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Optimization of ultrasounds assisted extraction of polysaccharides from cladodes of Opuntia ficus-indica using response surface methodology / Zamboi, A., Fraterrigo Garofalo, S., Tommasi, T., Fino, D.. - In: SUSTAINABLE CHEMISTRY AND PHARMACY. - ISSN 2352-5541. - 37:(2024). [10.1016/j.scp.2023.101348]

Availability:
This version is available at: 11583/2984921 since: 2024-01-09T10:12:32Z

Publisher:
Elsevier

Published
DOI:10.1016/j.scp.2023.101348

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24 November 2026

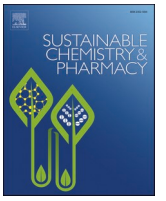
Sustainable Chemistry and Pharmacy 37 (2024) 101348



Contents lists available at ScienceDirect

Sustainable Chemistry and Pharmacy

journal homepage: www.elsevier.com/locate/scp



Optimization of ultrasounds assisted extraction of polysaccharides from cladodes of *Opuntia ficus-indica* using response surface methodology

Aurora Zamboi [a,b], Silvia Fraterrigo Garofalo [a,*], Tonia Tommasi [a], Debora Fino [a,b]

[a] Department of Applied Science and Technology (DISAT), Politecnico di Torino, Corso Duca Degli Abruzzi 24, Torino, TO, 10129, Italy
[b] Istituto Italiano di Tecnologia (IIT), Center for Sustainible Future Technologies, Via Livorno 60, Torino, TO, 10144, Italy

ARTICLE INFO

Handling Editor: Klaus Kümmerer

Keywords:
Ultrasound-asssited-extraction
Polysaccharides
Design of experiments
Opuntia ficus-indica
Green extraction

ABSTRACT

In this work, polysaccharides are extracted from cladodes of *Opuntia ficus-indica* using Ultrasounds-Assisted Extraction (UAE). UAE operating conditions are optimized by a Face Centered Central Composite Response Surface Design (FCCRD) with four variables at three levels. The optimal operating conditions are obtained using Response Surface Methodology (RSM) and are the following: Solid-Liquid ratio SL = 1:10 w/v, pH = 2.5, time t = 20 min, and temperature T = 65 °C. The predicted extraction yield (12.07 ± 1.7 % dw) is in line with the experimental one (11.32 ± 0.25 % dw). The resulting extract is composed mainly of galacturonic acid (25.55 ± 0.30 %), the primary constituent of pectic material, followed by arabinose (14.34 ± 0.01 %) and galactose (13.5 ± 0.22 %). The Esterification Degree (ED) is found 42.84 ± 0.48 %, indicating the extract is Low-Methylated (LM). The FT-IR spectrum demonstrates that the extract has several peaks typical of polysaccharides. Total Phenolic Content (TPC) is equal to 41.33 ± 3.53 μgGAE/g (dw) and the anti-radical ability against the DPPH radical scavenging activity achieves 95.56 % at a concentration equal to 2 mg/mL, demonstrating that the extract is a polysaccharide functionalized with polyphenols. These results can encourage the use of the extract as a new ingredient in nutraceutical cosmetic applications.

1. Introduction

The growth in industrial activities in the food and agricultural sectors is causing a continuous increase in waste production. These wastes could represent an important source of high-added value compounds. Valorization could allow reducing the use of raw materials, decrease the amount of effective waste to be disposed and to incentivize and support the economy of the food and agri-food sector (Bhat et al., 2020).

Opuntia ficus-indica ((L.) Mill., 1768) is a crop species native to Mexico (Griffith, 2004) belonging to the Cactaceae family (Silva et al., 2021). Nowadays Opuntia is abundantly found in other parts of the world, such as the Mediterranean basin, thanks to its relative ease of vegetative propagation and its ease of growth (Griffith, 2004). The easy spread of Opuntia is due to its adaptative ability to difficult conditions (Bayar et al., 2016), which also makes it an excellent supporter in the prevention of soil degradation and desertification (Le Houérou, 2002). Mexico is the first region per hectares of cultivated areas (at least 50.000–70.000 ha) and Italy follows with about 7.000–8.300 ha of intensive plantations, 90% located in Sicily (Di Bella et al., 2022).

* Corresponding author.
E-mail address: silvia.fraterrigo@polito.it (S. Fraterrigo Garofalo).

https://doi.org/10.1016/j.scp.2023.101348
Received 18 September 2023; Received in revised form 26 October 2023; Accepted 12 November 2023
Available online 20 November 2023
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Table 1
Literature main results in polysaccharides extraction from cladodes of Opuntia ficus-indica (fw = Fresh Weight; dw = Dry Weight).

Extraction technique	Final product	Yield (%)	Reference
Acidified hot water extraction (HCl)	Pectin	0.18–0.06 fw	Sevgi et al. (2022)
Enzymatic extraction using cellulase (C) and xylanase (X)[a]	Pectin	16.67 ± 0.30 dw	Bayar et al. (2018)
Acidified hot water extraction (HCl)	Mucilage (MC), Pectin (PC), Total Pectic Mucilage (TPC)	MC = 10.24 ± 0.69 dw PC = 6.13 ± 0.60 dw[a] TPC = 13.12 ± 2.19 dw	Bayar et al. (2016)
Mechanical extraction: extrusion of mucilage from cladodes	Mucilage	>30 dw	Procacci et al. (2021)
Hot water extraction + EDTA added water extraction + acid extraction (HCl)	Total Pectins Fraction (TPF)	6.07 dw	Lefsih et al. (2016)
Ultrasonic Assisted Extraction (UAE)[a]	Pectin	18.14 ± 1.41 dw	Bayar et al. (2017)
Ultrasonic Assisted Extraction (UAE)	Polysaccharides	18.48 ± 0.35 dw	Yılmaz (2020)
Microwave Assisted Extraction (MAE)	Mucilage	25.60 dw	Felkai-Haddache et al. (2016)

[a] After mucilage removal.

