Food Bioprocess Technol
DOI 10.1007/s11947-015-1640-7

ORIGINAL PAPER

Conventional and Microwave-Assisted Extraction of Mucilage from *Opuntia ficus-indica* Cladodes: Physico-Chemical and Rheological Properties

Lamia Felkai-Haddache[1] · Hocine Remini[1] · Virginie Dulong[2] · Kahina Mamou-Belhabib[1] · Luc Picton[2] · Khodir Madani[1] · Christophe Rihouey[2]

Received: 21 July 2015 / Accepted: 3 November 2015

Abstract Cactus pear cladodes processing has potential value for mainstream industries and is equally important for marginal rural communities in arid regions. This work is focused on physico-chemical and rheological properties of *Opuntia ficus-indica* (*OFI*) peeled cladodes extracted by conventional method "CE" and using microwave-assisted extraction "MAE." MAE gave the highest yield extraction (8.13 %, w/w) within the lowest extraction time (500 W/7 min) and provided more protein (×1.03) and carbohydrates (×1.51) than CE. The monosaccharides detected by gas chromatography were arabinose, galactose, rhamnose, xylose, and galacturonic acid. The dialyzed mucilage solution characterized by SEC/MALS/VD/DRI (size-exclusion chromatography coupled with online multi-angle light scattering, viscometer detectors, and differential refractive index) revealed fractions with molecular weight (M_w) ranging from 15.3–15.7×10^6 g mol^{-1} for the CE extracts and about 16.7–17.5×10^6 g mol^{-1} for the MAE extracts. Dynamic oscillatory testing has been used to study the rheological properties of mucilage solution within the concentration of 0.50–3.00 % (w/v) at 25 °C. The rheological profiles of CE and MAE showed similar behavior. At low frequency and low mucilage concentration (<2.00 %), the viscous component (G") predominated over the elastic component (G'), while for higher frequencies, the behavior is reversed (G'>G"). If safely controlled, alternative energies like microwaves could extract soluble polymers with comparable properties to commercial ones, conventionally extracted.

Keywords *Opuntia ficus-indica* · SEC/MALS/VD/DRI · Rheological properties · Mucilage · Conventional extraction · Microwave-assisted extraction

Introduction

Among the many plants of the Algerian flora, the cactus racket (*Opuntia ficus-indica* "*OFI*") is one that stands out by the unique feature of all its form and its abundance in the more arid lands (de Rosny 1857). The plant of the genus *Opuntia*, even though native to North America, is widespread all around the world (McGarvie and Parolis 1979). Wild cactus pear covers an estimated area of 3 million hectares (ha) (Vishwakarma et al. 2014). The Algerian cactus plantation is about 28,000 ha in 2015. Studies have found that one hectare of *Opuntia* above the age of five is able to produce up to 100 t of fresh cladodes each year in areas with little rainfall (150 mm or less) (Varnero and de Cortázar 2006). Several species are designated as harmful in many countries (McGarvie and Parolis 1979). Although, the plant is used mainly for fruit production, some countries used it also as a vegetable (nopalitos) for human consumption or for forage (Ribeiro et al. 2010; Sáenz et al. 2004). Cladodes are highly used in industrial food (Ammar et al. 2015). The main cactus pear products produced by food industries are tender leaves prepared in brine or pickled in vinegar, sauces, and other foods incorporating tender leaves, including jams, candies, drinks, and flour. Leaves kept in brine or vinegar are the most popular product and have been in production since the 1970s (Garcia and Saenz 2006).

✉ Hocine Remini
hocine.remini@univ-bejaia.dz

[1] Laboratoire de Biomathématiques, Biophysique, Biochimie, et Scientométrie (L3BS), Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algeria

[2] Normandie Université, Polymers, Biopolymers Surfaces Laboratory, UMR 6270 CNRS19, UFR des Sciences et Techniques, Université de Rouen, Mont Saint Aignan Cedex 76821,20, France

Published online: 14 November 2015

Scientists and food technologists are always seeking alternative sources of soluble fibers (gum, mucilage, pectin, hemicellulose, or polysaccharides), with new improved functional properties, associated with reduced glucose and cholesterol levels, and stabilization of gastric emptying (Sáenz et al. 2004). For that, polysaccharides from plant extracts are an interesting source of additives for several industries, in particular food and drug industry. Many of these polysaccharides, like those from *Opunita ficus-indica* (*OFI*) (a member of the cactaceae family), have been empirically used to adjust and improve the rheological properties of some products (Trombetta et al. 2006; Medina-Torres et al. 2000). Polysaccharides became common in use in food technology, not only for their thickness and/or gelling properties, but also as capturing agents that hold moisture and inhibit its evaporation from foodstuffs, as stabilizing foams and emulsions (Krystyjan et al. 2012), coating agents in confectionery and fried foods, adhesives in bakery glazes, clarifying agents in beer and wine, clouding agents in juices, flocculating agents in wine, encapsulating agents in powdered fixed flavors or some oils, and also as fat substitutes in meat and dairy products (Li and Nie 2015). Recently, in the treatment of turbid water, *Opunita ficus-indica* (*OFI*) cladodes juice as coagulant aid has potential to enhance turbidity removal efficiency (Adjeroud et al. 2015).

Composition studies on the mucilage of *Opuntia ficus-indica* cells (*OFI*) revealed the presence of mucilage, a polysaccharide containing a molecular structure up to 30,000 monosaccharides (McGarvie and Parolis 1981), as a high molecular weight polymers (HMWP) and low molecular weight polymers (LMWP) which acts as a polyelectrolyte (Majdoub et al. 2001; Medina-Torres et al. 2000). In the same way, the *Opunita ficus-indica* (*OFI*) cladodes are characterized by their complex polymeric substance of carbohydrate nature, with a molecular weight ranging from 2.3×10^4 to 300.0×10^4 g mol^{-1} (León-Martínez et al. 2010), that have a highly branched structure, mainly consisting of rhamnose, galactose, and enough galacturonic acid content (Cai et al. 2008; Majdoub et al. 2001; Matsuhiro et al. 2006). The first important step to recover polysaccharides from *Opuntia ficus-indica* (*OFI*) cladodes is "extraction;" it involves separation of these phytochemicals from the cellular matrix of *OFI*. Various researches have been devoted to mucilage extraction and purification methods from *Opuntia ficus-indica* (*OFI*) cladodes, while most of them homogenize the cactus cladodes with water; however, this method is effective but may be long and may prove to be costly (León-Martínez et al. 2010; Cai et al. 2008; Medina-Torres et al. 2000). Nowadays, an eco-friendly method like microwave-assisted extraction "MAE" is widely used to extract polysaccharides from various kinds of plant materials due to its enhanced extraction efficiency, reducing both extraction time and solvent consumption, compared to other conventional methods (Amin et al. 1970; Thirugnanasambandham et al. 2015).

