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Valued bioproducts from waste *Opuntia ficus-indica* peel via microwave-assisted hydrodiffusion and hydrodistillation

Rosaria Ciriminna,[1] **Carmelo Danzì**,[2] **Giuseppe Timpanaro**,[2] **Mattia Locatelli**,[3] **Diego Carnaroglio**,[3] **Alexandra Fidalgo**,[4] **Antonino Scurria**,[1] **Giuseppe Avellone**,[5] **Laura M. Ilharco**[4]* **Mario Pagliaro**[1]*

[1]*Istituto per lo Studio dei Materiali Nanostrutturati, CNR, via U. La Malfa 153, 90146 Palermo, Italy;* [2]*Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi di Catania, via Santa Sofia 100, 95123 Catania, Italy;* [3]*Milestone, via Fatebenefratelli, 1/5, 24010 Sorisole (BG), Italy;* [4]*Centro de Química-Física Molecular and IN-Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, University of Lisboa, Complexo I, Avenida Rovisco Pais 1, Lisboa, Portugal;* [5]*Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche, Università degli Studi di Palermo, Palermo, Italy*

Abstract

The integral extraction via microwave-assisted hydrodiffusion and hydrodistillation of water-soluble bioproducts contained in the peel of *Opuntia ficus-indica* white and red cultivars harvested in Sicily, affords red and stable aqueous extracts mostly containing valued betanin, pectin and biophenols. Potentially useful as nutraceutical products, these aqueous extracts are a source of valued ingredients in high demand for a number of important food, cosmetic, beverage and nutraceutical applications.

Keywords: *Opuntia ficus-indica*; nutraceutical; betanin; pectin; natural colorant

**Corresponding authors*
Prof. Laura M. Ilharco
Centro de Química-Física Molecular and IN-Institute of Nanoscience and Nanotechnology
Instituto Superior Técnico, University of Lisboa
Avenida Rovisco Pais 1, Lisboa, Portugal
E-mail: lilharco@tecnico.ulisboa.pt

Dr M. Pagliaro
Istituto per lo Studio dei Materiali Nanostrutturati, CNR
via U. La Malfa 153
90146 Palermo (Italy)
E-mail: mario.pagliaro@cnr.it

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Introduction

Aiding to close the material cycle in agriculture, in general, once economically viable and environmentally friendly industrial extraction processes will be developed, the extraction of valued bioproducts from agriculture and food processing waste will become a common bioeconomy practice in all world's countries hosting significant agricultural activities.[1] Ubiquitous in Mexico, North African countries and Sicily, as well as widely cultivated in Brazil, South Africa, Argentina, cactus pear *Opuntia ficus-indica* (OFI) is a perennial plant specie belonging to the *Cactaceae* family whose fruits and leaves (cladodes) afford a number of phytochemicals of significant nutraceutical importance.[2] Accommodating 88 wt% water in the cladodes,[3] the cactus pear is a water and livestock feed reserve that will play an important role to combat desertification.[4]

Concerning the fruit, we have recently shown that the fatty acid composition of Sicilian OFI seed oil is similar to that of fruits grown in Tunisia, while it has a completely different profile than the OFI fruits grown in Algeria and Morocco.[5] Like the oil obtained in Tunisia, the Sicilian oil has a higher vaccenic acid content, but it comprises significant amounts of other unsaturated fatty acids showing highly beneficial health properties. Rich in unsaturated fatty acids exerting unique skin and hair hydrating action, the OFI seed oil has antioxidant and anti-inflammatory properties which offer significant potential as functional ingredient of nutraceutical and food supplement products.[6]

In general, beyond the polyunsaturated fatty acids contained in the seeds, the OFI fruits are a source of nutritionally relevant compounds such as aminoacids, essential minerals including calcium, potassium and magnesium, vitamin C (20-40 mg/100 g)[7] and sterols,[8] as well as anti-inflammatory betalain pigments[9] (particularly betanin and indicaxanthin)[10] imparting them with neuroprotective, antiulcerogenic and hepatoprotective properties.[11] Recently, following a comprehensive review of safety,

