

Article

Nutraceuticals Obtained by SFE-CO₂ from Cladodes of Two *Opuntia ficus-indica* (L.) Mill Wild in Calabria

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Abstract: The aim of the present study was to evaluate the possibility to extract, by supercritical fluids, nutraceuticals as polyphenolic compounds, able in the prevention and in the treatment of a series of chronic-degenerative diseases, from plant matrices like the cactus pear. Supercritical fluid technology is an innovative method to extract nutraceuticals from natural matrices. This method offers numerous advantages that include the use of moderate temperatures, solvents with good transport properties (high diffusivity and low viscosity), and cheap and nontoxic fluids. Fresh cladodes from two different wild ecotypes of *Opuntia ficus-indica* (L.) Mill. were extracted both with methanol and with SFE-CO₂ using different samples preparations, to maximize the % yields and the selectivity of extraction of polyphenols. The biggest contents of phenolics, evaluated by *Folin-Ciocalteu* assay, has been observed with the sample dehydrated of *O. ficus-indica* cultivar that shows, as well, the best yield % (m/m) of extraction with both methanol and SFE-CO₂. Better results were obtained with the samples of *O. ficus-indica* cult. (*OFI cult.*), in spite of the *O. ficus-indica* s.l. (*OFI s.l.*); the two different ecotypes of *OFI* showed dissimilar phytochemicals profile. We noticed that the reduction of both quantity and quality of polyphenols was drastic with the increase of pressure at 250 bar; this shows that high pressures result in a loss of bioactive principles, like polyphenols. By changing the variables of extraction processes with SFE-CO₂ and by varying the preventive treatments of the natural matrices, it was possible to increase the selectivity and the purity of the products. Thus, the optimization of this useful and green technique allowed us to increase the value of the *Opuntia* cladodes, a by-product very diffused in Calabria, which is an extraordinary source of nutraceuticals. These extracts could be used directly as functional foods or as starting material in the pharmaceutical, nutraceutical or cosmetic companies; they are safe and without any solvents traces and it is possible to obtain it in a few hours respect to the conventional extraction that requires longer extraction time.

Keywords: cladodes; *Opuntia*; nutraceuticals; antioxidants; polyphenols; SFE-CO₂; rutin; iso-quercitrin; nicotiflorin; narcissin



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1. Introduction

Nowadays, the consumption of natural extracts from plants and seeds present in nature and the discovery of the many benefits connected to their intake, have favoured and promoted research in the field of extraction and use.

For this purpose, in recent years, extract of plant parts (seeds, cladodes, stem, fruits, etc.) of the family *Opuntiaceae* have been investigated, as for instance: *Opuntia joconostle* seeds for cholesterol-lowering properties [1]; *Opuntia humifusa* cladodes, for cytotoxic activity against the human breast cancer cell lines MCF-7 and human colon SW-480 cells, and stem and fruits able to inhibit the growth of U87MG glioblastoma cells [2]; *Opuntia*

stricta for antioxidant, anti-inflammatory and cytotoxic activities [3,4]. Instead, our attention was focused on the cladodes of the cactus pear (*Opuntia ficus-indica* (L.) Mill.), a tropical or subtropical invasive plant, widespread and well adapted to Mediterranean area due to the climate.

Opuntia was an important source of the agricultural economy and diet of the ancient Mexicans of the Aztec empire [5] and it was also traditionally used to differentiate the properties of the peasants and to curb fires; the cladodes have high water content, 95% in mass [6].

In some countries, like Mexico, Italy and South Africa, the most commercial variety *Opuntia ficus-indica* (L.) Mill. is cultivated on the considerable surface for industrial purposes; in Mexico, its culture stretches on a surface of 300,000 ha [7]. The immature cladodes of *Opuntia ficus indica* are a part of the edible plant and also used in Mexican cuisine for many traditional dishes and in folk medicine for certain diseases, such as obesity, gastrointestinal disorders [8], ulcers and healing of wounds [8].

Opuntia ficus indica fruits are used as a laxative in Turkey, to reduce kidney stones, rheumatism pains, and as a sedative [9]. It was also been reported that the extracts of fruits and stems exhibit hypoglycemic, anti-ulcer [10], anti-allergic, analgesic and antioxidant activity [11]. From the fruits and the stem extracts was isolated the β -sitosterol, an active anti-inflammatory principle [12], whereas the fruit and flower infusions significantly increase diuresis [13].

Several researches show that the prickly pear is a plant very rich in vitamins, minerals, amino acids and sugars [14]. In particular, its fruit, with a pleasant flavor, has a high content of potassium, phosphorus, magnesium, sodium, calcium, vitamins C and E, dietary fibers, pigments betalains (betacyanins and betaxanthins), polyphenols and their glycosides, glutamine, proline and taurine; the cladodes, consumed as vegetables and used also as animal feed too [15], are rich of mucilages, pectins, sterols, vitamins and polyphenols.

The blades of the prickly pear are however very perishable and can be stored for about 5 days at room temperature or up to 10 days in refrigerated environments [16]. They contain high quantities of pectins and fibers that are capable of increasing fecal mass and intestinal motility, which therefore improve plasma levels of cholesterol and glucose. [17].

The young cladodes of OFI, also known as nopalitos, contain functional polyphenols (Figure 1), like Ruthin, Iso-quercitrin, Narcissin and Nicotiflorin [18] and a series of polysaccharides with high molecular weight and important functional properties, as rheological [19], medicinal and nutritional [20].