So, the aim of this work is to compare for the first time mucilage extracts from *Opuntia ficus-indica* (*OFI*) cladodes, obtained by conventional "CE" and microwave-assisted extraction "MAE" based on: (i) the determination of the extraction yield; (ii) physico-chemical characterization using several techniques such as gas chromatography for sugar composition, viscosity in dilute solution, multi-angle laser light scattering (MALS), and steric exclusion chromatography; and (iii) characterization of rheological and functional properties.

Materiel and Methods

Plant Material

OFI cladode samples were harvested from Aokas (Bejaia) located in North East of Algeria during the period of March–April 2014. The cladodes were studied without thorns and epidermis; in this case, approximately 42 % of the epidermis in both sides was removed. The average physical dimensions of the selected cladodes were 420±3-mm-long, 256±25-mm-wide, and 25±5-mm-thick; the initial mass was 1250 ±42 g for the intact cladodes and 725±10 g when epidermis was cut. Cladodes were rinsed with distilled water (0.5 μS/cm) and disinfected with ethanol 70 % (v/v). After epidermis removal, cladodes were mixed in a domestic mill (Moulinex MASTERCHEF 370, Alençon, France). The obtained homogenates were stocked at 4 °C for further uses.

Mucilage Isolation

Conventional Extraction

Conventional extraction (CE) was carried out at temperatures of 20 and 80 °C during 60, 120, 180, and 240 min. The extraction consists of maceration of the mixed cladodes in 500-mL volumetric flask with three volumes of distilled water (0.5 μS/cm) under agitation (500 rpm).

Microwave-Assisted Extraction

Microwave-assisted extraction (MAE) from cladodes were performed in domestic microwave oven system (2.45 GHz, Samsung Model NN-S674MF, Kuala Lumpur, Malaysia). The apparatus was equipped with a digital control system for irradiation time and microwave power (the latter was linearly adjustable from 100 to 1000 W). The oven was modified in order to condense the vapors generated during extraction into the sample.

The MAE procedure was performed in a 500-mL volumetric flask with three volumes of distilled water (0.5 μS/cm) at different powers (500, 700, and 900 W) during 7 min with 1-min steps.

Mucilage Precipitation

The extracted mucilage was immediately cooled in ice bath (4 °C) and was filtered, through a double layer cheesecloth to remove pulp, before centrifugation at 4000×g during 15 min at 4 °C (Sigma 2–16 PK model, Osterode am Harz,Germany). The filtrate was precipitated in three volumes of ethanol (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 95 % (v/v) at 4 °C overnight. The precipitate was washed three times with ethanol 75 % (v/v) then desolvated by lyophilization at −55 °C for 12 h (MartinChrist, Freeze Dryer, Alpha 1–2/LD, Gefriertrockungsanlagen GmbH, Osterode am Harz, Germany) as reported by Matsuhiro et al. (2006).

Calculation of Extraction Mucilage Yield

Extraction yield was the ratio of the dry mass lyophilized extract (DMLE), on the dry weight of the dehydrated peeled cladodes (DWDPC) dried at 70±1 °C in a vacuum oven (Memmert, Model VO 200, GmbH, Schwabach, Germany) as outlined by Sepúlveda et al. (2007). All extraction yields were expressed as the means of at least three replications. The analysis of variance (ANOVA) was performed using XLSTAT Release 10 (Addinsoft, Paris, France). Tukey's multiple range test (HSD) was used to compare means of the calculated extraction yields. Evaluations were based on the $p<0.05$ significance level. In fact, only the highest extraction yields from "CE" and "MAE" were considered.

Water Soluble Extract Solution Preparation

To obtain final concentration of mucilage (1 g L^{-1}), a mass of dried water soluble extracts were dissolved in required distilled water volumes and stirred at 500 rpm for 24 h prior to their use for various dosage experiments.

A known volume of this solution was dia-filtred with KrosFlo® II Research tangential flow filtration (TFF) system (Spectrum Laboratories, Rancho Dominguez, Canada), using a cut-off hollow fiber column of 10 kDa. This is an alternative eco-technique for the purification, concentration, and size-selection of polydisperse nanoparticle suspensions or colloids (Dorney et al. 2014). The dialyzed solution was lyophilized; the lyophilizated mucilage was reconstituted in required distilled water volume (for a final concentration of 1 g L^{-1}) and stirred at 500 rpm overnight for further analysis.

Chemical Composition of Water Soluble Extracts

Protein Content Determination

Protein contents were determined with a micro-bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, Illinois, USA) and bovine serum albumin (Bayer, Puteaux, France) as a standard. One milliliter of working reagent was added to 1 mL of assay solution and incubated during 1 h at 60 °C. The mixture was immediately cooled, and the absorbance was read at 562 nm (PerkinElmer Lambda 7UV/vis spectrophotometer, Illinois, USA).

Neutral Sugar Determination

Total neutral sugar (NS) content was determined by using phenol sulfuric acid, as described by DuBois et al. (1956). Under the concentrated sulfuric acid action, polysaccharides are hydrolyzed to neutral monosaccharides (hexoses and pentoses) and uronic acids. The released neutral monosaccharides are converted into derivatives furfurals which condense with the phenol to give a yellow coloring. The intensity of the color depends on the concentration and nature of the monosaccharide, having maximum absorption at 490 nm. NS content was expressed as microgram equivalent to xylose per milligram of extracted soluble polymer.

Uronic Acid Content Determination

Uronic acid (UA) contents were determined according to the method of Blumenkrantz and Asboe-Hansen (1973). Under the concentrated sulfuric acid action, polysaccharides are hydrolyzed in neutral oses and uronic acids. The acid monomers released are transformed into derivatives *furfuriques* forming a pink complex with meta-hydroxydiphenyl (m-HDP) solution (0.15 % m-HDP in 0.50 % NaOH), having maximum absorption at 520 nm. UA content was expressed as microgram equivalent to galacturonic acid per milligram of extracted soluble polymer.

Monosaccharide Composition

Extracted soluble polymers were submitted to acidic hydrolysis. A volume of 25 μL of 4-mM (w/v) inositol aqueous solution (internal standard) and 182 μL of concentrated trifluoroacetic acid for 2-M final concentration were added to 1 mL of assay solution. The mixture was incubated at 4 °C overnight then 2 h at 110 °C. Samples were then freeze-dried.

Monosaccharide composition (MC) was determined as described by Ray et al. (2004). The liberated monosaccharides were converted to their methoxy sugars by incubation at 80 °C overnight with 200 μL of 1 M methanolic-

HCl. After evaporation of the methanol, the resulting methyl glycosides were dried and converted into their trimethylsilyl derivatives with 200 μL of a commercial derivatization kit (HMDS, TMDS, pyridine (1:1:10, v:v:v)) at 80 °C for 30 min, reagent excess was evaporated, and derivatives were dissolved in 1 mL of cyclohexane. One microliter of the resulting solution was analyzed by gas chromatography (Varian GC 3800, Walnut Creek, CA, USA) equipped with a flame ionization detector (FID), a WCOT fused-silica capillary column (L 25 m, i.d 0.25 mm, film thickness 0.25 μm) with (CP-Sil 5 CB, Santa Clara, California, USA) as stationary phase and helium as gas vector (constant pressure 20 psi). The oven temperature program was: 2 min at 120 °C, 10 °C min^{-1} to 160 °C, and 1.5 °C min^{-1} to 220 °C and then 20 °C min^{-1} to 280 °C. The quantification of monosaccharide content (MC) was done by peaks integration (Varian GC Star Workstation software, Walnut Creek, CA, USA), and determination of the corresponding molar values using response factors was established with standard monosaccharides, i.e., arabinose (Ara), fucose (Fuc), galactose (Gal), glucose (Glc), mannose (Man), rhamnose (Rha), xylose (Xyl) as well as the internal standard inositol (Ino). Galacturonic acid (GalA) that was also identified by its retention times even was not quantified by this method. All solvents used for gas chromatography analysis were analytical grade.

