Journal of Molecular and Pharmaceutical Sciences

Original Research Article

Determination of the chemical composition of the pectic fractions of myrtle (*Myrtus communis* L.)

Amina Chidouh[a], Saoudi Aouadi[b], Alain Heyraud[c], Maroua Cheribot Cherif[a*]

[a]*Laboratoire de Chimie, Physique et Biologie des Matériaux, Ecole Normale Supérieure d'Enseignement Technologique de Skikda, Algérie*

[b] *Laboratoire de Biochimie et Microbiologie Appliquée (LBMA), Département de Biochimie, Faculté des Sciences, Université Badji Mokhtar, Annaba, Algérie*

[c]*Centre de Recherches sur les Macromolécules Végétales (CERMAV-CNRS), Grenoble, Cedex 9, France*

Abstract

This work aims to determine the chemical structure of the pectic fractions of *Myrtus communis* L. (myrtle). This species' edible fruit, which was collected in the Annaba region (north-eastern Algeria), used for industrial, medicinal, and nutritional purposes. After 85% (v/v) ethanolic treatment, pectins were sequentially extracted using water at 80°C (water-soluble polysaccharide), EDTA solution at 60 °C (chelating soluble polysaccharide) and HCl solution at 80°C (acid soluble polysaccharide). High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) was used to determine the monosaccharide content. The results showed that the WSP fraction is composed of 3.3% (w/w) galacturonic acid and 27.7% (w/w) neutral sugars. While the CSP fraction contains 2.07% (w/w) neutral sugars and 1.2% (w/w) galacturonic acid. The results showed that the residue D is composed of 9.8% (w/w) neutral sugars, 0.07% (w/w) galacturonic acid, and 11.7% (w/w) neutral sugars after hydrolysis with TFA (2N) and H_2SO_4 (2N), respectively. Analysis by ^{1}H NMR spectroscopy showed that CSP and ASP are pectins.

Keywords:
Myrtle, *Myrtus communis*, HPAEC-PAD, ^{1}H NMR, Neutral sugars, Galacturonic acid, Pectins.

⋆Received October 17, 2022; accepted November 22, 2022
*Corresponding author
Email address: cheribotmaroua@gmail.com (Maroua Cheribot Cherif)
Cited as: Chidouh A, Aouadi S, Heyraud A, Cheribot M.C, Laidouni I. Determination of the chemical composition of the pectic fractions of myrtle (*Myrtus communis* L.). J. Mol. Pharm. Sci. 02 (01), 2023, 1-8.

1. Introduction

Over the last few years, many researchers have conducted the extraction and structural analysis of complex carbohydrates from consuming fruits that are utilized in traditional healthcare. This has made it possible to prepare biopolymers with well-defined functional properties: technological or biological, as for the fruit of myrtle (*Myrtus communis* L.). [1] were able to isolate and analyse the water-soluble fraction of myrtle fruit. This fruit is currently undergoing significant development, for example, in the industrial stage. It is utilized in the fabrication of jelly products and jams in Sicily (Italy) and Corsica (France) [2, 3].

Parietal residues of fruit juice industrial products can be isolated and used in various applications according to their various characteristics.

Pectins are substances of plant origin. They are complex polysaccharides found mainly in the middle lamina and higher plants' primary walls [4]. Their role is crucial in the adhesion and maintenance of tissue cells' plants by forming a cement that binds the cells together [5]. The major constituent of pectins is galacturonic acid (GalA) residues bonded together by α(1→ 4) partially acetylated or esterified with methyl groups.

Rhamnogalacturonans (RG), homogalacturonans (HG) and arabinans form a pectic network covalently bound to hemicelluloses. The pectic polysaccharides, derived from several plant matrices, have been widely studied and are still the subject of many studies, as is the case for sugar beet pectins [6, 7], apples [6], flax fibers [8] or hemp fibers [9]. However, in the case of myrtle fruits (*Myrtus communis* L.), these constituents have never been studied in detail. Therefore, this work aims to study the chemical composition of the pectins that were extracted from this fruit.

