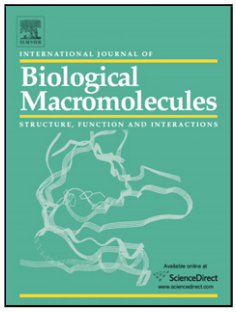

Title: Extraction, Characterization and gelling behavior enhancement of pectins from the cladodes of *Opuntia ficus indica*

Author: Khalef Lefsih Cédric Delattre Guillaume Pierre Philippe Michaud Tejraj M. Aminabhavi Farid Dahmoune Khodir Madani

PII: S0141-8130(15)30050-7
DOI: http://dx.doi.org/doi:10.1016/j.ijbiomac.2015.10.046
Reference: BIOMAC 5460

To appear in: *International Journal of Biological Macromolecules*

Received date: 29-7-2015
Revised date: 14-10-2015
Accepted date: 16-10-2015

Please cite this article as: K. LEFSIH, C. DELATTRE, G. PIERRE, P. MICHAUD, T.M. AMINABHAVI, F. DAHMOUNE, K. MADANI, Extraction, Characterization and gelling behavior enhancement of pectins from the cladodes of *Opuntia ficus indica*, *International Journal of Biological Macromolecules* (2015), http://dx.doi.org/10.1016/j.ijbiomac.2015.10.046

Extraction, Characterization and gelling behavior enhancement of pectins from the cladodes of *Opuntia ficus indica*

Khalef LEFSIH *[1,4], Cédric DELATTRE[2], Guillaume PIERRE[2], Philippe MICHAUD[2], Tejraj M. AMINABHAVI[3], Farid DAHMOUNE[1], Khodir MADANI[1]

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

[2]*Clermont Université, Université Blaise Pascal, Institut Pascal, UMR CNRS 6602 CNRS Polytech Clermont-Ferrand, 24 avenue des Landais, BP 206, Aubière Cedex, F-63174, France*

[3]*Department of Pharmaceutical Engineering and Chemistry, Soniya College of Pharmacy, Dharwad, 580 002, India*

[4]*Département de Biochimie et Microbiologie, Faculté des Sciences Biologiques et des Sciences Agronomiques. Université de Tizi ouzou. 15000 Tizi ouzou, Algeria*

* To whom correspondence should be addressed

Tel.: +213-793 438 779

E-mail: klefsih@yahoo.fr

Abstract:

Total Pectins Fraction (TPF) was extracted at room temperature from dried cladodes of *Opuntia ficus indica.* TPF is constituted of three pectic fractions WSP, CSP and ASP, which are made up of 66.6%, 44.3% and 81.1% (w/w) of galacturonic acid, respectively. The antioxidant ability of TPF increased with the concentration increasing. It scavenged hydroxyl radical by 90% and chelated 90% of ferrous ions at 5 g/l. FTIR study was carried out. Strong characteristic absorption peaks at 1618 cm^{-1} assigned to the vibration of COO^- group of galacturonic acid. In the fingerprint region, we noticed three well-defined peaks at 1054, 1085, and 1154 cm^{-1} characteristic of pectic polysaccharides. TPF are non-gelling pectins. The co-crosslinking of TPF with carrageenan was carried out and the gelling behavior was successfully improved. Thermo-sensitive hydrogel was obtained with 82% of TPF and 18% of carrageenan (w/w).

Keywords: Pectin; FTIR; antioxidant; gelling behavior; viscosity; carrageenan; co-crosslinking; Opuntia ficus indica.

1. Introduction

Opuntia ficus indica (OFI) is one of the widely cultivated species of the genus Opuntia in North Africa that is used for human consumption as natural fruits and animal food for its cladodes due to its rich nutrient content. It is widely cultivated in the semi-arid countries to feed goats, sheep and bovines as well as it is well-known as a medicinal plant to cure diarrhea and anti-inflammatory diseases. Cladodes of cactus contain important fibers viz., cellulose, hemicelluloses and pectins [12]. The major

water soluble polysaccharides extracted from *Opuntia ficus indica* cladodes consists of pectins [13] of which the main pectin component is a central linear backbone chain composed of α-D-galacturonic acid units linked by (1→4) glycosidic bonds. Neutral sugars, rhamnose, arabinose, galactose, xylose and glucose are usually present in about 5-10 wt% of galacturonic acid. Functional properties of pectin strongly depend on its structural and compositional parameters, such as galacturonic acid content, methyl-esterification level, molecular weight distribution and length of galacturonic blocks [14].