SEC/MALS/VD/DRI Analysis

The light scattered by a polymeric molecule in solution is proportional to its molecular weight and its concentration. Scattered light intensity variation as a function of the measuring angle is directly related to the molecule's size. The simultaneous determination of the concentration (C) and light scattering (I (θ)) to several angles was used to calculate the molecules molar mass (M) eluted with the light scattering law in conditions approximation of Rayleigh-Gans-Debye (Eq. (1)),

$$I(\theta) = f \times \left[M \times C \times \left(\frac{dn}{dC} \right) \right] \quad (1)$$

Where f is the optical constant and $\frac{dn}{dC}$ is the refractive index increment of the solution. Size-exclusion chromatography (SEC) coupled to multi-angle light scattering (MALS) was undergone in order to determine the molar number, average molar masses, and the intrinsic viscosity. The SEC/MALS/VD/DRI was used; it is size-exclusion chromatography (SEC) coupled online with multi-angle light scattering (MALS), differential refractive index (DRI), and viscometer detectors (VD).

The MALS detector is a DAWN Heleos-II (Wyatt Technology Inc, USA) fitted with a K5 cell of 50 μL and 18 photodiodes (normalized relative to the 90° detector using bovine serum albumin), the viscometer is a ViscoStar (Wyatt Technology. Inc, USA), and the DRI detector is a RID-10A (Shimadzu, Japan). The columns were OHPAK SB 804 HQ and OHPAK SB 806 HQ columns, from Shodex (USA). The eluted solvent (LiNO3 0.1 M) was filtered through 0.1-μm filter unit (Millipore, USA) and online degassed (Shimadzu DGU-20A3R, Japan); the flow is fixed at 0.5 mL min^{-1}. The solutions were prepared from dried dialyzed mucilage at 0.5 g L^{-1} in the eluent, filtered on 0.45-μm filter unit (Interchim, France), and 100 μL were injected in the line (SIL-20A auto sampler, LC10Ai pump, Shimadzu, Japan). $\frac{dn}{dC}$ of 0.14 L g^{-1} has been used (Majdoub et al. 2001).

The collected data were analyzed using Astra 6 software package from Wyatt Technology Inc. The R_h was determined according to the measurement of the intrinsic viscosity using the Einstein-Simha equation (Eq. 2)

$$V_h = \frac{[\eta] \cdot M}{\nu \cdot N_A} \quad (2)$$

where V_h is the hydrodynamic volume ($V_h = {R_h}^3 \ \pi.4/3$), N_A is Avogadro's number, M is the molar mass, $[\eta]$ is the intrinsic viscosity (g mL^{-1}), and ν is a conformational parameter that takes the value of 2.5 in the case of a spherical conformation, according to the expected random coil conformation of the polysaccharides in aqueous solutions.

Rheological Properties

Rheological measurements of mucilage were performed in sodium chloride aqueous solution (0.1 M with a pinch of sodium azide) within the 0.25–3.00 % (w/v) range of raw mucilage concentration, with an AR 2000 Rheometer from TA Instrument (U.K.), using standard-size double concentric cylinder geometry. Linearity domains of viscoelastic properties were checked at 1 Hz between 0.01 and 10 Pa. G' and G" were measured, the storage or elastic modulus (G') is a measure of the energy stored and recovered per cycle and the loss or viscous modulus (G") is a measure of the energy dissipated or lost as heat per cycle. Oscillation procedures for frequency sweep were performed between 0.01 and 10 Hz at a fixed stress depending on the *OFI* mucilage concentration (0.005 Pa for the 0.25 % (w/v) mucilage solution and 1 Pa for the 3.00 % (w/v) mucilage solution). The analyses of the results were performed with Rheology Advantage for Instrument Control AR V3.0.0 software. The study was performed for all samples, but only CE 80 °C/120 min and MAE 500 W/7 min are shown.

All extractions and characterization procedures are demonstrated in Fig. 1.

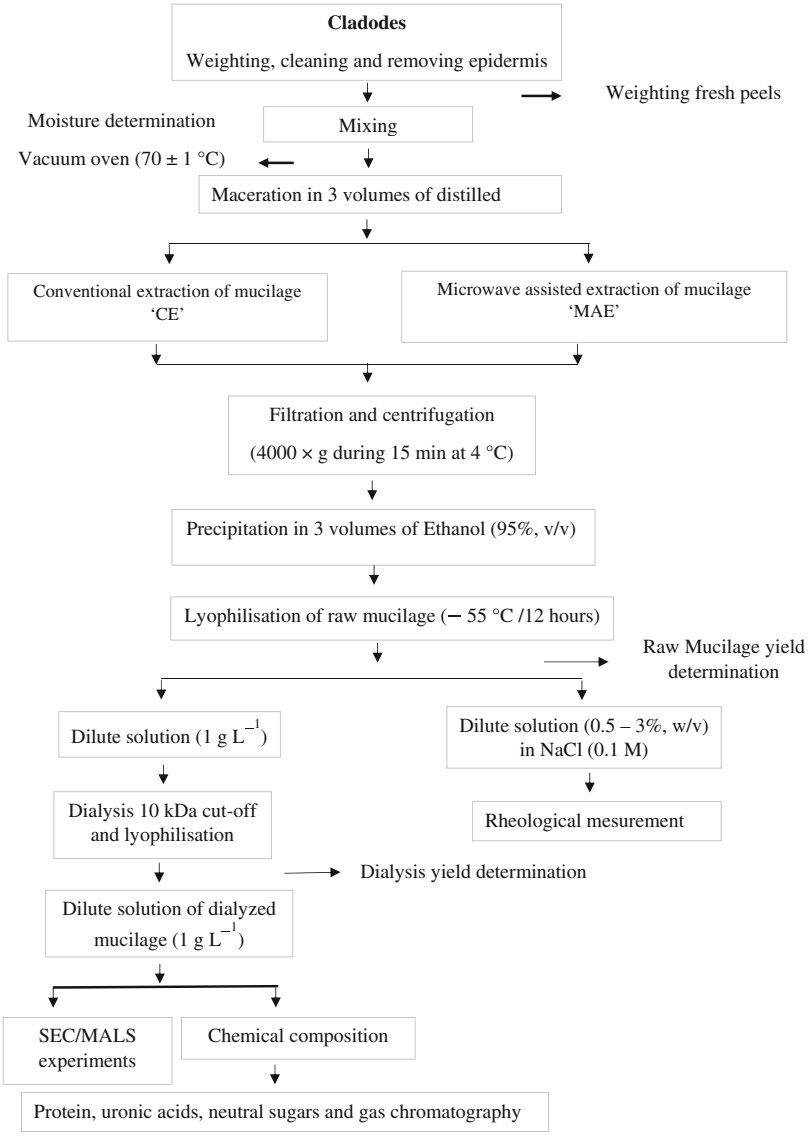

Fig. 1 Followed extractions and characterization procedures of mucilage from *Opuntia ficus-indica (OFI)* peeled cladodes