Opuntia ficus-indica is cultivated mainly for the fruit (known as prickly pear) while other cactus parts, such as cladodes (or Nopal), are generally undervalued and considered as pruning waste to exploit at most for feeding livestock. Annual pruning waste from Opuntia cultivation is about 6–8 tons/hectare, representing one of the main costs for farmers (Procacci et al., 2021).

Despite that, cladodes demonstrated to be a rich source of value-added compounds (Fawzy et al., 2021) such as dietary fibers, inorganic elements, mono, and polysaccharides (Albergamo et al., 2022). Many studies have been conducted in recent years to prove their content and possible uses (de Andrade Vieira and Tribuzy de Magalhães Cordeiro, 2023): Cladodes can be used as a source of polyphenols (Rocchetti et al., 2018), mono and polysaccharides such as pectin (Bayar et al., 2016, 2018; Sevgi et al., 2022), fibers for food applications (Guevara-Arauza et al., 2012), paper production (Sottile et al., 2021) or in biocomposites for building industry (Maderuelo-Sanz et al., 2022). The mucilage of opuntia cladodes can be used as a bio-coagulating agent for oil sands process-affected water (Choudhary et al., 2019) or heavy metals contaminated water (Vargas-Solano et al., 2022).

Cladodes are rich in pectin-based polysaccharides (Yılmaz, 2020).

Pectin is a water-soluble heteropolysaccharide found in the cell wall and middle lamellae of plants, that has many functional and nutritive uses within food and other industries (Adetunji et al., 2017). Commercial pectin is usually extracted from citrus peel and apple pomace and is mainly used in the food industry as an additive, indicated as E440, thanks to its gelling, stabilizing, and thickening properties. Depending on its origin, pectin has very varied macromolecular and microstructural properties (Adetunji et al., 2017). Its final use (in food and non-food sectors) and its health benefits depend on these properties.

While the classic procedure for pectin-based polysaccharides consists generally of acidified hot water extraction, the most innovative and green techniques include the use of subcritical fluids, enzymatic extraction, ultrasounds-assisted extraction (UAE), microwave-assisted extraction (MAE), use of pulsed electric field or high hydrostatic pressure.

For Opuntia cladodes, there is a rather vast literature in which different extraction processes of the polysaccharides are tested. A summary of the results found in other searches is shown in Table 1, with different results in terms of yield of extraction (Y%) and final product characteristics.

In the present work, the interest is focused on Ultrasound-Assisted Extraction as a novel method to obtain polysaccharides from cladodes of O. Ficus Indica. Ultrasounds offer a clean and environment-friendly technique, with the advantage of the ease of use, versatility, and relatively low budget compared to other innovative extraction techniques (Tiwari, 2015).

Ultrasonic waves generate cavitation bubbles that enhance the contact between solvent and plant materials, improving and accelerating the mass transfer of the target compounds (Adetunji et al., 2017; Moorthy et al., 2017). Many factors can influence the effectiveness of extraction while using UAE, such as the ultrasonic source, operating frequency and intensity, extraction time and temperature, the choice of solvent and its characteristics and the ratio between solvent and biomass to be treated (Tiwari, 2015). In this study, for the optimization of the procedure is necessary to determine which parameters to fix and which to vary. The process is therefore optimized based on four parameters (solid-liquid ratio, pH, temperature, and time), while the others (power, frequency, etc) are kept constant. The testing intervals are solid-liquid ratio (SL) 1:10–1:40 w/v (mL/g), pH 1.5–2.5, sonication time (t) 10–30 min, and temperature (T) 25–75 °C. These boundaries were determined based on common sense after consulting several studies that use ultrasound to extract polysaccharides from prickly pear peels (Hernández-Carranza et al., 2019) or other types of plant matrices, such as jackfruit, agave, and grapefruit (Bagherian et al., 2011; Maran and Priya, 2015; Moorthy et al., 2017).

After the optimization of UAE, properties of the extract at the best operational conditions are investigated, to determine polysaccharides composition, total phenolic content, degree of esterification, and anti-radical activity.

To the best knowledge of the authors, there are still no studies done on the Apulian Opuntia, as all the studies done in Italy mostly come from Sicily. The south of Apulia region is affected by a disease of olive caused by a bacteria called “Xylella fastidiosa”, which implies the death of trees, firstly isolated in 2013 and now widespread in the region for about 54.000 ha (Bajocco et al., 2023). This bacteria causes enormous damage in terms of economic and environmental disruption. The partial reconversion of olive groves into new types of plantations is therefore an alternative for the recovery of the affected areas, and *Opuntia Ficus-Indica* represents a perfect candidate. Furthermore, there is still a lack of literature regarding the influence of some extractive parameters in the UAE of polysaccharides in Opuntia cladodes: the combined effect of temperature, time, pH and solid-liquid ratio has not been studied yet. Finally,

currently, no one has ever thought of evaluating the content of polysaccharides functionalized with polyphenols for Opuntia cladodes. The present study therefore aims to fill these gaps.

2. Materials and methods

2.1. Material and sample preparation

All HPLC grade reagents, commercial standards for carbohydrates, TDF-100A kit for total dietary fiber determination, Folin-Ciocalteu reagent, 2,2′-diphenyl-1-picrylhydrazyle (DPPH), and other reagents, were purchased from Merck (Darmstadt, Germany). Ultrapure water (type 1) was obtained from a Direct-Q C9185 water purification system (Merck).