Commercially, pectin is commonly derived from fruit waste mainly apple and citrus peel. Pectins that are abundant, renewable and biodegradable have the capacity to associate through physical and chemical interactions with a wide variety of molecules; which would enhance their functionality [15]. In particular, pectins are used as thickening additives in foods, cosmetics and pharmaceutical preparation. Chemical modifications are, in some cases, needed to improve their functionality. Fully de-esterified pectin with excellent gelling properties in the presence of calcium was isolated from fresh nopal cactus pads [13]. For controlled crosslinking of pectin it is of interest to prolong the life-time over a desired time as required in many applications, especially as implantable drug delivery systems, for preparing stable gels, and membrane devices [16]. Intensive research efforts have focused on the mucilage value of OFI cladodes and fruits, but limited information is available as pectin composition of the whole cladodes. The main objective of this work is to characterize the extracted pectin from cladodes of OFI in terms of their monosaccharide composition, co-crosslinking and gelling capacity enhancement for further bioassays applications.

2. Material and methods

126

127 *2.1. Plant material*

128 Cladodes used in this work were harvested in July, 2013 in Kabylia, a region in
129 north Algeria, an area having semi-arid climate with cold and rainy winter as well as hot
130 and dry summer with temperatures ranging between 25°C and 40°C. This plant belongs
131 to the Cactaceae family, *Opuntia* genus and *ficus indica* species. About 50 kg of fresh
132 cladodes were dried under sun for one month, cut into small pieces and were grinded to
133 get fine powder using an industrial grinder. Finally, a step of sifting is carried out and a
134 powder of fine granulometry (< 125 µm) was recovered and preserved in a hermetic
135 bottle.

136

137 *2.2. Pectins extraction*

138 Pectic polysaccharides sequentially extracted from 300 g of the fine powder of
139 grinded and dried cladodes. The water-soluble pectins (WSP) were extracted from water
140 10% (w/v) at 60°C under stirring (500 rpm) for 1.5h. The solution was then centrifuged
141 at 10000 g / 15 min and the supernatant was neutralized with 5N NaOH, mixed with
142 three volumes of ethanol (96%), stirred vigorously, and left overnight at 4°C. Next,
143 chelating-soluble pectins (CSP) were extracted from residue I using an aqueous solution
144 of calcium chelating agent (EDTA 0.5%, 80°C, 1,5h). After centrifugation, the
145 supernatant was neutralized with NaOH (5N) and precipitated with ethanol. Residue II
146 was treated with HCl 0.05M at 50°C for 1h, from which the residue III and the ASP
147 fraction (acid soluble pectins) were obtained.

148

149 *2.3. Anti DPPH radical*

Antioxidant activities of Total Pectins (TPF) and ascorbic acid were evaluated using 2,2'-diphenyl-1-picrylhydrazyle (DPPH) procedures described by [17] as adapted from [18]. Briefly, the fraction was previously dissolved at various concentrations (0 to 10 g/L) in ultra-pure water. 1 mL of solution (sample or control) was added into 1 mL of DPPH solution at 0.1 mM in ethanol vigorously stirred and incubated for 30 min at room temperature (25°C). Absorbance was measured at the λ_{max} value of 517 nm using Shimadzu UV-1700 spectrophotometer (PharmaSpec). The DPPH inhibition (%) was calculated using Equation 1.

Eq. 1 $$DPPH\ inhibition\ (\%) = [1 - (A_{sample}/A_{control})] \times 100$$

Where A_{sample} and $A_{control}$ are, respectively absorbances at 517 nm of 1 mL of the sample (0-5 g/L) and 1 mL of ultra-pure water with 1 mL of DPPH at 0.1 mM in ethanol.

2.4. Anti-hydroxyl radical

For the adapted hydroxyl radical method [19], the fraction was previously dissolved at different concentrations (0 to 10 g/L) in ultra-pure water. A 0.2 mL of the solution (sample or control) was added into 0.2 mL of an aqueous solution of 5 mM $FeSO_4$. After stirring, 0.2 mL of aqueous solution of H_2O_2 at 1% (v/v) was added to the mixture solution was stirred and incubated at room temperature. After 60 min, 1 mL of ultra-pure water was added and the absorbance was measured at 510 nm using a Shimadzu UV-1700 spectrophotometer (PharmaSpec). The hydroxyl radical inhibition (%) was calculated using Equation 2.

Eq. 2 $$\textit{Hydroxyl radical inhibition}\ (\%) = [(Ac - As)/Ac] \times 100$$

where A_s and A_c are absorbances, of sample and control, at 510 nm of 0.2 mL of the sample (0-5 g/L) or 0.2 mL of ultra-pure water with 0.4 mL of a solution (v/v) of $FeSO_4$ (5 mM)/H_2O_2 (1%).