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antioxidant activity, clinical efficacy and bioavailability the first human daily intake of betanin and indicaxanthin was proposed at 100 and 50 mg, respectively.[12]

Labelled E 162 as approved food additive in the European Union, betanin is a valued violet-red betacyanin which colors food without altering the flavor. The latter property, adding to the pigment stability at pH between 3 and 7, makes it particularly well suited for use in processed food products such as beverages, confectionary, bakery, ice cream and dairy products. Though containing a far lesser betanin amount (50 mg/kg for OFI *vs.* 300-600 mg/kg for red beet roots)[13] sourcing this important colorant from *Opuntia* in addition to current industrial practice to extract the pigment from the roots of red beet (*Beta vulgaris* L.),[14] would provide significant advantages. Contrary to the red beetroot which slowly affords its high betanin content after two years of cultivation, for example, the *Opuntia* plant is ubiquitous and gives numerous fruits twice a year between mid June and the late November.

Similar arguments hold true in regard to pectin, the valued natural hydrocolloid currently mostly obtained from dried lemon peel and, to a lesser extent, from apple pomace.[15] The first study suggesting the use of OFI peel as a source of pectin to be used as thickening material goes back to 1994,[16] when scholars in Italy found out that hot acid extracted OFI pectin had a galacturonic acid content of 64%, and a low degree of methoxylation (10%). Being not edible, the peel is removed from the fruit in all companies selling pacakaged fresh fruits as well as from those producing jams and fruit juice. The production of waste *Opuntia* peel is thus significant with most peel currently used as animal feed, and a smaller fraction feeding methanogen bacteria in anaerobic digestors.

Ten years later, scholars in Morocco and in France reported the first structural analysis of the pectic material contained in the OFI peel.[17] Another ten years, and Rodríguez-Hernández and co-workers in Mexico,[18] relying again on conventional

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hydrolytic extraction in hot acidic water (treatment of the milled peel with 1 wt% ethylenediaminetetraacetic acid for 2 h at 70 °C and pH = 4.0), reported that pectin extracted from the peel of *Opuntia albicarpa Scheinvar 'Reyna'* fruits has a low methoxyl content (30,7%) and high molecular weight (M_w = 10.16 × 10^5 g mol^{-1} *vs.* M_w = 76 × 10^3 g mol^{-1} of lemon pectin) which imparts the *Opuntia*'s peel pectin the ability to form soft and elastic gels.

We now report the first outcomes of four separate tests aimed to extract pectin and natural pigment from the peel of white and red OFI fruits harvested in Sicily, Italy, via microwave-assisted hydrodistillation and microwave-assisted hydrodiffusion.

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Results and Discussion

All extractions were carried out on 1-2 kg scale using a Ethos X microwave extractor, since the latter instrument simulates exactly the process occurring in the semi-industrial extractor MAC-75. Containing a removable, rotating drum that allows to load and process up to 75 L of biological material, the latter device is able to process 30 kg of biological matrix per hour.[19]

Sicily's OFI fresh fruits were used in all tests. We attempted two extractions for each sample of white and red fruits. In both tests we only used the peel of the OFI fruits, getting rid of the edible pulp. Furthermore, the peel was milled prior to extraction in order to enhance the contact surface area during the microwave-assisted extraction.