Results and Discussion

Extraction Yield

Table 1 depicts the extraction yield of raw and dialyzed mucilage from conventional extraction "CE" and microwave-assisted extraction "MAE" at different extraction times. For conventional extraction, the maximum extraction yields of 6.30 and 6.91 % were obtained for 20 °C/180 min and 80 °C/120 min, respectively. However, beyond 120 min at 80 °C, the extraction yield was reduced significantly. This is likely due to the elevation of the temperature liable to a decrease in solvent viscosity, which improves the diffusion of the solute in the vegetal matrix and increases the polysaccharides solubility in the solution (Zheng et al. 2011; Wu et al. 2013). Yang et al. (2015) have obtained the same trends of the polysaccharide yield extraction from Chinese yam. Furthermore, they explain that excessive lengthening extraction time will induce polysaccharides gelatinization, which was not good for separation. For microwave-assisted extraction "MAE" (Table 1), the maximum extraction yields of 8.03, 8.81 and 8.95 % for 500 W/7 min, 700 W/5 min and 900 W/3 min, respectively. However, above 5 min at 700 W and 3 min at 900 W, the extraction yield was reduced significantly. Furthermore, over than 6 min at 500 W, the extraction yield remains constant. The performance of "MAE" depends on its operational mode of heating and provides pulsed microwave heating at certain power which is efficient in extracting thermal-labile compounds (Tumolo et al. 2004). The increasing microwave irradiation power

Table 1 Raw and dialyzed mucilage extraction yield from conventional "CE" and microwave-assisted extraction "MAE" at different extraction time

Extraction method	Extraction time (min)	Raw mucilage extraction yield (% DMLE/DWDC)	Dialysis yield[a] (%)
Conventional (°C)			
20	60	4.23±0.24a	4.22
	120	5.47±0.17b	
	180	6.30±0.15c	
	240	6.09±0.17c	
80	60	5.22±0.3ab	4.63
	120	6.91±0.3c	
	180	6.01±0.3b	
	240	4.87±0.3a	
Microwave (W)			
500	1	5.00±0.33a	5.38
	2	5.73±0.3ab	
	3	6.43±0.44bc	
	4	6.78±0.70cd	
	5	7.18±0.33e	
	6	7.84±0.23ef	
	7	8.03±0.25ef	
700	1	5.49±0.28a	7.05
	2	6.83±0.22bc	
	3	8.09±0.22cd	
	4	8.42±0.47de	
	5	8.81±0.22e	
	6	6.68±0.5b	
	7	5.57±0.55a	
900	1	7.46±0.23bc	7.60
	2	7.83±0.22c	
	3	8.95±0.34d	
	4	6.76±0.12b	
	5	4.81±0.22a	

Values with different letters (a, b, c, d, e, and f) were significantly different (Tukey, $p<0.05$) for each types of treatment

DMLE dry mass lyophilized extract, *DWDPC* dry weight of the dehydrated peeled cladodes

[a] Dialysis yield of raw mucilage extraction yield maxima (*g* of dialyzed and lyophilized DMLE/100 g of dried peeled cladodes)

enhances the penetration of solvent into the plant matrix, which allows the dissolution of components to be extracted (Yan et al. 2010). After obtaining higher extraction yield related to the time-power exposure, previous works have shown that the excessive time-power exposure under the microwave fields leads to degrade the polysaccharide molecules (Zheng et al. 2011).

The dialysis yields at extraction yield maxima ranged from 5.38 to 7.60 % for "MAE" and 4.22 to 4.63 % for "CE," relative to 100 g of dried cladodes. Considering raw mucilage extraction yield and/or dialysis yield, the results depict clearly that "MAE" extracts more efficiently polysaccharide molecules than "CE" and should be preferred based on the lower extraction time and extractability quality.

Chemical Composition of Dialyzed Extracted Soluble Polymer

Protein, neutral sugars, and uronic acid contents were measured through spectrophotometric method; the corresponding results are given in Table 2. No significant differences in proteins composition for the "CE" were observed whatever the temperature, whereas it rises significantly in "MAE," it ranged from 162 to 169 μg/mg of extract. Microwave thermal effects naturally match the requirements for the disruption process of tissues and, consequently, could be used to induce rupture of the cells for efficient extraction of useful components in plant tissues. Read and Gregory (1997) reported that "MAE" treatment resulted in significantly higher

Table 2 Proteins, uronic acids, and neutral sugars of dialyzed soluble mucilage of *OFI* peeled cladodes

Extraction method	Extraction time (min)	Protein µg/mg of extract	UA	NS
Conventional (°C)				
20	180	155.00±0.96a	45.0±0.6c	494±9a
80	120	156.00±1.01a	51.0±0.5e	558±5ab
Microwave (W)				
500	7	162.00±1.48b	48.0±0.1d	746±5b
700	5	164.00±0.57c	44.0±0.8b	744±8b
900	3	169.00±0.99d	39.0±0.3a	752±3b

Values with different letters (a, b, c, d, and e) were significantly different (Tukey, $p<0.05$) for each test

UA uronic acids, *NS* neutral sugars

concentrations of soluble protein. In the same way, carbohydrate content (neutral sugar+uronic acid) was higher in CE 80 °C/120 min than that in CE 20 °C/180 min; for "MAE," this finding was conducted by thermal effect (Yoshida et al. 2010). The MAE yielded the highest content of neutral sugar whatever the applied microwave power than CE. However, the variability of the obtained uronic acid amounts can be explained by its easy property to be decarboxylated by raising heat temperature (Lefèvre and Tollens 1907). Table 3 summarizes the experimental results on mucilage sugar composition. As it can be shown, the main sugar components of mucilage are arabinose, galactose, xylose, rhamnose, and galacturonic acid. These results are in agreement with those obtained for *OFI* mucilage sugar composition by (Medina-Torres et al. 2000; Majdoub et al. 2001). Table 3 shows no noteworthy differences in the monosaccharide composition between "MAE" and "CE," indicating that the solubilization rate was largely affected by the carbohydrates solubilization rather than thermal effect (Yoshida et al. 2010). However, rhamnose decreased significantly in all samples extracted with "MAE," while galacturonic acid increased significantly (Table 3). The decrease in rhamnose content in the solubilized fraction under microwaves effect was ascribed to their secondary degradation (Yoshida et al. 2010), and the increase of galacturonic acid content can be explained by the oxidation of reducing sugars to uronic acids at high temperature under the microwave effect.