2.5. Chelating activity

Ionchelating activity of polysaccharide on Fe^{2+} was measured as reported previously [20]. A2 mL of the sample was mixed with 3.7 mL of deionized water and reacted with ferrous chloride (5 mmol/L, 0.1 mL) to which 0.2 mL of 5 mmol/L ferrozine was added, the solution was mixed to measure the absorbance at 562 nm employing EDTA as a positive control. A lower level of absorbance indicated stronger chelating activity. The chelating rate of polysaccharide on Fe^{2+} (%) was calculated according:

Eq. 3 $$\textit{Chelating rate}\ (\%) = ((Ab - As)/Ab) \times 100$$

Where A_b is absorbance of the control (deionized water instead ofsample) and A_s is absorbance of the test sample mixed with reaction mixture.

2.6. Monosaccharide composition analysis

Polysaccharides from cladodes of OFI (10 mg) dissolved in 4 M TFA (1 mL) were heated at 100°C for about 8 hand hydrolysates were neutralized with ammonia solution (4 M). Monosaccharide composition of thepolysaccharides was evaluated by

High Pressure Anion Exchange Chromatography (HPAEC) on an ICS 3000 (Dionex, USA) column equipped withpulsed amperometric detection and AS 50 auto-sampler that was assembled with a guard CarboPac^{TM} PA1-column (4 × 50 mm) and analytical CarboPac^{TM} PA1-column (4 × 250 mm). Samples (10 mg/mL) were filtered using 0.2 µm membrane filter at the fixed injection volume of 25 µL. Before each injection, columns were equilibrated by running for 15 min with 18 mM NaOH. Samples were eluted with 18 mM NaOH for 30 min followed by linear gradient between 0 to 1 M sodium acetate in 200 mM NaOH for 20 min to elute the acidic monosaccharides. The column was then washed for 15 min with 200 mM NaOH by keeping eluent flow constant at 1 mL/min. Columns were thermostated at 25°C, results were collected and analyzed with Dionex Chromeleon 6.80 software (Sunnyvale, USA).

2.7. Gas chromatography /mass spectrometry experiments

A 10 mg of polysaccharide was dissolved in 2 M HCl (2 mL), heated at 90°C for 4 h. to evaporate undera nitrogen stream. Trimethylsilyl-O-glycoside residues were solubilized by adding 500 µL of dichloromethane and analyses were carried out by GC/MS-EI using an Agilent 6890 Series GC system coupled to an Agilent 5973 Network Mass Selective Detector. The solutions were injected into an Agilent HP-1 (30 m, 0.32 mm, 0.25 µm) at a Helium flow rate of 2.3 mL/min. The helium pressure was adjusted to 8.8 psi at the split ratio of 25:1. The rise in temperature was programmed for the first step at 100°C for 3 min, an increment of 8°C/min up to 200°C for 1 min and then a final increment of 5°C/min up to 250°C. The ionization was performed by Electronic Impact (EI, 70 eV), the trap temperature was set at 150°C and the target ion was fixed at 40-800 m/z.

2.8. *Degree of methylation (DM)*

From [21], it is inferred that the ratio of the area of the band at 1736 cm^{-1} (corresponding to the number of esterified carboxylic groups) over the sum of the areas of the bands at 1735 $cm^{-1}$and 1618 cm^{-1} (corresponding to the number of total carboxylic groups) should be proportional to the degree of methylation (DM) as given by:

Eq. 4 $$DM = [A_{1735} /(A_{1735} + A_{1618}) + 0.107] \times 100$$

2.9. Solid FTIR spectroscopy

The polysaccharide fractions were analyzed using Fourier transform infrared spectrophotometer. The dried polysaccharides were ground with spectroscopically pure potassium bromide (KBr) powder and pressed into pellets (150 mg of dried KBr and 1 mg of lyophilized samples)and spectra were recorded in the transmission mode at room temperature (mid-infrared region, 4000-600 cm^{-1}) using a Nicolet spectrometer coupled with the personnel computer loaded with OMNIC software. A total of 40 scans were measured with a resolution of 4cm^{-1}.

2.10. Viscosity analysis and polymer blend

The gelling behavior of the native pectin and saponified pectin was studied by monitoring the evolution of the viscosity over time. Viscosity measurements were performed by a SNB-1 digital viscometer (Princeton instruments). TPF (10 g/100mL) were saponified with NaOH (0.1N) for 24 h at 4ºC under vigorous stirring. Then the samples were neutralized with HCl (3N), precipitated with two volumes 96%ethanol and dried in oven at 40°C.

Polymer blends were prepared with TPF and different proportions of κ-carrageenan (10%, 12%, 14%, 16%, 18%, and 20%) (Sigma-Aldrich). Viscosity measurements were performed for each TPF/carrageenan blend over time.

Finally, the effect of pH (2, 4, 6 and 8) and temperature (5°C, 15°C, 25°C, 35°C and 45°C), on the viscosity of pectin/ carrageenan (18%) blend were monitored.