Rutin is found in many fruits and vegetables and has been used in over 130 therapeutic medicinal preparations that have been registered as drugs worldwide [21]. In literature, it has been found that Rutin improves the memory of mice in Alzheimer's as it reduces the levels of A β -oligomer and attenuates oxidative stress and neuroinflammation. [22].

Isoquercitrin, as glycosylated flavonoid, is rapidly absorbed and transformed, in the gastro-intestinal tract, in glucuronidated Quercetin [23,24] that was found to be the major form in plasma after oral administration of Isoquercitrin in rats [25]. Nicotiflorin, as well, shows protective effects on memory dysfunction in multi-infarct dementia model rats [26]. Narcissin is known for its biological proprieties, such as hepatoprotective, antioxidant, anti α -glucosidase, and cytotoxicity against human myelogenous erythroleukemia cells [27].

In general, flavonoids can be found in many varieties of fruit, vegetables and cereals [28–31]. They play a very important role in a series of pathologies, both in their control and in their prevention, such as in Alzheimer's, cancer, etc., perhaps because they act eliminating free radicals. [32–36].

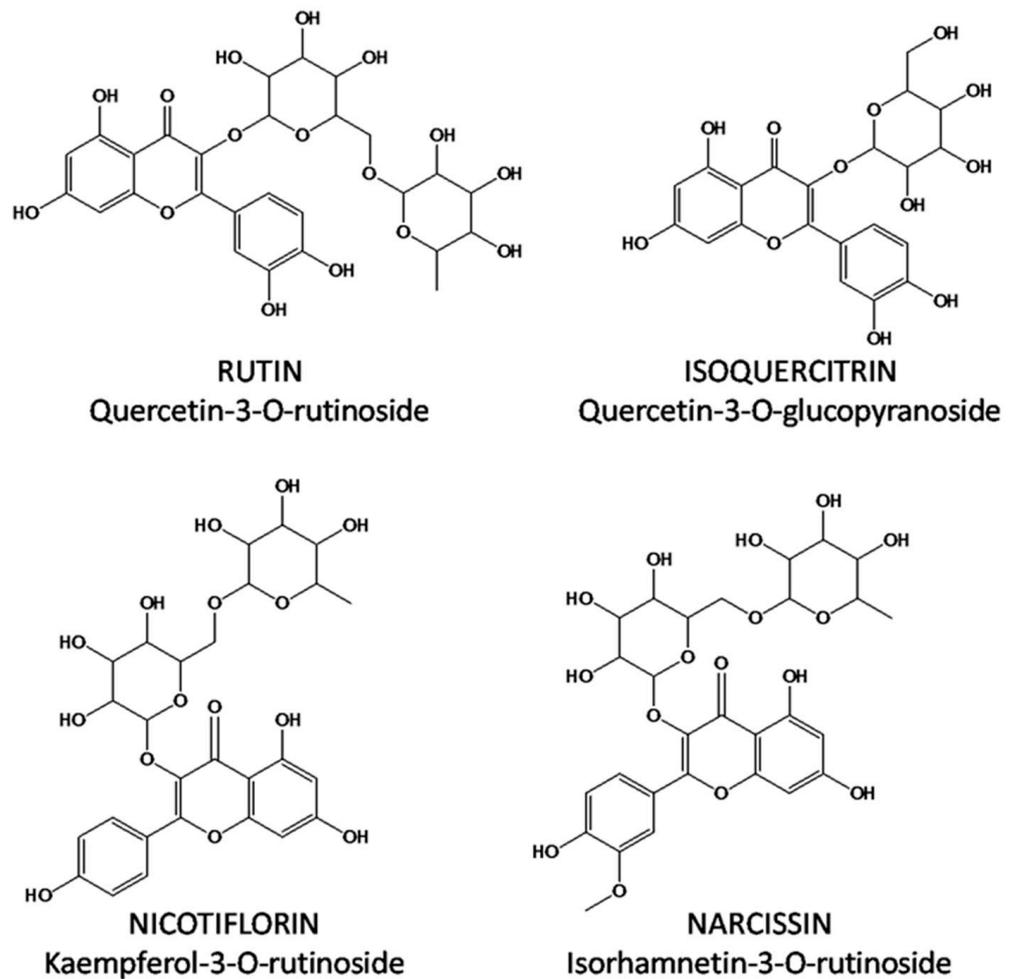


Figure 1. Polyphenols present in cladodes of *Opuntia ficus-indica*.

Conventionally the used techniques to obtain polyphenols extracts, such as Soxhlet, maceration [37], organic solvent extraction [38–40], autoclave treatment and microwave [37] or ultrasound-assisted extraction [39–44], usually require several hours or days and spending a large volume of solvents, frequently toxics, and with problematic garbage disposal. The correct separation between solute and solvent is important, as a possible degradation of the thermolabile components would compromise the benefits of the extract. Furthermore, any residual solvent could lower the quality and quantitative yield of the extraction.

Instead, for the extraction of polyphenols from cladodes, in this work it was used a green extraction technique: the supercritical extraction (SFE) with CO₂ [37], generally used to obtain oils from natural products [45–48].

This method has been used for the purpose of investigating the possibility of phytochemicals extraction without any solvent and to optimize the selectivity between phenolics and other substances in the cladodes of two ecotypes of *Opuntia ficus-indica* wild in Calabria, *O. ficus-indica* cult. (*OFI* cult.) and *O. ficus-indica* s.l. (*OFI* s.l.).

Supercritical fluid technology is the most innovative method for preparing bioactive products from plants used as supplements for functional foods [49] and it results as promising technology both in food farming and pharmaceutical industry. Supercritical fluids have high solvation capabilities, which are similar to those of liquids, as well as diffusion properties similar to those of gases. For these reasons, the extraction using supercritical fluids is particularly suitable for the extraction of biocompounds from plant matrices [50].