Molecular Mass Analysis of Mucilage Solution (Above 10 kDa)

Multi-angle light scattering coupled to SEC can provide an absolute means for measuring the molar mass, size, and distribution of the polymer. Figure 2a–c shows the evolution of the average molecular weight (M_w), light scattering (LS), intrinsic viscosity [η], and differential refractive index (DRI) as function of elution volume for "CE" (20 °C/180 min and 80 °C/120 min) and "MAE" (500 W/7 min), respectively. The solid line shows light scattering (LS) response, which is the function of polymer size (M_w) and concentration, the dashed line shows the concentration signal (DRI), whereas the dotted line and scatter curve represent the obtained mass

Table 3 Monosaccharide composition of dialyzed soluble mucilage of *OFI* peeled cladodes

Extraction method	Extraction time (min)	Ara[a]	Gal[a]	Xyl[a]	Rha[a]	GalA[b]
Conventional (°C)						
20	180	41.0±0.6c	31.0±1.1b	19.0±0.6b	7.0±0.6d	5.0±1.4a
80	120	39.0±2.0bc	31.0±0.4b	20.0±0.8c	7.0±0.4d	6.0±0.4b
Microwave (W)						
500	7	36.0±0.6a	32.0±0.8c	18.0±1.0a	6.0±1.2c	10.0±0.9e
700	5	38.0±0.4b	30.0±0.4a	20.0±1.3c	5.0±0.5b	9.0±0.6d
900	3	42.0±1.6d	30.0±1.3a	21.0±0.8d	4.0±1.7a	7.0±1.0c

Values with different letters (a, b, c, d, and e) were significantly different (Tukey, $p<0.05$) for each test

[a] Molar ratio of monosaccharides detected by GC

[b] Determined by the colorimetric method of (Blumenkrantz and Asboe-Hansen, 1973)

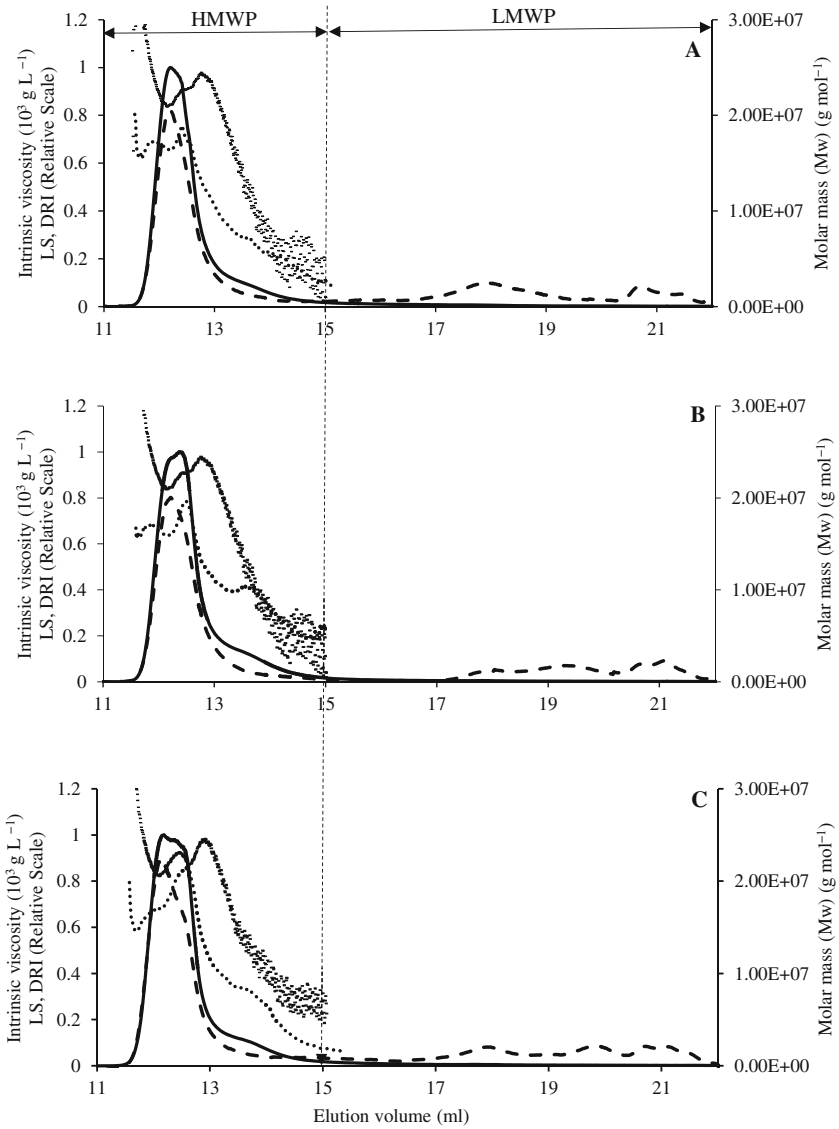

Fig. 2 Size-exclusion chromatogram: light scattering at 90° detection (*solid line*), differential refractive index detection (*dashed line*), intrinsic viscosity [η] (*scatter line*), and calculated molar mass distributions (*dotted line*) of CE 20 °C/180 min (**a**), CE 80 °C/120 min (**b**), and MAE 500 W/7 min (**c**). *HMWP* high molecular weight polymers, *LMWP* low molecular weight polymers

distribution (M_w) and intrinsic viscosity [η], respectively. The determination of molar mass (M_w) and sizes (Rh_w) was performed using Zimm fit method (Tumolo et al. 2004). The corresponding parameters were shown in Table 3.

Two distinct polymer populations appear for all samples (Fig. 2) due to a DRI chromatogram broad distribution. The first was eluted from 11.55 to 15.00 mL, representing the majority of polymers with high molecular weight polymers

Table 4 Average molar mass, intrinsic viscosity, and size calculated for high weight molecular polymers (HWMP) from *OFI* peeled cladodes eluted between 11.5 to 15 mL

Extraction method	Extraction time (min)	Mass recovery (%)	M_w 10^6 (g mol^{-1})	Rh_w (nm)	Intrinsic viscosity (mL g^{-1})
Conventional (°C)					
20	180	69	15.3±0.6a	126±2a	855±8ab
80	120	75	15.7±0.7a	132±2b	843±7a
Microwave (W)					
500	7	71	16.7±0.4b	130±1ab	862±7b
700	5	80	17.2±0.6c	132±2b	861±7b
900	3	83	17.5±0.6c	133±2b	868±7c