Opuntia cladodes 25–50 cm long were manually harvested from Apulia, in the south of Italy. After a washing phase, the thorns were removed and cladodes were cut into small slices (about 2 × 2 cm). The sample was dried in an air oven at 103 °C until constant weight and subsequently ground. The obtained powder was stored in a tightly closed protected place until further analyses.

2.2. Proximate composition

The proximate composition of cladodes was determined using AOAC official methods (AOAC, 2019). All the analyses were performed in triplicate. Moisture was determined using the AOAC Official Method 925.09 based on sample weight loss after a night passed in the air oven at 103 °C. For ash determination, the gravimetric method was used. Basically, 0.5 g of dry samples were heated in a muffle furnace at 550 °C for 6 h. Lipids were determined using the AOAC Official Method 920.39, where 5 g of sample are treated with petroleum ether in a Soxhlet apparatus. Protein content was determined using the Dumas method, where a CHNS analyzer (Vario MACRO cube, Elementar Italia Srl) was used to determine the % nitrogen. This value was then multiplied by a 6.25 conversion factor to obtain the % protein. Total Dietary Fibers (TDF) were determined using the Total Dietary Fiber Assay Kit TDF-100A which works with a combination of enzymatic and gravimetric methods. The dried sample is firstly treated with stable α-amylase and then digested with protease and amyloglucosidase. The fiber precipitation takes place with the addition of ethanol. Carbohydrates were determined by difference.

2.3. Polysaccharides extraction

The cladodes powder was mixed with acidified water in a beaker at a well-defined Solid-Liquid (SL) ratio and pH. After that, the beaker was placed on a magnetic stirrer equipped with a thermocouple. An ultrasonic tip (VCX750 Ultrasonic Processors – Sonic and Materials Inc), with 40 % amplitude, 750 W power intensity, and 20 kHz frequency, was used to improve the extraction efficiency. The extraction took place at a given temperature and time. After the extraction, the sample was centrifuged with an SL 16R centrifuge (Fisher Scientific Italia) at 4000 rpm for 15 min and then filtered with the aid of a vacuum pump using filter paper Whatman n. 1. Two volumes of 95% ethanol were added to the permeate liquid phase to allow polysaccharides precipitation and the sample was stored at 4 °C overnight to be sure precipitation occurs. The ethanol-added samples were centrifugated again at 4500 rpm for 15 min to make all the extract settle well. The supernatant was removed and the extract was dried at 50 °C until constant weight. The extraction yield Y was calculated as follow:

$$Y(\%) = 100 \times \frac{m_0}{m} \quad \text{Eq. 1}$$

where m $_0$ is the weight of dried extract (g) and m is the weight of dried cladodes powder (g).

2.4. Experimental design

A face-centered central composite response surface design (FCCRD) was used in this study to investigate the effect and optimize the process variables. Four factors in three levels were tested to obtain the maximum yield in polysaccharides: Solid-Liquid (SL) ratio (1:10–1:40 % w/v), pH (1.5–2.5), extraction temperature (25–75 °C) and sonication time (10–30 min). A total number of 27 experiments, including three replicates at the central point to validate the model, were performed. The experimental data were used to build a mathematical model which expresses the correlation between the four independent variables and the response. To do that, a second-order polynomial equation was used whose generalized form is the following:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2(i\neq j)}^{k} \beta_{ij} X_i X_j \quad \text{Eq. 2}$$

Where Y is the response; β_0 is the model intercept; X_i and X_j are the independent variables (where i and j vary from 1 to k); β_i, β_{jj}, and β_{ij} are respectively the linear, quadratic and second-order terms. k corresponds to the number of independent parameters ($k = 4$).

All data analyses, the model determination, and the selection of the best operational parameters were done with the help of CAT (Chemometric Agile Tool) software (Leardi et al., 2023).

2.5. Model validation

Optimal factor levels were obtained by studying the response surface plots produced by CAT software. After that, three experiments were performed at the optimal levels and the results were compared to the predicted one to validate the model.

Table 2
Proximate composition of cladodes from Opuntia ficus-indica. The results are expressed in terms of 1g/100g on dry weight (% dw) and as mean ± standard deviation of 3 replicates.

	moisture	ash	lipid	protein	TDF	carbohydrates
% dw	5.88 ± 0.56	21.99 ± 0.06	1.68 ± 0.34	7.06 ± 0.29	40.96 ± 0.04	22.43 ± 0.86

2.6. Extract characterization

2.6.1. Monosaccharide composition

High-Performance Liquid Chromatography with a refractive index detector (Shimadzu HPLC-RID 10A Prominence) was performed with a Rezex ROA-Organic acid H+ (8%) LC column 300 mm × 7.8 mm. H_2SO_4 5 mM in ultrapure water was used as the mobile phase, at a flow rate equal to 0.7 mL/min. The temperature of the column was 50 °C. HPLC analyses were used to define the monosaccharide composition of the extract following the method described by (Van Wychen and Laurens, 2013) with some modifications. Briefly, 25 mg of samples were hydrolyzed in 250 μL of sulfuric acid 72% w/w at 30 °C for 1 h. After that, 7 mL of ultrapure water was added to the sample. The sample was then well closed and maintained at 121 °C for 8 h with the aid of an oil bath. The pH was measured and the sample was neutralized. Monosaccharide identification (Galacturonic acid, Galactose, Glucose, Arabinose) was based on standards analyzed at equal conditions.