Although there are several usable supercritical fluids, supercritical CO₂ is the most commonly used. This application success is due to its low critical constants (T_c = 31.1 °C, P_c = 7.38 MPa), to the fact that it is non-toxic and non-flammable and because it is available at high purity at low cost. Moreover, other advantages are a high diffusion coefficient and low viscosity; being gas at atmospheric condition, the CO₂ immediately seeps out when brought to the environment, so the products obtained are free from the “extraction solvent” and thermal degradation compounds.

The extracts obtained by SFE-CO₂ are also considered as *Generally Recognized As Safe* (GRAS) for the *American Food and Drug Administration*, being possible to add them to all food without undesirable effects for health. Some data have also been reported on the application of SFE-CO₂ from vegetable by-products [51].

Today, new chemistry knowledge at the molecular level regarding the functional and structural properties of active principles may allow a better selection of the products and extracts that can satisfy the request of the market, also according to the specific needs of both food and pharmaceutical industries [52]. Thus, with this innovative and advantageous technique, we extracted the Rutin, Isoquercitrin, Nicotiflorin and Narcissin from hard to manipulate plant matrices, namely the cladodes, with the aim to use them as safe supplements in the food and pharmaceuticals companies.

2. Materials and Methods

2.1. Plant Material

Two cladodes ecotypes of *Opuntia ficus-indica* (L.) Mill. (Figure 2) were analysed in this study: one supposed to be a cultivar with the hybrid origin, which often escapes, from cultivation and behave like an invasive species, it is almost spineless and it was accepted with the name *Opuntia ficus-indica* cult. (*OFI* cult.); and the second one with white and hard spines, long about 3–4 cm, sharing some characters with *Opuntia amyclaea* Ten., *O. maxima* Miller and *O. ficus-barbarica*. Hereafter we will use for the spiny ecotype the name *Opuntia ficus-indica* (L.) Mill. s.l. (*OFI* s.l.), because the studied local area lacks of taxonomic and nomenclatural study of the genus *Opuntia*.

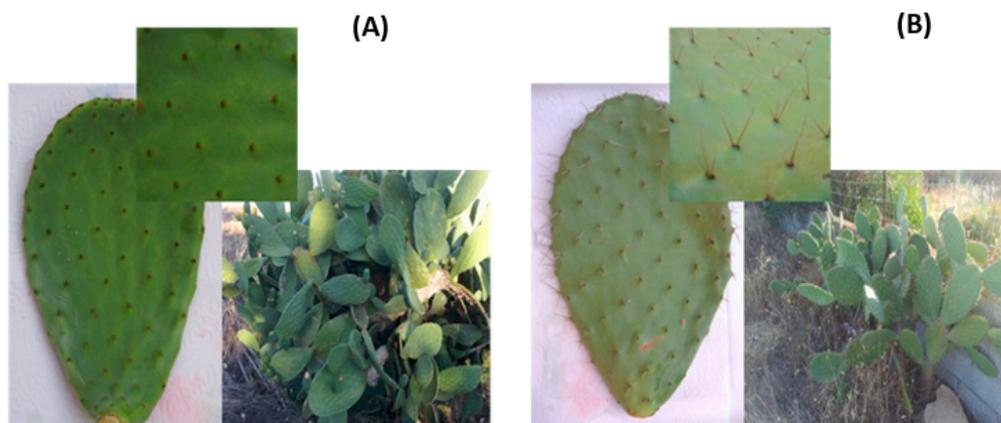


Figure 2. Two ecotypes of *Opuntia ficus-indica* (L.) Mill cladodes: (A) *Opuntia ficus indica* cult. and (B) *Opuntia ficus-indica* (L.) Mill s.l.

The cladodes from both *O ficus-indica* were collected between June–August 2012 in Calabria (South of Italy). All the cladodes, covered by spines and multicellular hairs or trichomes, were manually cleaned, cut into small pieces and then homogenized (Figure 3) with a TYPE HR 2064 PHILIPS-600 W and frozen until analysis.

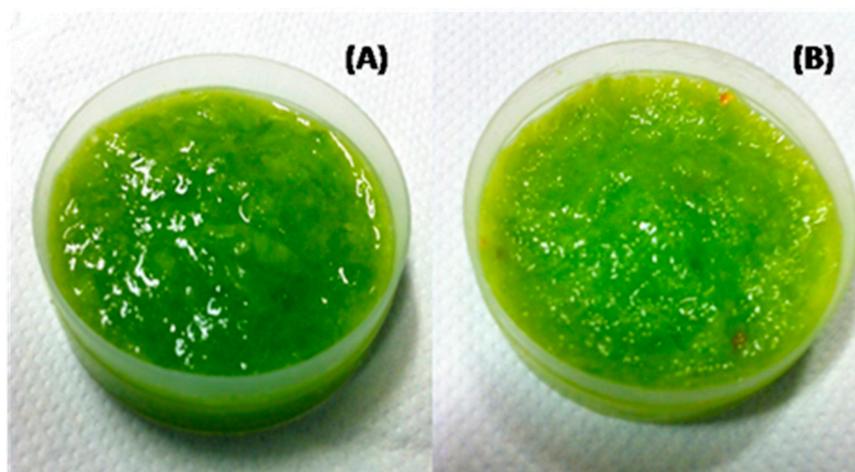


Figure 3. Homogenized from fresh samples of *OFI cult.* (A) and *OFI s.l.* (B).