3. Results and discussion

3.1. Antioxidant and chelating activities

The scavenging abilities of TPF increased with increasing concentration reaching a plateau of 85 ± 2% at concentrations higher than 2g/l. TPF showed high antioxidant activity as it scavenged hydroxyl radical by 90% at 5 g/l, by 70% at 0.5 g/l (**Fig. 1A)**, while it scavenged DPPH radicals by ≈ 90% at 2.25 g/l and by 85% at 1 g/l (**Fig. 1B)**. These values are very close to those given by the scavenging activity of the ascorbic acid used as a positive control. The TPF showed a good chelating ability compared to the chelating effect of synthetic metal chelator (EDTA). The TPF chelated 90% of ferrous ions at 5g/L and 85% and 55% at 2.2 g/L and 1g/L, respectively (**Fig.2**).

3.2. Monosaccharide composition and DM

In their work, [22] have shown that cladodes of OFI are mainly constituted of carbohydrates and that cladode's polysaccharides are mostly made up of pectins. Similarly, glucose and galacturonic acid are the main sugars of Opuntia cladodes [12]. Neutral sugars are from the highly branched chains of galactan and arabinan [23]. In this work, the monosaccharide composition obtained by HPAEC of the three soluble fractions is shown in **Table 1**. The mass yields (% w/w) of galacturonic acid in these

fractions are 66.6%, 44.3% and 81.06% for WSP, ASP and CSP, respectively suggesting the presence of an important amount of pectin polymers. The major pectin fraction referred here as water-soluble pectin (WSP) represents 5.14% of dry cladode weight. It also contains 11.4% of galactose, 9.2% of arabinose and 6.9% of glucose resulting from the ramified chains of arabinogalactan and contaminating glucan. The presence of 6.6% of rhamnose and 44.3% of galacturonic acid in ASP fraction suggests the occurrence of rhamnogalacturonan backbone for acid soluble pectin with homogalactan branched chains (40.2% of galactose and 0.8% of arabinose). The CSP fraction represents 0.21% of dry weight and is mostly constituted of homogalacturonan pectin type (81.1% of galacturonic acid and does not contain rhamnose residue). This high content of galacturonic acid is in accordance with the results of [24] who studied the structural characteristics of pectin polysaccharides obtained from OFI. Very low quantities of rhamnose and xylose are in accordance with those reported by [13] and corroborate the earlier findings [25]. This low yield of rhamnose was also explained [24] to confirm that Opuntia is rich in pectin polysaccharides.

The monosaccharide composition obtained by CG-MS from the final insoluble fraction (Residue III) and that of the whole cladode is presented in **Table 2**. By comparing the results of both the fractions, we can see the enrichment of the final residue with glucose and galactose i.e., 45.3% and 37.2%, respectively, suggesting a high content of the final residue in cellulose and hemicellulose.

The highest rates of galactose are found in the final residue and in the ASP fraction, suggesting that the chains of galactan are related to the insoluble parietal polysaccharides and are released by the acid treatment as was suggested before [26, 27]. In general, the overall monosaccharide composition of Opuntia cladodes is in concurrence with those of the earlier reports [23, 25, 28].

Table 3 shows the results of polysaccharide extraction yield for each pectic fraction as well as its rate in galacturonic acid and its degree of methylation. The results of DM show that three pectin fractions belong to the family of low-methylated pectins (DM < 50%). Our results are in agreement with those of [29], which showed that pectins resulting from the pulp of cactus cladodes are low-methylated as opposed to those extracted from the walls of the cladodes are high-methylated.

3.3. FTIR analysis

FTIR spectra of pectin fractions (CSP, WSP and ASP) are shown in **Fig. 3**. The results were analyzed in three characteristic regions: O-H stretching bands envelope (3200-3600 cm^{-1}); C-H (methyl) stretching bands (2800-3000 cm^{-1}) and the fingerprint region envelope (700-1800) cm^{-1}) [30]. A broad absorption band at 3434 cm^{-1} due to stretching frequency of O-H group and a band around 2850-2919 $cm^{-1}$are attributed to the C-H stretching vibration [31]. The Fingerprint region of the FTIR spectra for these three pectin fractions show practically identical parts with three bands that are characteristics of pectin polysaccharides at 1054, 1085 and 1154 cm^{-1}, assigned to -C-OH, -C-C- and -C-O- vibration mode, respectively [13, 30, 32]. Pectins belong to the class of carboxypolysaccharides, which differ from the neutral polysaccharides, with an intense band in the region 1750-35 cm^{-1} related to the vibration of esterified carboxyl group and in the regions of 1400-1450 cm^{-1} and 1600-1650 for free carboxyl group [33]. The absence of a band at 1750 cm^{-1} might be because it is covered by a strong peak at 1618 cm^{-1} (asymmetrical COO^{-} stretching vibration). In the ASP spectrum, the absence of a band at 1415 cm^{-1} present in WSP and CSP, spectra indicate that it is either overlapped with a strong band at 1318 cm^{-1} or due to the absence of O-acetyl-ester group.