2. Materials and methods

2.1. Treatment of myrtle fruits with ethanol (85%)

Fresh fruits of *Myrtus communis* L. were purchased from the market in Annaba region (north-eastern Algeria) during the ripening phase. They were separated by hand and chosen for their similarity of shape and color. Myrtle fruits were dispersed in ethanol 85% (v/v). After that, they were put in a bath of water and boiled for an hour under reflux. After filtering and washing them with the similar solution of ethanol, the remaining material was dried at 40 °C in a ventilated oven. The fruits were peeled by hand to extract the seeds once they had dried, and they were then crushed into a fine powder in a mill.

2.2. Extraction of pectic polysaccharides

These successive extractions were carried out to exhaust the material of all the pectic polysaccharides. Therefore, the pectins were hot-extracted sequentially with water, Ca^{2+} chelating agent, and hydrochloric acid. (Figure 1) shows the extraction procedures for the pectic fractions. Myrtle powder treated with ethanol (85%) was extracted with water for 24 hours at 80°C. After precipitation with 70% ethanol, dialysis with distilled water and lyophilization, the WSP (Water Soluble Polysaccharide) fraction was obtained.

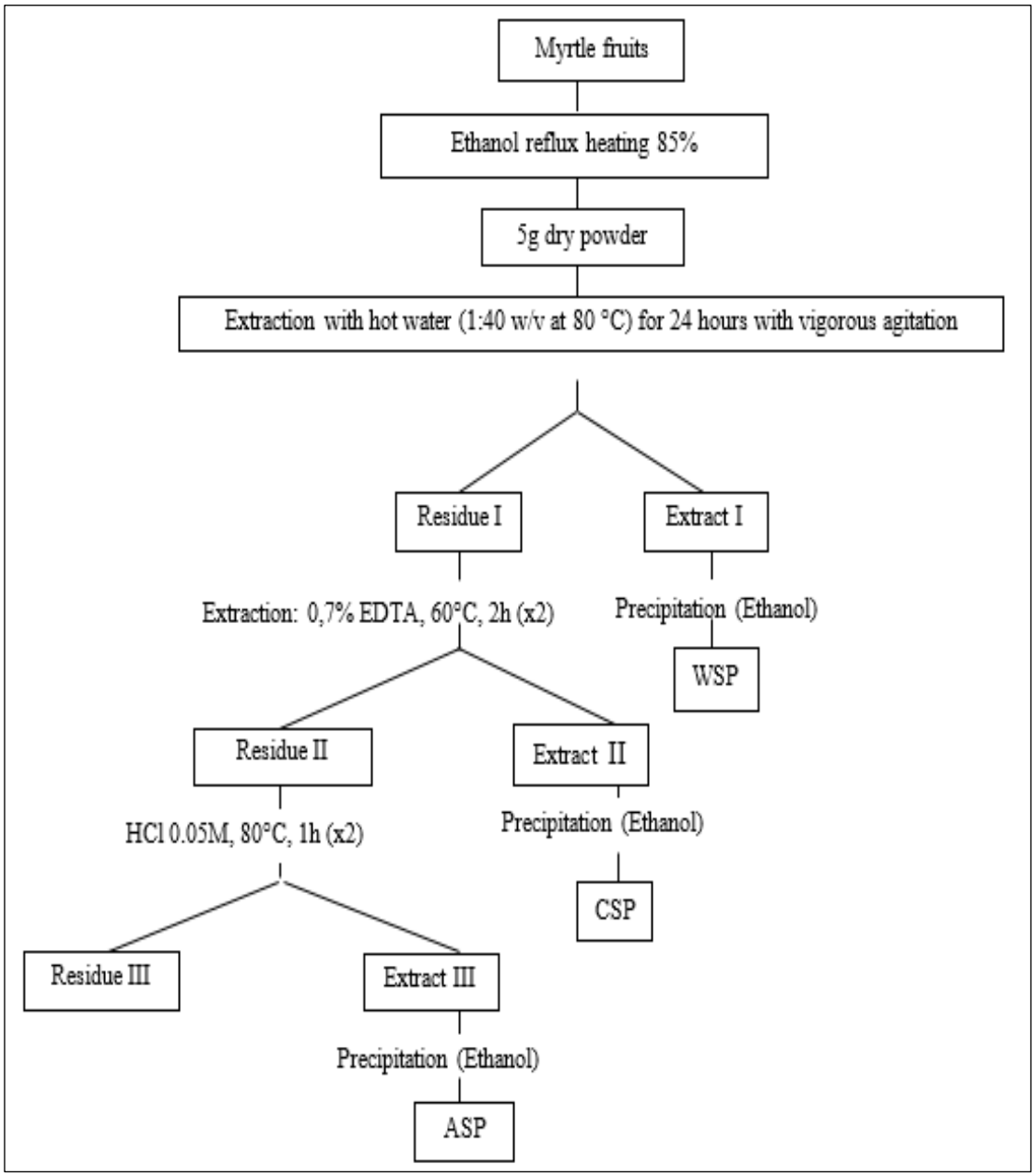

Figure 1: Schematic diagram of the extraction of pectic fractions from fruits (*Myrtus communis* L.).