2.6.2. Esterification degree

Esterification Degree (DE) was determined as described by (Bayar et al., 2017). Briefly, 200 mg of sample were wetted using 2 mL of ethanol and then 20 mL of 40 °C heated distilled water was added. The sample was kept at 40 °C under continuous stirring for 2 h. Three drops of phenolphthalein were added and the obtained solution was titrated with NaOH (0.1 M). The volume of used NaOH was recorded as V_1. After that, 10 mL of NaOH (0.1 M) was added and the solution was kept under continuous stirring for 2 h 10 mL of HCl (0.1 M) was added and the sample was then stirred until the pink color disappeared. Again, the solution was titrated with NaOH (0.1 M), and the volume used for the second titration was recorded as V_2. The Esterification Degree was calculated as follows:

$$DE(\%) = \frac{V_2}{V_1 + V_2} \times 100 \quad \text{Eq. 3}$$

2.6.3. Total Phenolic Content (TPC) quantification

The Total Phenolic Content (TPC) of the extract was determined using the Folin-Ciocalteu reagent, applying the standard method for Total Phenolic Content quantification from tea leaves (International Organization of Standardization, 2005). Briefly, 1 mL of diluted extract (30 mg/mL) was mixed with 5 mL of F–C reagent (10 % v/v). Within 3 min–8 min, 4 mL of sodium carbonate (7.5 % w/v) was added. After incubation at ambient temperature in the dark for 1 h, the absorbance at 765 nm with UV–Vis spectrophotometer DR 5000 (Hach) was measured. The results were expressed as mg Gallic Acid Equivalent (GAE) per g of dry matter (DM). To evaluate TPC a calibration curve of gallic acid was constructed.

2.6.4. Anti-radical ability

The anti-radical activity of the extract was evaluated using the method reported by (Lefsih et al., 2016) with some modifications. 1 mL of the extract solution at different concentrations (2–15 mg/mL) was mixed with 1 mL of 0.1 mM DPPH• solution in ethanol. The obtained samples were vigorously stirred and incubated at 25 °C for 30 min. A control sample was prepared with the same protocol, using 1 mL of ultrapure water and 1 mL of 0.1 mM DPPH• solution in ethanol. Ascorbic acid was used as a reference. A UV–Vis spectrophotometer DR 5000 (Hach) was used to determine the absorbance of the samples at 517 nm and anti-radical activity was obtained using the following equation:

$$DPPH\bullet \text{ radical scavenging activity } (\%) = \left[1 - \left(\frac{A_{sample}}{A_{control}}\right)\right] \times 100 \quad \text{Eq. 4}$$

2.6.5. Infrared spectroscopy analyses

Infrared spectroscopy analyses were carried out using FTIR-ATR Bruker Equinox 55 (Attenuated Total Reflectance Infrared Spectroscopy) in the wavenumber range 400–4000 cm^{-1} at a resolution of 2 cm^{-1}. A sample of commercial citrus peel pectin was also analyzed to make a comparison.

3. Results and discussion

3.1. Proximate composition

Moisture, ash, lipid, protein, TDF, and carbohydrates contents in dried cladodes are shown in Table 2.

Cladodes composition generally depends on various factors such as maturity stage, cultivar, season, or cultivation site (Stintzing and Carle, 2005) therefore, it is generally difficult to establish an unambiguous trend of the results of other studies. Nevertheless, the results obtained in the present study are compatible with many other researches (Di Bella et al., 2022; López-Cervantes et al., 2011; Stintzing and Carle, 2005). The samples show a high content of TDF, carbohydrates, and ashes. Similar to what is obtained from (Albergamo et al., 2022; Di Bella et al., 2022), cladodes can be considered a “natural source of fiber” since their content in TDF exceeds 3 %, as established in the Regulation (EC) 1924/2006 (Zicari et al., 2007).

Table 3
Experimental results of face centered central composite response surface design.

Exp No	SL (g/mL) X_1	pH X_2	t (min) X_3	T (°C) X_4	Experimental Yield %
1	10	1.5	10	25	10.14
2	40	1.5	10	25	3.22
3	10	2.5	10	25	9.76
4	40	2.5	10	25	6.40
5	10	1.5	30	25	9.40
6	40	1.5	30	25	3.57
7	10	2.5	30	25	8.81
8	40	2.5	30	25	7.61
9	10	1.5	10	75	10.59
10	40	1.5	10	75	4.11
11	10	2.5	10	75	10.47
12	40	2.5	10	75	9.70
13	10	1.5	30	75	11.23
14	40	1.5	30	75	6.83
15	10	2.5	30	75	11.96
16	40	2.5	30	75	8.54
17	10	2	20	50	12.89
18	40	2	20	50	7.19
19	25	1.5	20	50	8.68
20	25	2.5	20	50	9.52
21	25	2	10	50	7.20
22	25	2	30	50	9.06
23	25	2	20	25	8.29
24	25	2	20	75	8.33
25	25	2	20	50	8.07
26	25	2	20	50	7.61
27	25	2	20	50	8.36

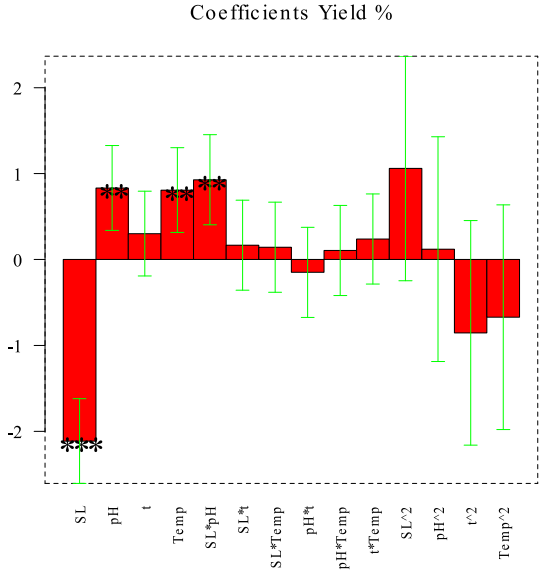

Fig. 1. Coefficients graph. The green lines represent the error. * visually indicates the significance of the coefficients (p-value): *** = $p < 0.01$; ** = $p < 0.001$.