2.2. Chemicals

Solvents as methanol (analytical grade and for HPLC), trifluoroacetic acid, water (analytical grade and for HPLC) and reagents such as *Folin-Ciocalteu* and Chlorogenic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Ottawa Sand and Spe-edTM PSE Matrix-Hydroscopic Samples Dispersing Agent (Diatomee Sand), used as SFE-CO₂ accessories, were purchased from Labservice Analytica S.R.L. (Italy).

Standards, for HPLC analysis and quantification, including Rutin, Isoquercitrin, Nicotiflorin and Narcissin were purchased from Extrasynthese (Lyon, France).

2.3. Preliminary Analysis

The pH value of the homogenized system (pH 700, Eutech Instruments, Germany), the activity level (Novasina AW Sprint-TH 500, Switzerland), the Brix degrees (ATAGO, Hand Refractometer N Type Series, Japan) and humidity (Mettler Toledo Moisture Analyzer HB43-S, Switzerland) were measured. The analyses were repeated in triplicate and the results are reported as mean value and standard deviation.

2.4. Exhaustive Extraction and Total Phenolics Content

The fresh cladodes of *Opuntia ficus-indica* both ecotypes were extracted with methanol (48 h × 3 times) at 4 °C. The same procedures were followed for a sample of *OFI cult.* and *OFI s.l.* Moreover, the exhaustive analysis was performed on samples dried in the oven for 360 min at 32 ± 1 °C; removing the 30% of the weight.

The extraction solutions were filtered in synthetic cloth, concentrated and dried under *vacuum* at 35 ± 1 °C for the thermolability of the polyphenols compounds.

The total phenolic content of the cladodes extracts was quantified using *Folin-Ciocalteu* reagent and chlorogenic acid used as standards. The absorbance was measured at 726 nm (Perkin Elmer Lambda 40 UV/VIS spectrophotometer) and the total content was expressed (mean ± S.D. of three determinations) as mg of chlorogenic acid equivalents on 100 g of fresh raw material (Singleton Rossi, 1965).

2.5. Extraction of Polyphenols with Supercritical Fluids

The supercritical extractions by CO₂ were performed on a *Spe-ed* SFE 4 extractor (Applied Separation, Allentown, PA, USA) and following the necessary steps: loading in the 50 mL stainless steel vessel, pressurization with CO₂ and waiting for a transitory state of temperature, which is kept constant by an oven module. After the reaching of target temperature and pressurization, in order to guarantee the intimate contact between the CO₂ and the matrix, the system was kept closed for 20 min (static phase), and only after

this time the micrometric valve was opened until a constant flow of 1 was reached. 5 L/min (dynamic phase), measured by a ball float rotameter. A graphical scheme of *Spe-ed* SFE 4 extractor is given in Figure 4.

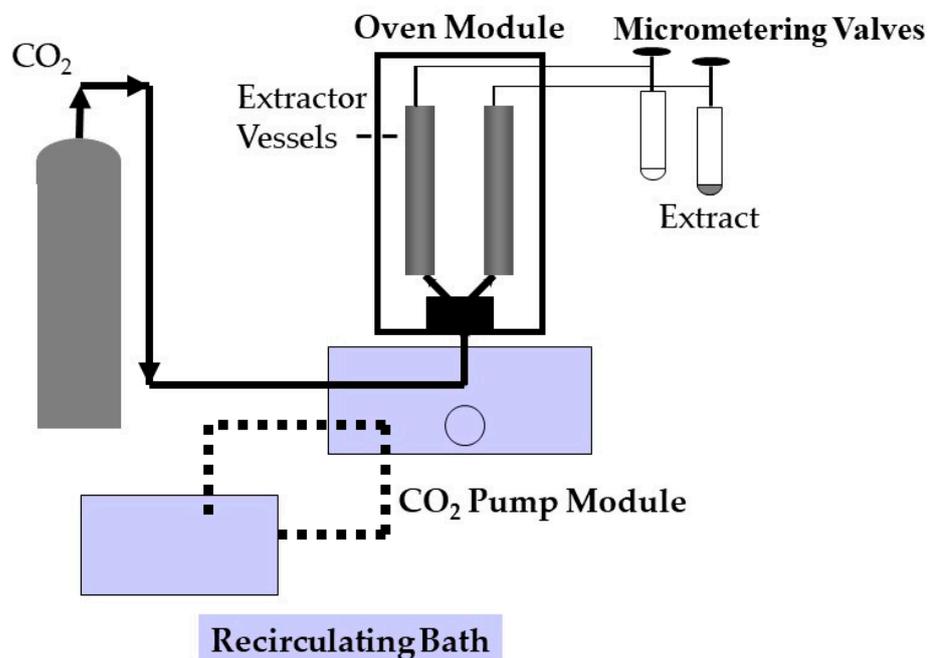


Figure 4. Scheme of *Spe-ed* SFE 4 extractor unit.

The extract left the vessel through a valve, which was in advance thermostated to avoid CO₂ solidification, due to the expansion. Experiments were carried out at 40 °C, 110 bar and 250 bar by varying the preventive sample preparation with PSE accessories, as Ottawa Sand and an *Spe-ed*TM PSE Matrix (Hydroscopic Samples Dispersing Agent). All the extracts were stored at −18 °C before HPLC analysis.

This extraction method has always attracted because it is safe and eco friendly. In addition, the supercritical fluid, in the specific case CO₂, has properties similar to those of gas in the supercritical state so that it can extract in a very short time compared to the classic extraction types, returning an extract already separated from the solvent after depressurization. Supercritical fluids have a high diffusion coefficient and a low viscosity which favours their intimate contact with the matrix. On the other hand, however, this method has limits, especially in relation to the use of the supercritical solvent because CO₂ has low polarity. Therefore this type of extraction technique is generally used for the extraction of oils, fats or in any case polar substances. Only the use of other solvents, such as ethanol or water, increases the solvent power of CO₂ in the supercritical state.