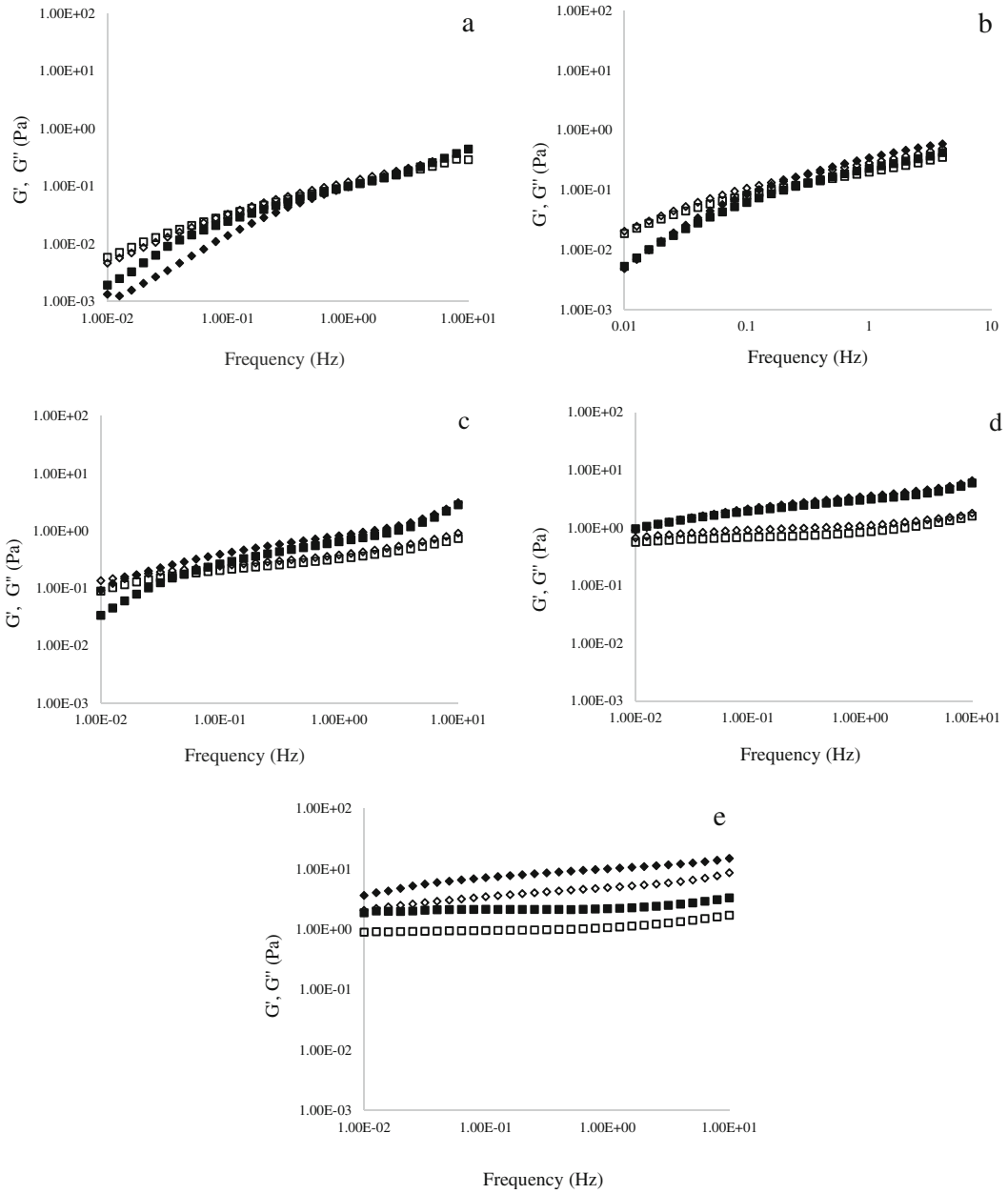

Fig. 3 Mechanical spectra as function of frequency from $CE_{80\ °C}$ and $MAE_{500\ W}$ of *OFI* mucilage. 0.50 (**a**), 0.75 (**b**), 1.00 (**c**), 2.00 (**d**), and 3.00 % (w/v) (**e**) mucilage concentration. *G′* elastic moduli, *G″* viscous moduli. (♦) G' $CE_{80\ °C}$, (◊) G" $CE_{80\ °C}$, (■) G' $MAE_{500\ W}$, and (□) G" $MAE_{500\ W}$

(HWMP), for which one can access to the molar masses, size, and intrinsic viscosities regarding to the sufficient light scattering and viscometric signal (Fig. 2). This first fraction represents an average of 69 and 75 % for CE 20 °C/180 min and CE 80 °C/120 min, respectively, and about 71, 80, and 83 % for MAE 500 W/7 min, 700 W/5 min and 900 W/3 min, respectively. The second populations from 15 to 22 mL, corresponds to low weight molecular polymers (LWMP) fractions, as represented by the low deferential refractive index (DRI) signal and for which, light scattering becomes too low to be measured at these concentrations. The molecular weight (M_w) ranged from 15.3–15.7×10^6 g mol^{-1} for the "CE" extracts and about 16.7–17.5×10^6 g mol^{-1} for the "MAE" extracts. The M_w differences of the two processes may be explained by the enzymatic and/or bacterial contamination from "CE" extracts as well as to high exposure time/microwave power in "MAE." The huge variability of M_w in "MAE" compared to that of "CE" process is related to thermal runaway as a consequence of microwave exposure. According to Ridley et al. (2001), inactivation of the pectolytic activity by "MAE" treatment leads to increase polysaccharides extractability from *OFI* cladodes, and the extracted soluble polymers

obtained after a microwave exposure have a higher molecular mass (M_w).

Conventional extraction "CE" shows significant differences between intrinsic viscosity values of 20 °C/180 °C and 80 °C/120 min (Table 4), which corroborate with the former finding of molecular weight parameter. For "MAE" (Table 4), narrow range of intrinsic viscosity values was obtained. Table 4 shows that the hydrodynamic radius (Rh_w) increases in harmony with the molecular weight. The Rh_w values obtained from CE 80 °C/120 min and "MAE" are close. According to Frank and Belfort (2003), the calculated values for the hydrodynamic radius of the polymer assume that the molecule is spherical since the actual three-dimensional structure is unknown.

Rheological Properties

Several authors found that mucilage has a viscoelastic behavior described by storage or elastic (G') and loss or viscous (G") modulus (Read et al. 1999; Read and Gregory 1997). G' is defined as the stress in phase with the strain, divided by the strain; G" is defined as the stress 90° out of phase with the strain, divided by the strain (Read et al. 1999). Certain flow experiments have been conducted showing that the high level of organization in some solutions, having high molar masses of polymers, leads to complex fluids with classical yield stress behavior (O'Shea and Tallon 2011). These behaviors are difficult to characterize in the flow mode and traduce the viscoelasticity of the solutions. Consequently, the best way is to use the dynamic mode (oscillatory mode) after checking the linearity domain.

Figure 3 shows the mechanical spectra describing the viscoelastic behavior of two mucilage solutions from CE 80 °C/120 min and MAE 500 W/7 min (selected from the raw and dialyzed mucilage extraction yield, Table 1) at concentrations 0.50, 0.75, 1.00, 2.00, and 3.00 % (w/v) under low-amplitude oscillatory deformation tests. As depicted in Fig. 3a–c, at low frequency and low mucilage concentrations (0.50–1.00 %, w/v), mucilage solutions show mainly viscous properties. While, the predominant response of the polymer to the imposed deformation is dissipative viscous flow of energy (G">G') (Read and Gregory 1997).