3.2. Polysaccharides extraction

3.2.1. Experimental design and model building

The UAE experimental results are shown in Table 3. These results are processed with the multiple regression analysis using the CAT software (Leardi et al., 2023) in order to obtain a model expressed as a second-order polynomial equation. The resulting model is the following:

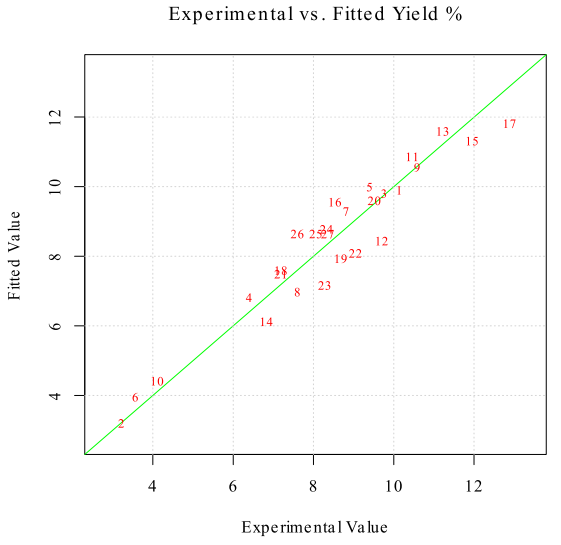

Fig. 2. Experimental vs Fitted yield. The red values represent the experimental data while the green line represents the fitted values obtained through the model.

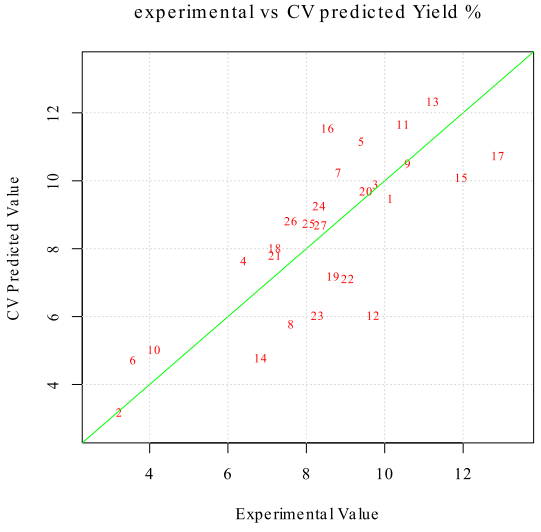

Fig. 3. Experimental data vs Cross-Validated (CV) predicted data. The red values represent the experimental data while the green line represents CV predicted values.

$$Y(\%) = 8.658 + (-2.114 * X_1) + (0.833 * X_2) + (0.300 * X_3) + (0.808 * X_4) + (0.929 * X_1 * X_2) + (0.167 * X_1 * X_3) + (0.142 * X_1 * X_4) + (-0.149 * X_2 * X_3) + (0.104 * X_2 * X_4) + (0.239 * X_3 * X_4) + \left(1.060 * X_1^2\right) + \left(0.120 * X_2^2\right) + \left(-0.854 * X_3^2\right) + \left(-0.672 * X_4^2\right)$$

Eq. 5

As can be seen from the equation, the parameter that most influences the process is the solid-to-liquid ratio, followed by pH and temperature. It seems that the sonication time has not a great influence on the tested interval. From the equation, it can be concluded also that the process is influenced by the correlation between the SL ratio and pH. The coefficient graph in Fig. 1 shows graphically the effect of each single variable ad their interactions on the system response. As expected, the most important parameter is the SL ratio with an inversely proportional correlation with the response. All the other relevant parameters are directly proportional to the response.

The explained variance of the model is equal to 82.63 %. This value expresses the accuracy with which the model describes the

