Optimization of Ultrasounds Assisted Extraction of pectin from cladodes of Opuntia ficus-indica (L.) Mill using Response Surface Methodology

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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 and their valorization could allow the reduction of the use of raw materials and the waste amount.

Opuntia ficus-indica (L.) Mill. is a crop species native to Mexico belonging to the Cactaceae family. Nowadays opuntia is abundantly found in other parts of the world, for example in the Mediterranean basin, thanks to its relative ease of vegetative propagation and its ease of growth. Actually, O. Ficus-indica is cultivated mainly for the fruit (prickly pear). Other cactus parts such as cladodes (or Nopal) are generally undervalued and considered as pruning waste to exploit at most for feeding livestock. Annuals pruning waste from opuntia cultivation are about 6 - 8 tons/hectare, representing one of the main costs for farmers.

Cladodes demonstrated to be a rich source of value-added compounds such as dietary fibers, inorganic elements (Mg, K, Mn, Fe), mono and polysaccharides. Many studies have been done in recent years to prove their content and possible uses: 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 uses (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).

As just mentioned, Opuntia ficus-indica contains a good quantity of polysaccharides. In literature, different extraction techniques are tested, with different results in terms of Yield of extraction (Y%). A summary of the main literature results is shown in Table 1.

Table 1: Opuntia polysaccharides extraction. The yield of extraction is based on fresh weight (fw) or dry weight (dw) of the initial sample

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)*	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* 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)*	Pectin	18.14 ± 1.41 dw	(Bayar et al., 2017)
Microwave Assisted Extraction (MAE)	Mucilage	25.60 dw	(Felkai-Haddache et al., 2016)

*After mucilage removal

Pectin is a water-soluble heteropolysaccharide found in the cell wall of plants, that has many functional and nutritive uses within the food and other industries. It is mainly used in the food industry as an additive (E440) thanks to its gelling, stabilizing, and thickening properties. The demand for pectin is equal to 30.000 tons/year but an increase in demand (4 – 5 % per year) is expected (Sevgi et al., 2022): the search for new sources of pectin is necessary.

In this work, the optimization of pectin extraction using ultrasounds through a design of experiments (DoE) is conducted. Ultrasounds generate cavitation bubbles that enhance the contact between solvent and plant materials, improving mass transfer and then pectin yield. The process variables are optimized by the Face Centered Central Composite Response Surface Design (FCCRD) with four variables at three levels: solid-liquid ratio (S/L) 10 – 40 g/mL, pH 1.5 – 2.5, time of extraction (t) 10 – 30 min, and temperature (T) 25 – 75 °C.

Briefly, 3 grams of dried and milled cladodes are mixed in acidified water. Temperature is adjusted using a water bath. Extraction is assisted by an ultrasonic probe and it takes place under continuous stirring. After the extraction phase, the samples are centrifuged at 4000 rpm for 15 minutes and then filtered using Whatman filter paper n. 1 to remove the solid fraction. Two volumes of ethanol 95% are added to the liquid phase to precipitate pectin and then the samples are left at 4°C overnight. Finally, the samples are centrifuged at 4500 rpm for 15 minutes to better precipitate pectin, the liquid is removed and pectin is left at 50°C overnight. The Yield of extraction is expressed as follows:

$$Y\% = \frac{P}{S} \cdot 100$$

Where P is the pectin weight (in grams) and S is the sample weight (in grams).

The extract is then characterized. The structural characteristics are investigated by the FTIR spectra, HPLC-RID is used to determine the monosaccharides composition, total phenolic content is determined using Folin-Ciocalteu method and antioxidant activity is determined using α, α-diphenyl-β-picrylhydrazyl (DPPH•) free radical scavenging method. The esterification degree (DE) is determined by the potentiometric titration method and rheological properties are evaluated using a viscosimeter.

After the extraction of the polysaccharides, the analysis of the solid residue of the opuntia is foreseen. It should consist predominantly of fiber that could be extracted for use alone or in combination with polysaccharides.

Although the progress of research, Opuntia remains a little cultivated plant, used mostly for fruit. Despite that, it represents a source of high-added value molecules of great commercial interest. Moreover, its interesting composition and its ability to grow in arid climates must lead the scientific community to investigate its uses.

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