Both samples (CE 80 °C and MAE 500 W, Table 5) show a crossover point corresponding to the characteristic frequency value (ω_c) which is related to the inverse of the characteristic time (t_c), reliable to the dynamic of the entangled polymer solution, which means the chain relaxation time (G"=G'). Viscoelastic properties of mucilage in different concentrations were depicted in Table 5, which shows that the CE 80 °C and MAE 500 W mucilage solutions can clump a gel structure in the concentrations of 0.50, 0.75, and 1.00 % (w/v) as their elastic modulus G' were much greater than the corresponding viscous modulus G" at frequencies from 0.1 to 10 Hz.

After the crossover point, the tendency is reversed, a predominance of elastic moduli (G"<G') was observed. The mechanical response observed is characteristic of an entangled network of polymer coils. Notice that at low frequency, there is sufficient time for substantial chain disentanglement and rearrangement within the timescale of the oscillation period. At higher frequency, as the oscillation period of the applied stress decreases, the time needed for chain rearrangements exceeds the timescale of the stress rate; hence, elastic deformation of the entangled network becomes progressively more significant. At such high frequency (ω), the system behaves as an elastic solid (G'>G") (Read and Gregory 1997).

Table 5 Rheological experiments data of raw mucilage extraction yield maxima at different concentrations from *OFI* peeled cladodes mucilage solution

Extraction method	Extraction time (min)	Mucilage Conc. (%, w/v)	ω_c (s^{-1})	t_c (s)	G'$_c$ (Pa)
Conventional (°C)					
80	120	0.50	4.00	0.25	0.22
		0.75	0.25	4.00	0.16
		1.00	0.02	50.00	0.17
		2.00	<0.01	>100	–
		3.00	<0.01	>100	–
Microwave (W)					
500	7	0.50	2.00	0.50	0.14
		0.75	0.30	3.00	0.13
		1.00	0.05	20.00	0.18
		2.00	<0.01	>100	–
		3.00	<0.01	>100	–

ω_c is the relaxation frequency at cross point (when G'=G")

t_c is the relaxation time at cross point (when G'=G")

G'$_c$ is the elastic moduli at cross point (when G'=G")

As shown in Fig. 3a–c, at low concentrations (0.50–1.00 %, w/v), the higher the concentration is, the greater were the values of modulus (G' and G"). Consequently, the dynamic of the system is logically slowed when the polysaccharides concentration increases as shown in Table 4 (0.25–50.00 s at 0.50–1.00 %, w/v, for CE 80 °C and 0.50–20.00 s at 0.50–1.00 %, w/v, for MAE 500 W). Similar behaviors were found for *Opuntia ficus-indica* (León-Martínez et al. 2011; Medina-Torres et al. 2000) and *Aloe vera* mucilages (Cervantes-Martínez et al. 2014).

For higher concentrations (2.00–3.00 %, w/v), the behavior appears clearly elastic with G' always higher than G" (Fig. 3d, e). G' tends to reach a plateau which is characteristic of strongly organized structures of the solution (sometimes called pseudo-gels). Logically, it is observed that the higher the polysaccharide concentration is, the higher is the value of G' plateau. For such concentrations, the polysaccharides chains cannot move in the studied time of the experiment, and t_c is higher than 100 s (Table 5).

The rheological profiles of MAE 500 W/7 min sample for polysaccharide concentrations from 0.50 to 3.00 % (w/v) (Fig. 3) appear very similar to those obtained for CE 80 °C/120 min sample. The other samples (CE 20 °C/180 min, MWE 700 W/5 min, and MWE 900 W/3 min) also behave this way with pseudo-gels above 2.00 % in all the cases (data not shown).

Conclusion

The efficiency of microwave-assisted extraction "MAE" compared to conventional extraction "CE" of mucilage from *Opuntia ficus-indica* (*OFI*) cladodes shows clearly that:

- "MAE" extracts more efficiently polysaccharides molecules than "CE" and should be preferred based on the lower extraction time and extractability quality;
- "MAE" allows to release more protein and carbohydrates compared to the "CE;"
- "MAE" extracts compounds with higher molecular weight as revealed by SEC/MALS/VD/DRI; and
- Rheological properties were reported to depend on concentration and frequency. The rheological profiles of "CE" and "MAE" showed similar behavior.

If safely controlled, alternative energies like microwaves could extract soluble polymers with comparable properties to commercial ones, conventionally extracted. The analysis of variable process and functional properties led to provide precise and relevant information in the identification of potential industrial applications of theses polysaccharides.

For industrial exploitation, it is interesting to note that for a ton of peeled racket, "MAE" allows to extract about 90 kg of dehydrated mucilage for 900 W/3 min, against an average of 70 kg for 80 °C/120 min, with an energetic consumption of 162 and 810 kJ, respectively.

Acknowledgments The authors gratefully acknowledge the Algerian Ministry of Higher Education and Scientific Research for funding the study. We thank also Professor Patrice Lerouge, EA 6218 GlycoMEV Laboratory of University of Rouen for free access to gas chromatography facilities.

References

Adjeroud, N., Dahmoune, F., Merzouk, B., Leclerc, J.-P., & Madani, K. (2015). Improvement of electrocoagulation–electroflotation treatment of effluent by addition of *Opuntia ficus indica* pad juice. *Separation and Purification Technology, 144*, 168–176.

Amin, E. S., Awad, O. M., & El-Sayed, M. M. (1970). The mucilage of *Opuntia ficus-indica* mill. *Carbohydrate Research, 15*(1), 159–161.

Ammar, I., Ennouri, M., & Attia, H. (2015). Phenolic content and antioxidant activity of cactus (*Opuntia ficus-indica* L.) flowers are modified according to the extraction method. *Industrial Crops and Products, 64*, 97–104.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry, 54*(2), 484–489.

Cai, W., Gu, X., & Tang, J. (2008). Extraction, purification, and characterization of the polysaccharides from *Opuntia milpa alta. Carbohydrate Polymers, 71*(3), 403–410.

Cervantes-Martínez, C. V., Medina-Torres, L., González-Laredo, R. F., Calderas, F., Sánchez-Olivares, G., Herrera-Valencia, E. E., Gallegos Infante, J. A., Rocha-Guzman, N. E., & Rodríguez-Ramírez, J. (2014). Study of spray drying of the Aloe vera mucilage (*Aloe vera barbadensis Miller*) as a function of its rheological properties. *LWT - Food Science and Technology, 55*(2), 426–435.

de Rosny, L. (1857). *L'Opuntia, ou Cactus raquette d'Algérie*. Paris: Université de Harvard.

Dorney, K. M., Baker, J. D., Edwards, M. L., Kanel, S. R., O'Malley, M., & Pavel Sizemore, I. E. (2014). Tangential flow filtration of colloidal silver nanoparticles: a "green" laboratory experiment for chemistry and engineering students. *Journal of Chemical Education, 91*(7), 1044–1049.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*(3), 350–356.