The different methods to prepare the samples and pressure are listed in Table 1.

Table 1. Different preparation of samples before SFE separation.

Sample	Ref.	Preventive Preparation	P (bar)
<i>OFI cult.</i>	CULT_20D	20% Diatomee Sand	110
<i>OFI cult.</i>	CULT_20P	20% Diatomee Sand	250
<i>OFI cult.</i>	CULT_90E_20O	Dried until 90% + 20% Ottawa Sand	110
<i>OFI cult.</i>	CULT_30E_20O	Dried until 30% + 20% Ottawa Sand	110
<i>OFI cult.</i>	CULT_C_20D	Centrifuged + 20% Diatomee	110
<i>OFI s.l.</i>	SL_30E_20D	Dried until 30% + 20% Diatomee Sand	110
<i>OFI s.l.</i>	SL_90E_20O	Dried until 90% + 20% Ottawa Sand	110
<i>OFI s.l.</i>	SL_90E_20D	Dried until 90% + 20% Diatomee Sand	110

All samples were cleaned, homogenized and frozen after collection, to be subsequently analysed.

Some samples analysed were centrifuged after defrosting at 3500 rpm for 10 min and only the precipitated part was used to extract the polyphenols while other samples were partially dried at 32 °C in the oven to reduce the amount of water.

The extraction time was 1 h. The final extracts were collected in glass tubes covered with an aluminium foil and frozen until analysis. Each extraction was carried out in duplicate.

2.6. HPLC Analysis

The analysis of all the extracts, both from solvent extraction and belonged from SFE, was carried out by means of HPLC using a Smartline HPLC system (Knauer, Germany). Chromatographic separation was carried out using a 2.0 mm ID × 150 mL, with pre-column, C-18 TSKgel ODS-100 V, 21810 (TOSOH BIOSCIENCE), both thermostatically at 40 °C. The operative conditions: mobile phase, flow rate and gradient of elution utilized are reported in Table 2. For the mobile phase, methanol and water with trifluoroacetic acid (TFA) were used.

Table 2. The mobile phase, flow rate and gradient of elution utilized.

Time (min)	Methanol [%]	Water + 0.1% TFA [%]	Flow (mL/min)
0	0	100	0.2
2	20	80	0.2
55	100	0	0.2
65	0	100	0.2

Absorbance spectra were recorded every 2 s, between 200 and 450 nm, with a bandwidth of 4 nm, and chromatograms were acquired at 254 and 280 nm. HPLC analysis was performed in duplicate.

A wavelength of 280 nm was used for quantification [18], while the calibration line was obtained from the integration of the absorption peaks obtained from a series of dilutions of Rutin, Isoquercitrin, Nicotiflorine and Narcissin.

3. Results and Discussion

3.1. Preliminary Analysis

Cladodes by *Opuntia* genus showed a weakly acid pH and this allows easier conservation of the homogenized system. The results are shown in Table 3.

Table 3. Level of acidity, water activity, Brix degrees and humidity of fresh samples of *FI cult.* and *OFI s.l.*

Plant	pH (-)	a _w (-)	°Brix (°B)	Humidity (w/w)
<i>OFI cult.</i>	4.45 ± 0.03	0.935 ± 0.03	6.5 ± 0.1	96.7 ± 1.5
<i>OFI s.l.</i>	4.50 ± 0.02	0.933 ± 0.02	7.8 ± 0.2	96.8 ± 1.3

The higher amount of saccharides was found in the sample of *Opuntia ficus-indica* s.l., than that one of *Opuntia ficus indica* cult., and this is possible due to different factors such as the variety and age of the plant, soil, and climate. The pH was measured, because it can influence the viscosity of the mucilage and it could hamper the extraction, and it is similar for the two types of plant. The water activity and humidity values are quite similar, guarantying the same times of exposition to airflow and heat during the dehydration.

3.2. Exhaustive Extraction, Total Phenolics Content and HPLC Analysis

The high content of total phenolics was observed with the *Folin–Ciocalteu* assay in the samples dried in the oven, Cult_AD and Sl_AD; these show as well the better quantitative yield of extraction, calculated using the following equation [45]:

$$\text{Yield \%} = \frac{[W_{d.e.} (g)]}{[W_m (g)]} * 100 \quad (1)$$

where $W_{d.e.}$ is the weight of the dry extract obtained and W_m the mass of the plant macerated. The data for the yield of extraction at different sample preparation and the total phenolics content are shown in Table 4.

Table 4. Data for the yield of extraction at different sample preparation and total phenolic content expressed as mg of phenols on 100 g of fresh raw material.

Plant and Time of Collection	Ref.	Sample Preparation	Yield of Methanolic Extraction (%w/w)	Total Phenolics Content (mg/100 g Raw Material)
<i>OFI cult.</i> (June)	Cult_J	Fresh macerated	2.79 ± 0.29	170.01 ± 2.98
<i>OFI cult.</i> (August)	Cult_AD	Dried macerated	5.01 ± 0.63	320.13 ± 6.54
<i>OFI cult.</i> (August)	Cult_AF	Fresh frozen macerated	2.14 ± 0.22	170.01 ± 2.65
<i>OFI s.l.</i> (August)	Sl_AD	Dried macerated	4.39 ± 0.48	414.90 ± 9.58
<i>OFI s.l.</i> (August)	Sl_AF	Fresh frozen macerated	2.19 ± 0.25	221.29 ± 5.93

In Table 4 it is possible to point out that samples dried give a higher yield of extraction and total phenolics content with respect to the fresh macerated ones, showing that the total polyphenol contents vary depending on the type of treatment and extraction.