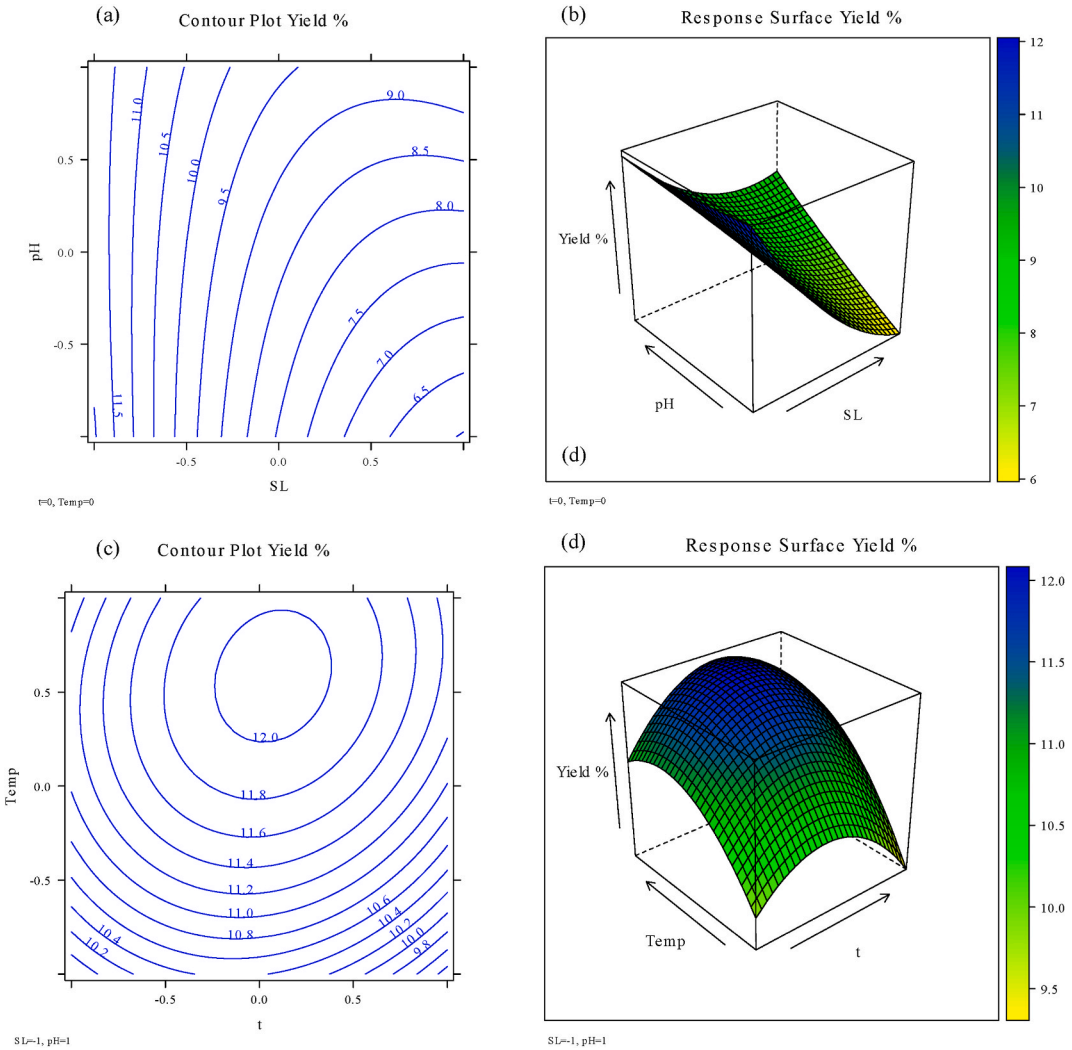

Fig. 4. Response Surfaces. (a) and (b) shows the correlation between pH and SL ratio in 2D and 3D respectively, keeping t and T at their middle values. (c) and (d) shows the correlation between t and T in 2D and 3D respectively, keeping the SL ratio at its lowest value and pH at its highest value.

variability of the data.

The model accuracy is graphically represented in Fig. 2, where experimental data are very close to the line representing the fitted values.

In Fig. 3 the experimental vs Cross Validation (CV) predicted data are reported. The CV is a procedure that allows to obtain important information regarding the stability and generalizability of the regression model. The CV procedure splits randomly the data into two sets: training data for developing the model and validation data for evaluating the model's predictive ability. To evaluate the consistency of the model, the fit of the model to the training dataset can be compared with the refit of the model to the validation dataset. The software also reports the mean squared error of CV (RMSECV), which is equal to 1.553. In the figure the experimental values are close to the green line representing the CV predicted values. All these observations lead to the conclusion that the model is stable and generalizable.

3.2.2. Response surfaces

Response Surface Methodology (RSM) allows to describe the interaction between data to obtain statistical forecasts. It is applied to investigate the influence of each variable on the response of the system. As already mentioned, the adopted design is the FCCRD, with four variables in three levels (−1, 0, 1). The response surfaces allow us to visualize the first-order interaction between two variables, keeping the others constant.

Fig. 4 shows the 2D-RSM and 3D-RSM diagrams that demonstrate what has already been concluded in the previous paragraph. In Fig. 4 (a) and (b) the response of the system to variations in the SL ratio and pH, keeping t and T at their middle values, is illustrated. The SL ratio is confirmed as the most important parameter. At tested conditions, its variation leads from extraction yields of a minimum of 6 % (when SL is equal to 1) to yields of a maximum of 12 %, where SL is equal to −1. The inverse proportionality between working

volume and extraction yield is probably due to the limited range of action of the used ultrasonic probe, whose waves have difficulty spreading in a large working space. The effect of SL ratio variation is more marked when pH is kept low. The effect of pH is more marked when the SL ratio is low, while when SL is high, the pH is practically irrelevant. From these two graphs, it is possible to optimize the first two variables: SL is kept as low as possible (SL = −1) and pH is kept as high as possible (pH = 1). Based on this result, the graphs shown in Fig. 4 (c) and (d) were made. Keeping in constant SL ratio and pH at −1 and 1 respectively, the combined effect of t and T is evaluated. Temperature is slightly more influential than time, as previously predicted. From the paraboloid surface, it can be concluded that the optimized values for time and temperature are 0 and 0.5, respectively.

It can therefore be concluded that the optimized model is SL 1:10 w/v, pH 2.5, t 20 min, T 65 °C, giving a predicted yield equal to 12.07 ± 1.7 % dw.

Yılmaz et al. (Yılmaz, 2020), optimized ultrasound-assisted extraction of polysaccharides from opuntia, studying the effect of power intensity, temperature, and time variations. The maximum yield (18.58 % dw) was founded at power intensity 345.5 W, T 31.35 °C (304.5 K), t 28.5 min, Solid-Liquid ratio SL 1:15 (w/v), and pH 2.8. These results confirm the possibility of using less solvent and chemicals for the modification of pH. Bayar et al. (2016) carried out UAE setting the parameters as follow: SL 1:15 (w/v), pH 2.8, T 90 °C, t 2 h. The resulting yield was equal to 13.12 ± 2.19 % dw, similar to what was obtained in the present study. Nevertheless, no attention was paid to optimizing the extraction parameters, resulting in higher values of operational temperature and time. Sevgi et al. (2022) performed acidified hot water extraction with SL 1:15 w/v, pH 2.8 %, T 90 °C, t 4 h, obtaining an extraction yield equal to 0.18–0.06 % fw (with a moisture content equal to 92 ± 5 %). This result is quite low if compared with results obtained using ultrasound, confirming that ultrasounds are more efficient in extracting polysaccharides when compared to conventional methods.