Frank, B. P., & Belfort, G. (2003). Polysaccharides and sticky membrane surfaces: critical ionic effects. *Journal of Membrane Science, 212*(1–2), 205–212.

Garcia, J. L., & Saenz, C. (2006). Uso de los cladodios de nopal en productos alimenticios. In C. Saénz (Ed.), *Utilización agroindustrial del nopal. Boletín de Servicios Agrícolas de la FAO.* 162, (pp. 45–55).

Krystyjan, M., Sikora, M., Adamczyk, G., & Tomasik, P. (2012). Caramel sauces thickened with combinations of potato starch and xanthan gum. *Journal of Food Engineering, 112*(1–2), 22–28.

Lefèvre, K. U., & Tollens, B. (1907). Untersuchungen über die Glucuronsäure, ihre quantitative Bestimmung und ihre Farbenreaktionen. *Berichte der Deutschen Chemischen Gesellschaft, 40*(4), 4513–4523.

León-Martínez, F. M., Méndez-Lagunas, L. L., & Rodríguez-Ramírez, J. (2010). Spray drying of nopal mucilage (*Opuntia ficus-indica*):

effects on powder properties and characterization. *Carbohydrate Polymers, 81*(4), 864–870.

León-Martínez, F. M., Rodríguez-Ramírez, J., Medina-Torres, L. L., Méndez Lagunas, L. L., & Bernad-Bernad, M. J. (2011). Effects of drying conditions on the rheological properties of reconstituted mucilage solutions (*Opuntia ficus-indica*). *Carbohydrate Polymers, 84*(1), 439–445.

Li, J.-M., & Nie, S.-P. (2015). The functional and nutritional aspects of hydrocolloids in foods. *Food Hydrocolloids*.

Majdoub, H., Roudesli, S., Picton, L., Le Cerf, D., Muller, G., & Grisel, M. (2001). Prickly pear nopals pectin from *Opuntia ficus-indica* physico-chemical study in dilute and semi-dilute solutions. *Carbohydrate Polymers, 46*(1), 69–79.

Matsuhiro, B., Lillo, L. E., Sáenz, C., Urzúa, C. C., & Zárate, O. (2006). Chemical characterization of the mucilage from fruits of *Opuntia ficus indica. Carbohydrate Polymers, 63*(2), 263–267.

McGarvie, D., & Parolis, H. (1979). The mucilage of *Opuntia ficus-indica. Carbohydrate Research, 69*(1), 171–179.

McGarvie, D., & Parolis, H. (1981). The acid-labile, peripheral chains of the mucilage of *Opuntia ficus-indica. Carbohydrate Research, 94*(1), 57–65.

Medina-Torres, L., Brito-De La Fuente, E., Torrestiana-Sanchez, B., & Katthain, R. (2000). Rheological properties of the mucilage gum (*Opuntia ficus indica*). *Food Hydrocolloids, 14*(5), 417–424.

O'Shea, J.-P., & Tallon, C. (2011). Yield stress behaviour of concentrated silica suspensions with temperature-responsive polymers. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 385*(1–3), 40–46.

Ray, B., Loutelier-Bourhis, C., Lange, C., Condamine, E., Driouich, A., & Lerouge, P. (2004). Structural investigation of hemicellulosic polysaccharides from *Argania spinosa*: characterisation of a novel xyloglucan motif. *Carbohydrate Research, 339*(2), 201–208.

Read, D., & Gregory, P. (1997). Surface tension and viscosity of axenic maize and lupin root mucilages. *New Phytologist, 137*(4), 623–628.

Read, D., Gregory, P., & Bell, A. (1999). Physical properties of axenic maize root mucilage. *Plant and Soil, 211*(1), 87–91.

Ribeiro, E. M. D. O., Silva, N. H. D., Lima Filho, J. L. D., Brito, J. Z. D., & Silva, M. D. P. C. D. (2010). Study of carbohydrates present in the cladodes of *Opuntia ficus-indica* (fodder palm), according to age and season. *Food Science and Technology (Campinas), 30*, 933–939.

Ridley, B. L., O'Neill, M. A., & Mohnen, D. (2001). Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry, 57*(6), 929–967.

Sáenz, C., Sepúlveda, E., & Matsuhiro, B. (2004). *Opuntia spp* mucilage's: a functional component with industrial perspectives. *Journal of Arid Environments, 57*(3), 275–290.

Sepúlveda, E., Sáenz, C., Aliaga, E., & Aceituno, C. (2007). Extraction and characterization of mucilage in *Opuntia spp. Journal of Arid Environments, 68*(4), 534–545.

Thirugnanasambandham, K., Sivakumar, V., & Maran, J. P. (2015). Microwave-assisted extraction of polysaccharides from mulberry leaves. *International Journal of Biological Macromolecules, 72*, 1–5.

Trombetta, D., Puglia, C., Perri, D., Licata, A., Pergolizzi, S., Lauriano, E. R., De Pasquale, A., Saija, A., & Bonina, F. P. (2006). Effect of polysaccharides from *Opuntia ficus-indica* (L.) cladodes on the healing of dermal wounds in the rat. *Phytomedicine, 13*(5), 352–358.

Tumolo, T., Angnes, L., & Baptista, M. S. (2004). Determination of the refractive index increment (dn/dc) of molecule and macromolecule solutions by surface plasmon resonance. *Analytical Biochemistry, 333*(2), 273–279.

Varnero, T., & de Cortázar, V. G. (2006). Producción de bioenergía y fertilizantes a partir de los nopales. *Utilización agroindustrial del nopal Boletín de Servicios Agrícolas de la FAO, 162*, 113–120.

Vishwakarma, R., Chavan. R. S., Shivhare, U., & Basu, S. (2014). Textural and rheological properties of fruit and vegetables: Methods in food analysis. 1.

Wu, Z., Li, H., Tu, D., Yang, Y., & Zhan, Y. (2013). Extraction optimization, preliminary characterization, and in vitro antioxidant activities of crude polysaccharides from finger citron. *Industrial Crops and Products, 44*, 145–151.

Yan, M.-M., Liu, W., Fu, Y.-J., Zu, Y.-G., Chen, C.-Y., & Luo, M. (2010). Optimisation of the microwave-assisted extraction process for four main astragalosides in *Radix astragali*. *Food Chemistry, 119*(4), 1663–1670.

Yang, W., Wang, Y., Li, X., & Yu, P. (2015). Purification and structural characterization of Chinese yam polysaccharide and its activities. *Carbohydrate Polymers, 117*, 1021–1027.

Yoshida, T., Tsubaki, S., Teramoto, Y., & Azuma, J.-I. (2010). Optimization of microwave-assisted extraction of carbohydrates from industrial waste of corn starch production using response surface methodology. *Bioresource Technology, 101*(20), 7820–7826.

Zheng, X., Yin, F., Liu, C., & Xu, X. (2011). Effect of process parameters of microwave assisted extraction (MAE) on polysaccharides yield from pumpkin. *Journal of Northeast Agricultural University (English Edition), 18*(2), 79–86.