Comparing the same fresh sample collected in two different months, it is possible to observe that the extraction yield was slightly higher in the sample Cult_J with respect to the Fresh frozen macerated of August, but the total phenolic content is almost unchanged between samples of Cult_J and Cult_AF. This indicates that the different maturation period does not influence the content of secondary metabolites, such as phenols.

Moreover, the total phenolic content is higher in the dried samples Cult_AD and Sl_AD, probably because drying process is designed to dehydrate the matrix in order to stop the common enzymatic processes; the aqueous environment of the cytoplasm of plant cells could damage the active compounds [52].

The highest content of total phenols was found in the sample of Sl_AD, perhaps because being a wild ecotype is less affected by climate change, more adaptable than a cultivated plant.

In Figure 5 are reported the data, obtained by the HPLC analysis, of extractions with solvent.

Rutin is one of the polyphenols presents in greater amount in the analysed species. Moreover, it is possible to observe that by dring the sample it is possible to obtain extracts with a greater amount of some polyphenols (Cult_AD and Sl_AD).

As reported in literature rutin is already described to be present in cladode extracts of different *Opuntia* species [8,20].

Isoquercitrin is another bioactive compound present in our extract in similar quantity in the two species analysed while the quantity of Nicotiflorin and Narcissin are very low in the extracts. The different exposure to the sun or climate change can cause the secondary metabolism of the plant. As a consequence, the antioxidant profile can be influenced.

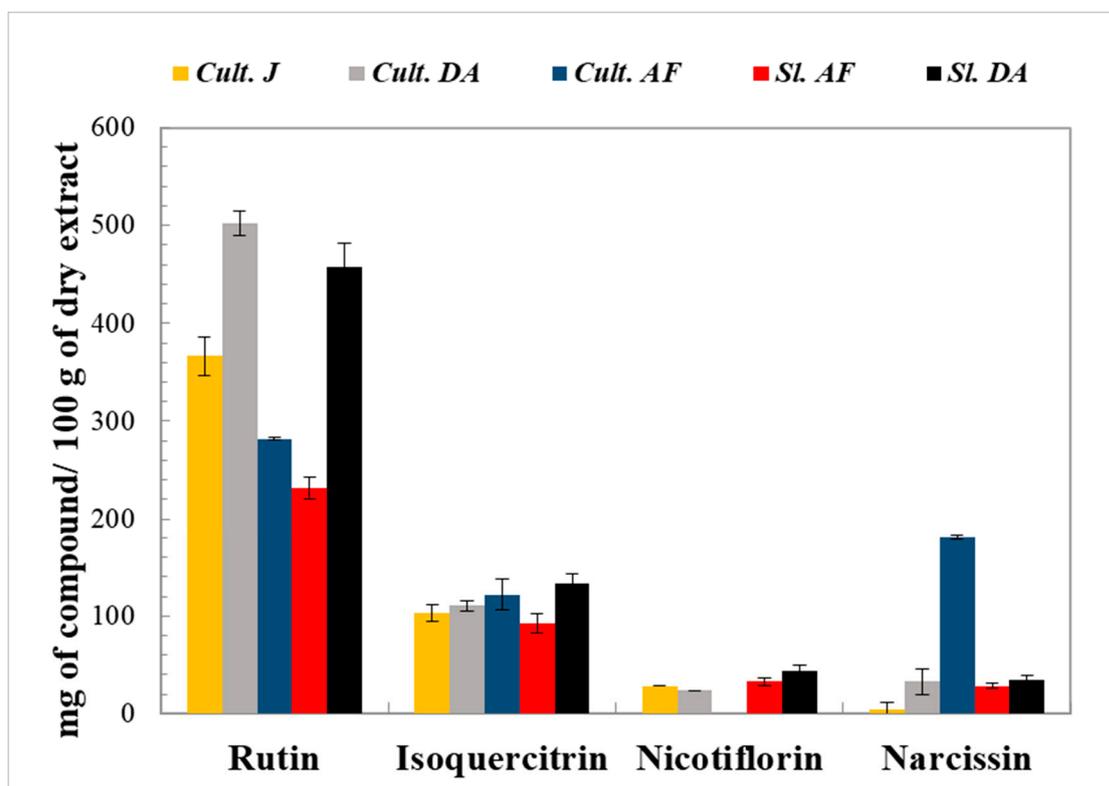


Figure 5. Quantification in mg of standards in 100 g of dry extract.

3.3. Extraction with Supercritical Fluids and HPLC Analysis

The yields extractions are calculated by using the following equation according to Yieddes et al. [45]:

$$Yield \% = \frac{[W_{extract} (g)]}{[W_{load} (g)]} * 100$$

where $W_{extract}$ is the weight of the extract obtained, which is the difference between the mass of glass trap with extract and the mass of empty glass trap and W_{load} is the mass of sample load in the column before the extraction. The results are reported in Table 5; instead, the results of HPLC quantification on mg of the compound for 100 g of material loaded (mean \pm S.D. of two determinations) for *OFI cult.* are shown in Figure 6, instead of *OFI s.l.* in Figure 7.

Table 5. Yield % of extraction (mean \pm S.D. of two determinations) of different samples by SFE-CO₂.