3.2.3. Model validation

To verify the validity of the model, three experiments at the optimal conditions (SL of 1:10 w/v, pH of 2.5, t equal to 20 min, and T equal to 65 °C) were performed. The predicted extraction yield was equal to 12.07 ± 1.70 %. This result is validated through the three repetitions at the optimal conditions (11.18 %, 11.17 %, and 11.61 %), whose average is equal to 11.32 ± 0.25 %. It can therefore be concluded that the validation result is included in the model error.

3.3. Extract characterization

3.3.1. Monosaccharide composition

The main monosaccharide was Galacturonic Acid (25.55 ± 0.30 %) which is the main component of pectin (Adetunji et al., 2017). The other detected monosaccharides are Glucose (1.12 ± 0.03 %), Galactose (13.5 ± 0.22 %) and Arabinose (14.34 ± 0.01 %). α-1, 4-linked D-galacturonic acid (commonly known as galacturonic acid, GalA) is the main constituent of pectin followed by D-galactose, L-arabinose, L-rhamnose and others (Chan et al., 2017). The α-1,4 linkages combine the GalA units. These units have carboxyl groups that are esterified by methyl groups causing variation in the degree of esterification (DE) (Raj, 2012).

Commercial pectin has usually more than 65 % Galacturonic Acid (Bayar et al., 2018) so the extract cannot be classified as pectin. This is in contrast with other results: (Yılmaz, 2020) found a GalA content equal to 72.75 ± 6.8 % using UAE, (Sevgi et al., 2022) got 56.16 ± 5.25 % using acidified hot water extraction, (Bayar et al., 2018) achieved 66.66 ± 2.46 % using enzymatic extraction. Despite that, the high content in GalA may imply a good content of polyphenols, since polyphenols are usually involved in methylation conjugation (Brglez Mojzer et al., 2016).

3.3.2. Esterification degree

The esterification degree (ED) is a parameter used to determine the degree of methylation of the extract. It can be Low Methylated (LM) if DE ≤ 50 % or High Methylated (HM) if DE > 50 %. The extract obtained with UAE has DE equal to 42.84 ± 0.48, which means it is LM. This result is in line with other research on cladodes of O. ficus-indica (Bayar et al., 2017, 2018; Sevgi et al., 2022; Yılmaz, 2020) even if it is slightly higher than the others.

The degree of esterification is one of the fundamental parameters for having information on the ability of the extract to form gels. While HM material requires sugars for gel formation, in LM gel formation depends on the presence of divalent ions. Indeed, the gelling mechanism of LM extract is based on the alignment of sequences of GalA monomer, linked through the electrostatic and ionic bond of carboxyl groups. Moreover, the presence of small quantities of sugars (10–20 %) can favor the gel formation (Raj, 2012).

3.3.3. Total Phenolic Content and DPPH radical scavenging activity

Polyphenols are secondary plant metabolites frequently present in vegetables and fruits. In this study, it was decided to estimate the TPC and the DPPH radical scavenging activity, to evaluate if the extract could be of interest for its nutraceutical, biomedical, or cosmetic functionalities.

The Total Phenolic Content (TPC) of the extract is equal to 41.33 ± 3.53 mgGAE/g (dw). This result is higher than what is found in the literature. (Procacci et al., 2021) have found a TPC equal to 19.40 ± 0.02 mgGAE/g. This significant difference in the obtained results is probably due to the extraction method used by the authors which uses mechanical-physical forces. The use of ultrasound, as well as the extraction temperature of the UAE process, are indeed responsible for the release of polyphenols from the raw material and in conclusion for the increase in yield of phenolic content.

However, the extraction conditions could have a negative effect on antioxidant compounds, since the latter could be altered causing a loss in antioxidant activity (Zeghbib et al., 2022). The antioxidant activity is one of the most important characteristics to determine while studying polyphenols, due to its beneficial effects in preventing disease and in the healing process (Brglez Mojzer et al., 2016). An antioxidant is a stable molecule able to neutralize free radicals by donating an electron. Antioxidants can inhibit cellular damage thanks to their free radical scavenging property (Geremu et al., 2016). For these reasons, the DPPH radical scavenging activity assay

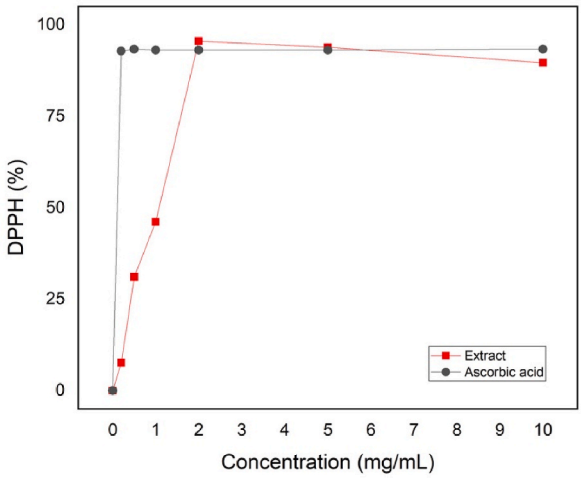

Fig. 5. DPPH anti-radical activity of the extract and the reference ascorbic acid.

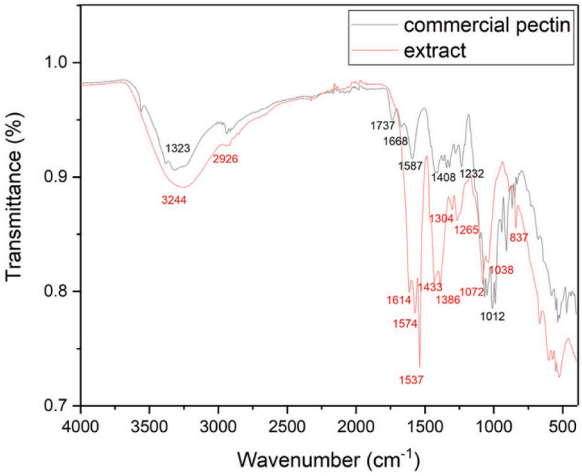

Fig. 6. FTIR spectra of extract and commercial pectin.

was performed.