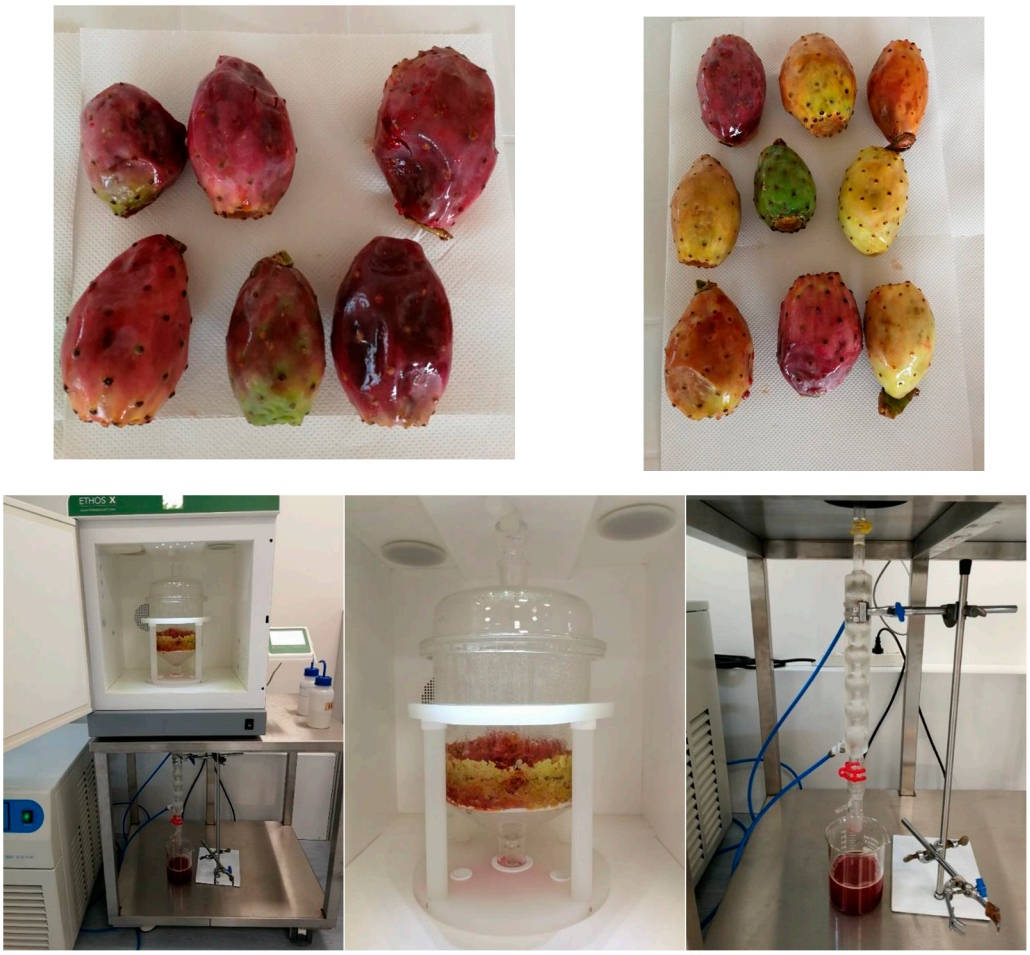

Figure 1. *Top*: OFI red fruits (*left*) and mixed red and white fruits (*left*).
Fresh fruits from Sicily, treated as received; *Bottom*: The Ethos X extractor (left) whose vessel was filled with milled OFI peel (*middle*) affording the aqueous extract (*right*).

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The current distribution of the OFI cultivars grown in Sicily has completely changed with respect to the early 2000s. Today, farming companies cultivate all three main cultivars (yellow, red and white; with OFI fruit colors due to different combination of purple-red betanin and yellow-orange indicaxantin) with the red and white cultivars in Sicily now accounting for about 40% of the plantations.

Test 1: Extraction of white OFI fruit peel via microwave-assisted hydrodistillation. A 2.4 kg sample of white fruit peel was milled and added with water (150 mL) in the extractor vessel. The extraction program used microwaves irradiated by a magnetron powered by 1500 W for 1 h. The volume of the final solution collected at the end of the extraction was 310 mL. All the colored solution contained in the extraction vessel was recovered by simple filtration and made available for analysis. A picture of the aqueous extract is provided in Figure 2.

Test 2: Extraction of white OFI fruit peel via microwave-assisted hydrodiffusion. A 1.15 kg sample of white fruit peel was milled and inserted in the extraction vessel. No water was added. The extraction program used microwaves generated by the magnetron powered by 1200 W for 40 min. Under said irradiation, the extraction temperature rapidly reached 70 °C, and did not further change. The final volume collected amounted to 365 mL. All the colored solution contained in the extraction vessel was recovered by simple filtration and made available for analysis. A picture of the aqueous extract is provided in Figure 2.