The most obvious difference between WSP, CSP and ASP spectra is the presence of well resolved peaks in ASP spectrum at 1318 cm^{-1}, assigned to C-H vibration [1] in haired zones which are liberated by acid treatment, and galactose absorption peak at 782 cm^{-1} [2]. This is in conformity with the monosaccharide composition of these fractions.

We can notice well-defined peaks of C-H methyl stretching around 2850-2919 cm^{-1} for CSP fraction, which may be dependent on the presence of traces of lipids in this fraction. It is highlighted by the disappearance of these peaks by degreasing treatment of CSP by toluene-ethanol (40:60 v/v) mixture.

For a more explicit study of the vibrations in the area of 400-900 cm^{-1}, we have carried out microfiltration of the fraction WSP against deionized water on a membrane of 0.2µ thickness. The microfiltration gave a fraction WSP-HM, with a high molecular weight and a second fraction WSP-LM with low molecular weight. The spectral analysis by FTIR of fractions WSP, WSP-HM and WSP-LM is shown in **Fig. 4,** wherein the vibrations in the around 400-900 cm^{-1} are in the shaded part. We observe a broad band in this part of the spectrum for WSP-LM, whereas it is hardly perceptible in the spectra of WSP and WSP-HM. These vibrations are essentially due to monosaccharide and oligosaccharide molecules [34], except the peak at 690 cm^{-1}that is assigned to the stretching vibration of C-Br formed during the preparation pallets in KBr.

3.4. Spectral subtraction

The most straightforward method of analysis for complex spectra is Difference Spectroscopy (Spectral Subtraction**)** that was carried out by simply subtracting the infrared spectrum of one component of the system from the combined spectrum to leave

the spectrum of other component. If the interaction between components results in the change of spectral properties of either one or both the components, these changes will be observed in the difference spectra which manifest via the appearance of positive or negative peaks in the spectrum. Spectral subtraction may be used for the data collected for solutions and solids [35]. Since the three extracted pectic fractions result sequentially from the same sample of whole cladode (WC), therefore the technique of FTIR spectral subtraction enables us to check the quality and purity of each fraction compared to the whole cladode sample (WC).

Fig. 5 shows the fingerprint region of WC spectrum as well as the subtracted spectra of each fraction from that of WC. Subtraction of WSP spectrum from that of WC give the subtraction spectrum as shown in **Fig. 5A,** where only two peaks are observed in the positive region. On the other hand, the peak at 1318 cm^{-1} that is characteristics of cellulosic substances (insoluble in water) as well as, the peak at 782 cm^{-1} is attributed to galactose confirm that the ramified chains of galactans are not easily extractable. This suggests that the ramified chains of galactans are in connection with the parietal polysaccharides. Otherwise, all the peaks allotted to the pectic substances are in the negative area, which proves that extraction of water-soluble pectins was effective. The connection of sidechains of pectins to other cell wall materials such as hemicelluloses and cellulose was reported in other works [3-5]. In fact, the arabinogalactan sidechains of pectins may act as bridges to connect the rhamnogalacturonan backbone of pectins and the other cell wall materials suchas cellulose and hemicellulosic xyloglucans [3].

Difference between WC and CSP is shown in the subtraction spectrum **(Fig. 5B)**; it is noticed that the major part of the spectrum is in the negative area confirming that CSP fraction was effectively extracted and is independent of the parietal

polysaccharides. This can be explained by the fact that chelating soluble pectins belong to the mucilage. In addition, mucilage polysaccharides in Opuntia do not seem to be chemically associated, either covalently or otherwise, to the structural cell-wall pectins [14].

Subtraction spectrum (**Fig. 5C**) illustrates that only the acid pectins are majority in ASP fraction as proved by the presence of a peak at 1650 cm^{-1}, which is characteristics of the COOH group in the negative area. The occurrence of a peak at 1320 cm^{-1} in the negative area highlights the poor ASP fraction in the cellulosics, which may reflect the association of acid pectin with parietal polysaccharides.

The results of spectral subtraction of the three pectic fractions from the final residue (RIII) give the spectra A, B, C of **Fig. 6**. All the three spectra are entirely in the negative area, which implies that RIII does not only contain traces of the components present in the fractions WSP, CSP, ASP, proving that RIII is completely exhausted in pectins present in the fractions WSP, CSP and ASP.

3.5. Viscosity measurement of TPF and TPF/Carrageenan blend

Pectins from various sources do not have the same capacity for gelling since the ability of pectins to form a gel depends on its degree of polymerization, the degree of esterification, degree of branching [36].

Fig. 7 demonstrated the effect of saponification on the viscosity of the TPF. In fact, saponification causes physical and chemical changes which tend to increase the solubility of the pectin, thereby decreasing the local crystallizations that promote increase in viscosity or gelation [37]. Gelation consists in the association of the polygalacturonate chains by forming the junction zones [38].