Plant	Ref.	Yield of Extraction (%)
<i>OFI cult.</i>	CULT_20D	0.48 \pm 0.07
<i>OFI cult.</i>	CULT_20P	1.00 \pm 0.13
<i>OFI cult.</i>	CULT_90E_20O	0.88 \pm 0.11
<i>OFI cult.</i>	CULT_30E_20O	2.14 \pm 0.35
<i>OFI cult.</i>	CULT_C_20D	1.81 \pm 0.15
<i>OFI s.l.</i>	SL_30E_20D	0.10 \pm 0.03
<i>OFI s.l.</i>	SL_90E_20O	0.25 \pm 0.05
<i>OFI s.l.</i>	SL_90E_20D	0.02 \pm 0.01

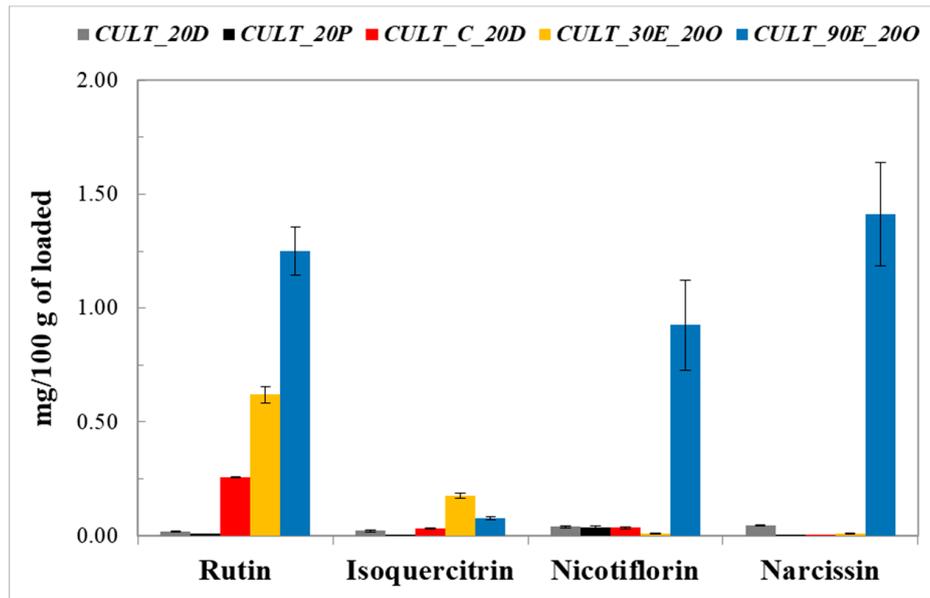


Figure 6. Quantification in mg of four different polyphenols for 100 g of loaded material for *OFI cult.*

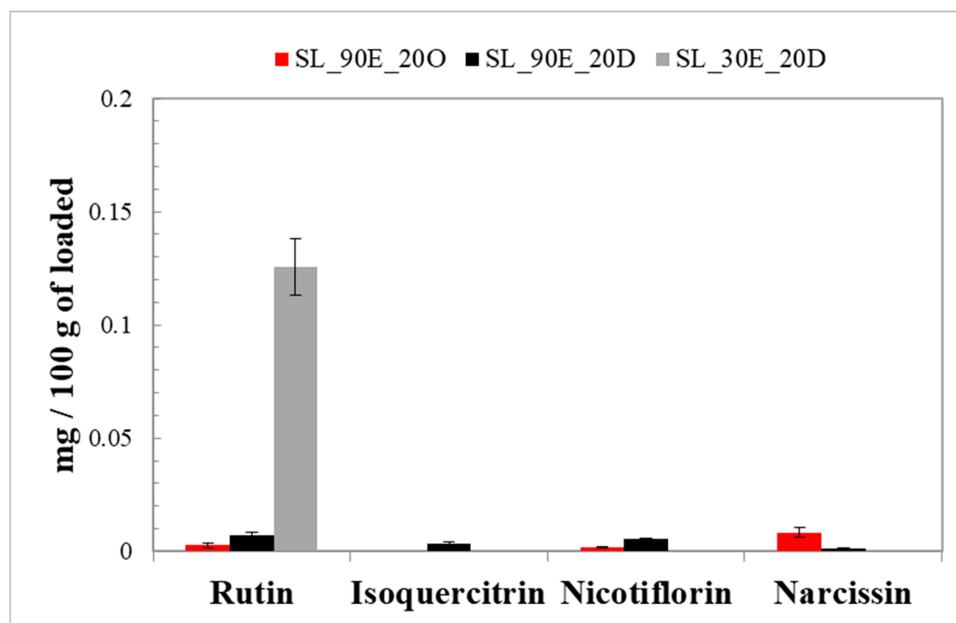


Figure 7. Quantification in mg of four different polyphenols for 100 g of loaded material for *OFI s.l.*

The yields of extraction with SFE-CO₂ are lower than that one with solvent as well as the amount of polyphenols, but the resulting extracts do not need to be separated from solvent, they are purer and cleaner.

It is possible to observe that the better results were obtained with the samples of *OFI cult.* despite the *OFI s.l.* In particular, the best yield of extraction was 2.14% ± 0.35, obtained with the sample CULT_30E_20O of *OFI cult* dehydrated at 30% and added with 20% (of the total weight loaded for the extraction) of Ottawa Sand at 110 bar. Given the results obtained from the extractions, it is possible to assume that Ottawa sands, hydrophobic natural silica particles, guarantee a better dispersion than diatomaceous earth, which also acts as a drying agent, being hydrophilic. The Ottawa sands, therefore, by favoring a homogeneous dispersion, under the same initial conditions (type of sample and initial drying conditions), guarantee a tighter

contact between the matrix and the supercritical fluid. At the increase of the dehydration to 90% for the sample CULT_90E_20O, a decrease in the yields' % follows, but an improvement is obtained concerning the selective extraction of polyphenols. In this sample, it is possible to observe the highest quantities of Rutin, Narcissin and Nicotiflorin, in accordance with the extractions by maceration, where the greatest quantity of polyphenols was obtained for the samples first dried and then macerated in methanol (Table 4).