Test 3: Extraction of red OFI fruit peel via microwave-assisted hydrodistillation. A 2,1 kg peel sample of red OFI fruit peel was milled and inserted in the extraction vessel along with 150 mL of water. The extraction program used microwave irradiated by the magnetron powered by 1500 W for 1 h. Under said irradiation, the extraction temperature rapidly reached 70 °C, and did not further change. The volume of the final solution collected at the end of the extraction was 265

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mL. All the solution contained in the extraction vessel was recovered by simple filtration and made available for analysis. A picture of the aqueous extract is provided in Figure 2.

Test 4: Extraction of red OFI fruit peel via microwave-assisted hydrodiffusion. A 1.34 kg sample of red OFI fruit peel was milled and inserted in the extraction vessel. No water was added. The extraction program used microwaves generated by the magnetron powered by 1200 W for 40 min. Under said irradiation, the extraction temperature rapidly reached 70 °C, and did not further change. The final volume collected amounted to 490 mL. All the solution contained in the extraction vessel was recovered by simple filtration and made available for analysis. A picture of the aqueous extract is provided in Figure 2.

Figure 2. Aqueous extracts from the peel of fresh *Opuntia-ficus indica* white fruits (*left*) and from the peel of red fruits (*right*). The larger volume samples were obtained via microwave-assisted hydrodiffusion. The others via microwave-assisted hydrodistillation.

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The colorimetric quality of the aqueous extract obtained is self-evident from the pictures in Figure 2. No chemical additive was added at any step of the extraction process nor for storing the extract at room temperature. The aqueous extract itself, mostly containing betalains, pectin and proteins, could be used as such, as a value-added nutraceutical product: an use that will be shortly evaluated in our Laboratories. The extracts are currently being analyzed in our Laboratories following separation and recovery of the pectic polymer via dialysis, as well as of betalains (betanin and indicaxantin) via lyophilization and subsequent chromatographic separation.

Ferreira and co-workers have lately emphasized how, in light of betalain use as functional ingredients and health enhancers/promoters in nutraceutical, pharmaceutical and cosmetic products, it will be "very important to establish optimum processing conditions to maximize the stability of for betalain natural pigments and their extraction yields".[20] This is one of the two main outcomes of the microwave-assisted extraction processes described herein.

The presence of stabilizing polyphenol compounds, abundantly contained in the peel (three-fold higher than in the pulp, up to 457 µg/mg gallic acid equivalents with flavonols, and isorahamnetin derivatives in particular, being the most abundant biophenol),[21] protects the betalains which are prone to O_2-induced degradation requiring best preservation by excluding air.[22] Stored at room temperature in semitransparent plastic bottles for over a month, the present aqueous extracts have shown no change in color tonality nor in intensity.

We also briefly remind here that the OFI peel is mostly composed (in wt%) of ash (11.5%), fat and wax (11%), lignin (2.4%), protein (8.6%), mucilage (4.1%), pectin (35%) and cellulose (27%).[17] Under the present extraction conditions no pretreatment is needed to get rid of the water-insoluble peel components which are easily filtered while recovering the aqueous filtrate.

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Figure 4. Detailed picture of the aqueous extracts from the peel of *Opuntia-ficus indica* red fruits obtained via microwave-assisted hydrodistillation (*left*) and microwave-assisted hydrodiffusion (*right*).

These results are surprising and indicate enhanced betanin content in the fruit peel when compared to the fruit. It is known from the first thorough study on the betalain composition of OFI fruit,[13] that the amount of purple-red betanin extracted from the

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pulp with methanol was negligible in the Sicilian white fruit, while the betanin/indicaxanthin ratio was 2:1 (w:w) in the red one.