The effect of divalent ions (Ca^{++}) on the gelling of the TPF is illustrated in **Fig. 8**. In fact, there is an increase in viscosity, reaching the maximum values for concentrations of Ca^{2+} from 40 mM to 80 mM; beyond this range, there is a significant drop in viscosity. These low concentrations of Ca^{++} are dependent of the weak dissociation of carboxylic groups, due to low pH, reducing the binding probability [39]. It is very important to note that despite the increase in viscosity, in the presence of Ca^{++}, there is no gel formation, although TPF are low methylated. This can be explained by a high degree of branching of these pectins. This means that despite the low degree of methylation, polygalacturonate zones, which may interact with Ca^{++} are very limited due to the high degree of branching. The effect of branching on pectin gelation may be more relevant in HM pectin, as intermolecular association by hydrogen bonding is the primary gelation mechanism. In LM pectin, the interactions between carboxyl groups of pectin and divalent ions are more important for gelation than intermolecular interactions, thus the effect of branching may not be pronounced in this case [6]. Two contradictory arguments have been issued for the role of sidechains of pectins in gelling. Selvendran [7] stated that the arabinose and galactose sidechains of pectins could give a positive contribution to gelling by holding water molecules within the gel framework. In contrast, BeMiller [8] stated that the sidechains of pectins might tend to limit the extent of interchain association, and thus formation of junction zones for gelling may be inhibited. Matthew *et al.* [9] reported that sugarbeet pectins treated with an enzyme prepared by *Aspergillus niger* significantly improved gelation. This was due to the combined effects of deacetylation, demethoxylation and the significant reduction in arabinose residues of sidechains.

Pectic molecules in solution are highly hydrated and the total negative charge depends on the dissociation of carboxylic functional groups. The decrease of hydration

is accomplished by the addition of carrageenan, which plays the role of water scavenger; on the other hand, it can form as copolymer with pectin, a three-dimensional physical network promoting gelation. The presence of carrageenan increases the viscosity and to induce a good gelation. Carrageenan plays a potential role in the formation of gel, in **Fig. 9**. The minimum ratio of carrageenan giving a perfectly solid gel is 18%.

The effect of pH and temperature on the mixing viscosity TPF (82%) / Carrageenan (18%) is illustrated in **Fig. 10** and **Fig.11,** respectively. It is clearly seen in **Fig. 10** that the viscosity of the hydrogel is not very sensitive to changes in pH closer to neutrality. The increase in viscosity with elevated pH is related to the increase of the dissociation rate of the carboxyl groups (COO^-) of pectin and ester sulfate groups (OSO_3^-) of carrageenan which promote interaction with the divalent ions present in the medium. Conversely, decreasing the pH decreases the dissociation of the previous ionizable groups, which induces an increase in the hydration of the TPF and carrageenan, as well as their solubility, which results in a decrease in viscosity.

The viscosity of pectin/carrageenan is very influenced and sensitive to the temperature. More the temperature is low, more the viscosity is increased (**Fig. 11**). The sol-gel transition appears, in the case of pectins, during cooling. Our results are in conformity with previous findings [10, 11], they attributed the decrease in viscosity to the depolymerization of pectin with increasing temperature.

The most important result here is that the gelation of *TPF*/*Carrageenan blend* is thermo-reversible. At cooling temperature, a hard hydrogel is obtained, by heating there is a significant decrease in viscosity until hydrogel become completely liquid. This thermo-reversibility is a promising property for the prospective use of this hydrogel as drug delivering system and in microencapsulation.

4. Conclusion

Chemical characterization gave us important information about pectin fractions extracted from the whole cladodes of OFI. The results of monosaccharide's composition obtained by HPAEC of the pectin fractions indicated that they were mostly made up of galacturonic acid. TPF shows high scavenging and chelating activities.

The general aspect of the spectra can be influenced by the purity and monosaccharide composition. However, the overall shape of pectin polysaccharide spectra showed the same characteristic bands at 1054 cm^{-1}, 1085 cm^{-1} and 1154 cm^{-1} assigned to pyranose cycle vibrations, -C-OH, -C-C- and -C-O-, respectively, while that at 1618 cm^{-1} is attributed to the $-COO^{-}$ stretching vibration. FTIR spectral subtraction enabled us to check the effectiveness of the extraction of each pectic fraction to control its purity and to reveal the possible interactions with other the components. Ca^{2+} has no effect on the gelling behavior of TPF. Therefore, its mechanism of gelling is not the egg-box model. Finally, the co-crosslinking of TPF and carrageenan was successfully performed, which also improved the capacity to gel. The gelling of TPF/carrageenane mixture is thermo-sensitive and occurs at low pH, this fact is so important for farther use of OFI pectins in drug delivering system and in microencapsulation.