The treatment of *OFI cult.* with 20% of Diatomee Sand (sample CULT_20D) implies a reduction of both quantity and selectivity of extraction of polyphenols, perhaps because the Diatomee Sand is hygroscopic. This sand absorbs water from the surrounding environment reducing the function of co-solvent of water naturally present in the matrix.

Being polyphenols polar compounds, the pressure was then raised to 250 bar for the sample CULT_20P, maintaining the same sand, trying to increase the polarity of CO₂, but the effect of the sand was stronger. It was obtained a reduction of both yield % and selectivity of polyphenols; this shows that high pressures result in a loss of bioactive principles sensitive as polyphenols.

When the sample is centrifuged (sample CULT_C_20D) to remove water, it was obtained a good increase of the yield % of extraction and a little improvement on the selectivity of polyphenols.

The yields % of extractions of the *OFI s.l.* were very low, ranging between 0.02 and 0.25%; but the selectivity and purity of the extracts derived therefrom are very high, as can be seen in Figure 8 for the sample SL_30E_20D.

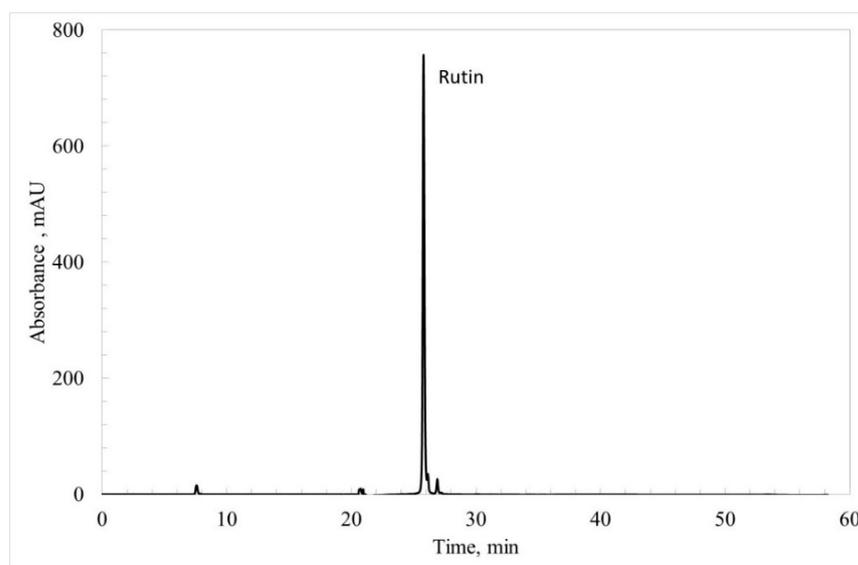


Figure 8. Chromatogram resulting from the analysis of the extract by SFE-CO₂ of *OFI s.l.* dried to 30% and added 20% of Diatomee Sand.

4. Conclusions

There is a global trend toward the use of natural flavonoids present in fruits, vegetables, oilseeds and herbs as antioxidants or functional foods.

In the present work, two different ecotypes of *O. ficus-indica*, showing dissimilar phytochemicals profile, were studied. The total phenolic content in the extracts with solvents is almost unchanged between the fresh samples collected in June and in August. This indicates that the different maturation period, if the plant still did not produce the fruit, does not interfere with the content of secondary metabolites, such as phenols, that is almost the same. The polyphenol present in greater amount in all species is Rutin; the dried samples (Cult_AD and Sl_AD) contain a greater amount than the fresh.

Being polyphenols polar compounds, the yields % quantitative of extraction with SFE-CO₂ are not high even if the extractions are very selective and the extracts nearly pure.

Concerning the SFE-CO₂ better results were obtained with the samples of *OFI cult.* in spite of the *OFI s.l.*, probably because the ecotype *OFI s.l.* was much mucilaginous, impeding to complete some extractions.

The better SFE-CO₂ extraction results were obtained with samples preventively dried, as the sample CULT_90E_200. Thus, the reduction of water, responsible for the degradation of active compounds, in presence of the Ottawa sand, produces highly selective extracts.

The treatment with Diatomee Sand implies a reduction of both quantity and selectivity of extraction of polyphenols, perhaps because the Diatomee Sand is hygroscopic and it adsorbs water from the surrounding environment, reducing the function of co-solvent of the water naturally present in the matrix. By changing the % hydration, it is possible to modulate the selectivity of extraction.

The polyphenols are polar compounds, therefore the pressure was increased to 250 bar to increase the polarity of CO₂. Nevertheless, a reduction of both the % yield quantitative and selectivity of extraction was obtained, demonstrating that the high pressures result in a loss of bioactive principles, like polyphenols. Our results evidenced as, for the first time, by improving the parameters of SFE-CO₂, which is an innovative, safe and cheap technique, it is possible to extract qualitatively and quantitatively the polyphenolic fraction from plant matrices hard to treat, namely the *Opuntia* cladodes. These results increase their value as good nutraceuticals sources, instead to be considered only a by-product from prickly pear cultivation in Calabria.

Finally, the cladodes extracts could be used as food supplements or starting materials in the pharmaceuticals industries.

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