We briefly remind here that the high antioxidant (free radical-scavenging) activity of betanin is molecularly associated with its ability to act as hydrogen and electron donator, which is particularly high at neutral and basic pH, namely going from the cationic form to mono-, di- and tri-deprotonated species present in increasingly basic solution.[14]

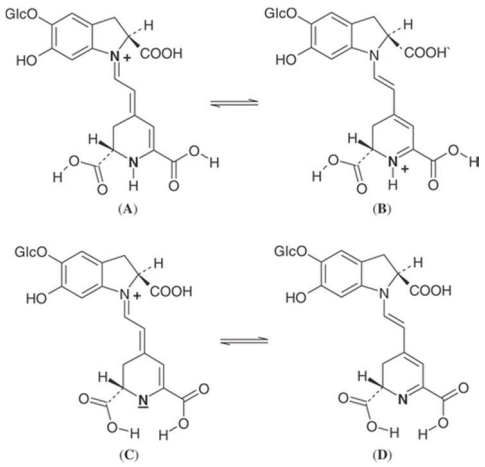

Figure 5. Resonance structures of betanin in cationic state (**A**, **B**) and in 16N^{-} deprotonated form (**C**, **D**). [Reproduced from Ref.14, with kind permission]

A betanidin 5-*O*-β-glucoside containing a phenolic and a cyclic amine groups, betanin shows superior anti-radical activity thanks both to the good electron donor properties of its two main molecular moieties (betalamic acid and with cyclo-3,4-dihydroxyphenylalanine), and to strong electronic conjugation between said betalamic acid and cyclo-DOPA-5-*O*-*β*-D-glucoside moieties (Figure 5).

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Conclusions

The global trend driving the market growth for pectin and for natural colorants is the ever increasing health and environmental consciousness of consumers across the world that demand health beneficial natural ingredients in food, beverage, cosmetic, personal care and now even in medicinal products. Using an entirely clean process requiring microwaves only, we have shown how the peel of the cactus pear *Opuntia ficus-indica* could shortly become an important source of pectin and betanin, namely the most important natural hydrocolloid and a valued red natural colorant for both of which shortage in the last few years has led to price increase and delay in product delivery.

Accounting for $1.31 billion in 2015 the global market of natural food colors (derived from a fruits, vegetables, seeds, algae, insects and minerals) is expected to grow at over 5% annual rate between 2016 and 2021.[23] Betalains are still a relatively small fraction of this market, but their growth potential is significant. For example, recent stability over time studies of red extracts from *Opuntia dillenii* added to foodstuff at different pH and stored at different temperatures by scholars in Colombia point to promising utilization of these betanin extracts.[24]

Though more costly than synthetic red colorants widely used to increase the appearance of beverage and food and make it attractive such as Red 40 (a red azo dye associated, along with other synthetic colorants, to child hyperactivity),[25] the use of betanin, for example formulated along with lycopene,[26] has the potential to transform a debated health issue into an opportunity for enhancing public health exactly as it would happen when replacing synthetic food antioxidants with olive biophenols.[27] Betanin, indeed, has an exceptionally high free radical scavenging activity being (at pH > 4) 1.5-2 times more active than anthocyanins considered excellent free radical scavengers.

In conclusion, extending our recent studies on microwave-assisted extraction of pectin and essential oils from the citrus (lemon, orange and grapefruit) fresh peel,[28] we

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have used microwave-assisted hydrodistillation and microwave-assisted hydrodiffusion to obtain highly promising aqueous extracts from the peel of both red and white OFI fresh fruits harvested in Sicily. Under mild conditions (1 h heating under microwaves at 70 °C) both fruit wastes afford red natural extracts of pronounced stability under ambient conditions. Requiring no addition of water, hydrodiffusion affords a more concentrated aqueous integral extract which is particularly promising in light of forthcoming utilizations. Alternative to conventional solvent extraction, the industrial extraction of natural products based on microwave irradiation of plant material and volumetric heating is now an industrial reality, though in its early days, offering important economic and environmental benefits.[29] We are currently investigating the composition and properties of the Sicilian OFI peel new aqueous extracts. Results will shortly be reported.

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