Acknowledgment

I thank Pr. Philippe Michaud for accepting me in his research unit and the University Mouloud Mammeri for financing this modest work.

471

472 References

473

474 [1] K.K. Pandey, A.J. Pitman, International Biodeterioration & Biodegradation, 52
475 (2003) 151-160.

476 [2] L. Yang, L.-M. Zhang, Carbohydrate Polymers, 76 (2009) 349-361.

477 [3] J. Hwang, Y.R. Pyun, J.L. Kokini, Food Hydrocolloids, 7 (1993) 39-53.

478 [4] M. McNeil, A.G. Darvill, P. Albersheim, The Structural Polymers of the Primary
479 Cell Walls of Dicots, in: W. Herz, H. Grisebach, G.W. Kirby (Eds.) Fortschritte der
480 Chemie organischer Naturstoffe / Progress in the Chemistry of Organic Natural
481 Products, Springer Vienna, 1979, pp. 191-249.

482 [5] B.S. Valent, P. Albersheim, Plant Physiol., 54 (1974) 105-108.

483 [6] V. Urias-Orona, A. Rascón-Chu, J. Lizardi-Mendoza, E. Carvajal-Millán, A.A.
484 Gardea, B. Ramírez-Wong, International journal of molecular sciences, 11 (2010) 3686.

485 [7] R.R. Selvendran, J. Cell Sci. Suppl., 2 (1985) 51-88.

486 [8] N.B. James, An Introduction to Pectins: Structure and Properties, in: Chemistry and
487 Function of Pectins, American Chemical Society, 1986, pp. 2-12.

488 [9] J.A. Matthew, S.J. Howson, M.H.J. Keenan, P.S. Belton, Carbohydrate Polymers, 12
489 (1990) 295-306.

490 [10] G.A. Morris, T.J. Foster, S.E. Harding, Carbohydrate Polymers, 48 (2002) 361-
491 367.

[11] G.W. Pilgrim, R.H. Walter, D.G. Oakenfull, The Chemistry and technology of pectin / edited by Reginald H. Walter, Academic Press, San Diego, 1991.

[12] E.M.O. Ribeiro, N. Silva, H., , J.L. Lima Filho, J.Z. Brito, & , M.P.C. Silva, Ciência Tecnologiade Alimentos Campinas, 30 (2010) 933-939.

[13] A. Cárdenas, F.M. Goycoolea, M. Rinaudo, Carbohydrate Polymers, 73 (2008) 212-222.

[14] F.M. Goycoolea, A. Cárdenas, Journal of the Professional Association of Cactus Development, 5 (2003) 17-29.

[15] W. Ciesielski, C.-y. Lii, M.-T. Yen, P. Tomasik, Carbohydrate Polymers, 51 (2003) 47-56.

[16] M. Rinaudo, European polymer journal, 46 (2010) 1537-1544.

[17] C. Delattre, G. Pierre, C. Gardarin, M. Traikia, R. Elboutachfaiti, A. Isogai, P. Michaud, Carbohydrate Polymers, 116 (2015) 34-41.

[18] T. Yamaguchi, H. Takamura, T. Matoba, & , J. Terao, Biosci. Biotechnol. Biochem., 62 (1998) 1201–1204.

[19] A.X. Luo, X.J. He, S.D. Zhou, Y.J. Fan, A.S. Luo, Z. Chun, carbohydrate Polymers, 79 (2010) 1014–1019.

[20] Y. Zheng, Y. Li, W.D. Wang, Carbohydr Polym, 111 (2014) 315-323.

[21] M. Monsoor, A. Kalapathy, U. Proctor, J. Agric. Food Chem., 49 (2001) 2756-2760.

512 [22] C.V.L. Giosafatto, P. Di Pierro, P. Gunning, A. Mackie, R. Porta, L. Mariniello,
513 Carbohydrate Polymers, 106 (2014) 200-208.

514 [23] A. Oosterveld, G. Beldman, M.J.F. Searle-van Leeuwen, A.G.J. Voragen,
515 Carbohydrate Polymers, 43 (2000) 249-256.

516 [24] Y. Habibi, A. Heyraud, M. Mahrouz, M.R. Vignon, Carbohydr. Res., 339 (2004)
517 1119-1127.

518 [25] E. Forni, M. Penci, A. Polesello, Carbohydrate Polymers, 23 (1994) 231-234.

519 [26] M.C. McCann, K. Roberts, in: The Cytoskeletal Basis of Plant Growth and Form.,
520 Academic Press, London., 1991, pp. 109–129.

521 [27] M. O'Neill, P. Albersheim, A.G. Darvill, In Methods in Plant Biochemistry,
522 Carbohydrates, ed. Dey P.M ed., Academic Press London, 1990.