Employing the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a common method to determine the antioxidant ability of phenolic compounds contained in natural tissues and food sources. The antiradical activity of antioxidants is determined using DPPH free radical, which has an absorption band at 517 nm in the UV–Vis spectrum. The absorbance decrease is directly proportional to the antioxidant ability and to the concentration of the added compound (Brglez Mojzer et al., 2016). The DPPH radical assay was done using ascorbic acid as a standard antioxidant. According to Fig. 5, the anti-radical ability increases with the concentration up to 95.56% at a concentration of 2 mg/mL. This considerable result could be due to the high TPC, the degree of methylation, and the high galacturonic acid content of the extract (Bayar et al., 2018). Moreover, it can be concluded that the extraction conditions do not alter the antioxidant ability of polyphenols in the extract. The extract can therefore be defined as a heteropolysaccharide functionalized with polyphenols. In recent years, interactions between polyphenols and polysaccharides have attracted interest since they play a role in the physiological properties, bioavailability, and stability of compounds (Guo et al., 2022).

3.3.4. FTIR analyses

FTIR spectra of the extract are compared with the spectra of commercial pectin in Fig. 6. Both samples show a peak in the region between 3550 and 3200 cm^{-1} which is due to the O–H stretching frequency (Pasandide et al., 2017). Between 3000 and 2840 cm^{-1} there is the C–H stretching band, in which both samples have a peak at 2926 cm^{-1}, typical of sugars (Niu et al., 2021), that indicates the symmetric stretching vibration of the C–H group of the methyl ester of galacturonic acid (Bayar et al., 2016). Wavenumbers lower than 1800 cm^{-1} are characteristic of the fingerprint region while the carbohydrates fingerprint is in the region between 1200 and 800 cm^{-1} (Bayar et al., 2016). More differences between the extract and pectin can be seen in these areas. Commercial pectin has a peak at 1737 cm^{-1} related to the vibration of the esterified carboxyl group C═O (Méndez et al., 2021). The extract does not have this peak maybe

because it is covered by the peaks at 1614 cm^{-1} and 1574 cm^{-1} representative of asymmetrical COO^- stretching vibration typical of the carboxyl group of galacturonic acid (Manrique and Lajolo, 2002). The peak at 1537 cm^{-1} is probably due to the presence of C═C bonds in the aromatic ring of feruloyl groups (Soltani and Madadlou, 2015). Both samples show the presence of peaks in the range 1400–1450 cm^{-1}, caused by the presence of free carboxyl groups COO^-. These peaks, along with those in the range 1600–1650 cm^{-1} (also caused by free carboxyl groups), are usually used to determine the esterification degree (Lefsih et al., 2016). In the extract, the peak at 1304 cm^{-1} is caused by the C–H vibration generated from the acid treatment (Lefsih et al., 2016). Peaks at 1265 cm^{-1} and 1232 cm^{-1} in the extract and pectin, respectively, are probably due to the presence of o-acetyl ester (Nejatzadeh-Barandozi and Enferadi, 2012). In the extract, the peaks at 1072 cm^{-1} and 1038 cm^{-1} are due to galactose and glucan units, respectively (Nejatzadeh-Barandozi and Enferadi, 2012). Peaks between 850 cm^{-1} and 900 cm^{-1} are typical of a-glycosidic linkage (Bayar et al., 2016). Peaks in the region between 900 cm^{-1} and 400 are representative of vibration due to monosaccharides and oligosaccharides molecules (Lefsih et al., 2016).

4. Conclusion

In this work, Ultrasound-Assisted Extraction was successfully employed to obtain polysaccharides from cladodes of *Opuntia ficus-indica*. The process parameters were investigated using Face Centered Central Composite Response Surface Design (FCCRD). A second-order polynomial equation was built to study the correlation between independent variables and the response. The variables were optimized using Response Surface Methodology (RSM) and the obtained optimum conditions were: SL = 1:10 w/v, pH = 2.5, t = 20 min, and T = 65 °C. Under these conditions, the experimental extraction yield was 11.32 ± 0.25 % dw, close to the predicted one that was equal to 12.07 ± 1.7 % dw. The extract showed an Esterification Degree (ED) equal to 42.84 ± 0.48 %, a content of galacturonic acid of 25.55 ± 0.30 %, arabinose of 14.34 ± 0.01 % and galactose 13.5 ± 0.22 %. The extract also had several peaks typical of polysaccharides in FT-IR spectrum. Total Phenolic Content (TPC) was equal to 41.33 ± 3.53 μgGAE/g (dw) and the anti-radical ability has achieved 95.56 % at a concentration equal to 2 mg/mL, demonstrating that the extract is essentially composed of polysaccharides functionalized with polyphenols. This study provides a novel and more efficient extraction technique if compared to conventional ones for the recovery of polysaccharides from cladodes of *Opuntia ficus-indica* harvested in Apulia region. Moreover, the possibility to extract functionalized polysaccharides has not studied yet for Opuntia. The obtained results can encourage the use of the extract as a new ingredient in nutraceutical cosmetic applications.

Author statement

Aurora Zamboi: Conceptualization, Methodology, Writing - Original Draft, **Silvia Fraterrigo Garofalo**: Validation, Writing - Review & Editing, **Tonia Tommasi**: Supervision, **Debora Fino**: Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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