfur species (ROS, RNS and RSS, respectively) (Ambigaipalan, 2015; Carocho and Ferreira, 2013).

Additionally to phenolic compounds, other relevant components in the cactus pear are the betalains pigments, which are present in most of the Caryophyllales family including the *Cactaceae* and replace the anthocyanins in flowers and fruits of plants within this family. Betalains are water soluble nitrogen-containing pigments situated in the vacuoles of the plant tissue responsible for the red-violet (betacyanins) and yellow-orange (betaxanthins) colors, showing a stable appearance in the range of pH 3–7 (Stintzing et al., 2002). Therefore, betalains could be used as a natural colorant alternative to synthetic dyes used in a broad range of food products.

The present study intends to contribute to the characterization of the bioactive compounds profile, antioxidant and antimicrobial activities of by-products, such as peels, of the cactus pear from the species *Opuntia ficus-indica* var. sanguigna (OS) and gialla (OG) and *Opuntia engelmannii* (OE). The results of this study might be useful to maximize the potential of the fruits by-products for their colorant and functional added value.

2. Material and methods

2.1. Samples preparation

Cactus pear fruits (*Opuntia ficus-indica* var. sanguigna −OS and gialla −OG) were collected in July-August 2016 in Sicily, Italy and purchased from a local market in Bragança, Portugal. Fruits from this species were separated regarding the maturation colour orange-red (*Opuntia ficus-indica* var. gialla) and red-violet (*Opuntia ficus-indica* var. sanguigna), obtaining two different samples. Prickly pear wild fruits (*Opuntia engelmannii*- OE) were collected in Bragança, Portugal (GPS location: 41.797344, −6.772735) on early September 2016. Voucher specimens were deposited in a herbarium.

Within 24 h, fruits were washed with distilled water in order to remove glochids and then further air-dried. Afterwards, all the fruits were peeled and the resulting peel was lyophilized (LabConco, Frezone −105 °C, 4.5 L Cascade Benchtop Freeze Dry System, Kansas, MO, USA) and stored in a cool and dry place until use.

2.2. Extraction procedure

The hydroethanolic (ethanol: water, 80:20 *v/v*) extract was obtained from the lyophilized peels. The sample (1 g) was extracted twice by stirring with 25 mL of hydro-alcoholic solution (25 °C at 150 rpm) for 1 h and subsequently filtered through the Whatman no. 4 paper. The obtained extracts were frozen, lyophilized and re-dissolved in: (i) hydroethanolic solution (ethanol: water, 80:20 *v/v*) for phenolic characterization (final concentration 5 mg/mL) and antioxidant activity evaluation (final concentration 40 mg/mL); or (ii) water for cytotoxicity evaluation (final concentration 8 mg/mL) and betalain characterization (final concentration 30 mg/mL); or (iii) 5% DMSO in distilled water (final concentration 10 mg/mL) for antimicrobial assays. The final solutions were further diluted to different concentrations to be submitted for distinct *in vitro* bioactivity evaluation assays.

2.3. Phenolic compounds

The lyophilized hydroethanolic extracts were analysed for their content in phenolic compounds, re-dissolve in ethanol:water (80:20, *v/v*) to a final concentration of 5 mg/mL. LC-DAD–ESI/MSn analyses were