523 [28] J. Deng, Z.J. Shi, X.Z. Li, H.M. Liu, BioRessources, 8 (2013) 405-419.

524 [29] H. Majdoub, L. Picton, D. Le Cerf, S. Roudesli, J. Polym. Environ., 18 (2010) 451-
525 458.

526 [30] A.A. Kamnev, M. Colina, J. Rodriguez, N.M. Ptitchkina, V.V. Ignatov, Food
527 Hydrocolloids, 12 (1998) 263-271.

528 [31] A. Sawut, M. Yimit, W. Sun, I. Nurulla, Carbohydrate Polymers, 101 (2014) 231-
529 239.

530 [32] M.A. Coimbra, A. Barros, D.N. Rutledge, I. Delgadillo, Carbohydr. Res., 317
531 (1999) 145-154.

532 [33] F. Nejatzadeh-Barandozi, S. Enferadi, Organic and Medicinal Chemistry Letters, 2
533 (2012) 33.

534 [34] W. Zhang, Biochemical technology of carbohydrate complexes, Zhejiang
535 University Press, Hangzhou, 1994.

536 [35] B. Stuart, Infrared Spectroscopy: Fundamentals and Applications, John Wiley &
537 Sons, Ltd. © (2004), ISBNs: 0-470-85427-8. , 2004.

538 [36] V. Evageliou, R.K. Richardson, E.R. Morris, Carbohydrate Polymers, 42 (2000)
539 245-259.

540 [37] C.D. May, Carbohydrate Polymers, 12 (1990) 79-99.

541 [38] A. Ström, E. Schuster, S.M. Goh, Carbohydrate Polymers, 113 (2014) 336-343.

542 [39] H. Kastner, U. Einhorn-Stoll, B. Senge, Food Hydrocolloids, 27 (2012) 42-49.

543

1. TPF shows a very promising scavenging and chelating abilities
2. Solid FTIR spectral subtraction for testing the efficacy of extractions and interactions between different extracted fractions.
3. HPAEC monosaccharide analyses indicated that WSP are mostly made up of galacturonic acid.
4. TPF are non-gelling low-methylated pectins even in the presence of Ca^{2+}.
5. Co-crosslinked TPF/carrageenan blend gives thermo-sensitive hydrogel.

Table 1

HPAEC determination of monosaccharide composition of pectic fractions extracted from the cladodes of *Opuntia ficus indica*

Constituents	Mass yield (%w/w)		
	WSP	ASP	CSP
Galactose	11.4	40.2	8.2
Glucose	6.9	7.3	1.9
Galacturonic Acid	66.6	44.3	81.1
Arabinose	9.2	0.8	4.1
Xylose	/	/	0.5
Rhamnose	2.1	6.6	/
Mannose	3.8	0.8	4.0
Sum	100	100	99.8

Table 2

CG-MS determination of monosaccharide composition of the whole cladode and residue III

Constituents	Mass yield (%, w/w)	
	Whole cladode	Residue III
Galactose	31.8	45.3
Glucose	25.1	37.2
Galacturonic Acid	23.2	7.9
Arabinose	18.8	8.4
Xylose	1.1	1.4
Rhamnose	/	/
Mannose	/	/
Sum	100	100.02

Table 3

Degree of methylation and yield of extraction of WSP, CSP and ASP

Fraction	Yield from dry mater (%, w/w)	Galacturonic Acid (%, w/w)	DM (%)
WSP	5.75	66.6	30.6
CSP	0.21	81.1	33.5
ASP	0.11	44.3	28

Figures captions

Fig. 1: Scavenging effects on (A) hydroxyl radical and (B) DPPH radical for (●): TPF and, (■): ascorbic acid.

Fig. 2: Chelating effects of (■): TPF and, (●): EDTA.

Fig. 3: FTIR spectra of CSP, WSP and ASP

Fig. 4: FTIR spectra of WSP, WSP-HM and WSP-LM

Fig. 5: Subtraction spectra, [A]: WSP from WC; [B]: CSP from WC; [C]: ASP from WC [WC = Whole Cladode]

Fig. 6: Subtraction spectra, [A]: WSP from RIII; [B]: CSP from RIII; [C]: ASP from RIII

Fig. 7: Viscosity of TPF 5% (w/v) (●) and saponified TPF 5% (w/v) (■)

Fig. 8: Effect of Ca^{++} on the viscosity of TPF

Fig. 9: Effect of carrageenan ratio (10%=0.11g/20ml; 12%=0.13g/20ml; 14%=0.16g/20ml; 16%=0.2g/20ml; 18%=0.22g/20ml; 20%=0.25g/20ml) on the viscosity of TPF (1g/20ml).

Fig. 10: Effect of pH on the viscosity of TPF (82%)/carrageenan (18%) blend

Fig. 11: Effect of temperature on the viscosity of TPF (82%)/carrageenan (18%) blend