Among Ca^{2+} chelating agents, EDTA was chosen. It has the advantage of not releasing Ca^{2+} ions into the solution once it has returned to room temperature. Indeed, the free Ca^{2+} ions could form insoluble aggregates by bridging polygalacturonic chains (egg-box) [10]. Therefore, 1g of the hot extraction pellet was treated with an EDTA solution (0.7%) at 60°C for two 2-hour cycles. The collected supernatants were added to ethanol (70%). The final result was kept overnight at 4°C to promote precipitate production. After vacuum filtration, the resulting material was washed with progressively higher ethanol

solutions (70%, 80%, 90% and finally 96%) and then dried on the sintered glass before being freeze-dried. The precipitate thus constitutes the CSP (Chelating Soluble Polysaccharide) fraction.

Treatment with hydrochloric acid solution (HCl, 0.05M), for two one-hour cycles at 80°C led to extracting the pectic polysaccharides strongly bound to cellulose microfibrils [10]. In this way, the protopectins associated with the plant wall were extracted, and pectin-free residue was recovered.

The hydrochloric acid-extracted fraction was precipitated with ethanol (70%) and freeze-dried. It is known as ASP (Acid Soluble Polysaccharide) [10]. The pellet obtained after this extraction is called residue III.

2.3. Sugar composition analysis by HPAEC-PAD

The determination of neutral and acidic sugar compositions was accomplished using HPAEC-PAD. In a Pyrex tube, 2 mL of sulfuric acid (H_2SO_4:2N) were added to 4 mg of the sample. For 6 hours, the suspension was heated on a heating block at 105 °C. Tap water was used to cool the hydrolysed product, then, with vigorous stirring, barium carbonate was used to neutralize the product, which was then filtered and concentrated under vacuum using a rotary evaporator. Each sample was diluted to 1:6, 1:2 and 1:6 for the WSP fraction, the CSP fraction and residue III, respectively. After filtration through a 0.45 µm filter, the HPAEC-PAD system was injected with 20 µL of each sample. A Dionex LC system was used to chromatograph the samples.

2.4. Nuclear Magnetic Resonance (NMR)

The ^1H NMR spectrum was recorded on BRUKER Avance 400 MHz NMR Spectrometer operating at 400.13 MHz in D_2O (5 mg of samples were dissolved in 500 mL of solvent at 80 °C). The proton spectrum was captured with a spectral width of 4006 Hz, 32768 data points, and an acquisition time of 4.089. The relaxation time was 0.1 up to 16 scans.

3. Results and discussion

3.1. Composition of sugar and uronic acid

Neutral sugars and uronic acids were identified from the pectic fractions (WSP and CSP) and Residue III. Table 1 shows the results obtained for the WSP and CSP fractions. The various fractions present a content of galacturonic acid (the WSP and CSP fractions present 3.3% and 1.2%, respectively.), compared to the composition of galacturonic acid (GalA) of the skin's pectic polysaccharides of *Opuntia ficus-indica* fruits (WSP and CSP fractions present 52.0% and 64.5%, respectively) [11].

Table 1: Sugar content of fractions obtained by sequential extraction of myrtle fruits.

Compound (%)	WSP*	CSP*
NS	27.705	2.075
UA	3.33	1.27
Rha	2.46	0.255
Ara	12.465	0.99
Gal	8.94	0.41
Glu	2.805	0.22
Man	0.57	0.075
Xyl	0.465	0.125
GalA	3.33	1.27

NS= neutral sugars, UA= uronic acids.
* HPAEC-PAD was used to acquire the data.

The results, presented in Table 1, show that the WSP fraction contains 27. 7% (w/w) neutral sugars. It is rich in arabinose (12. 4%) and galactose (8. 9%). It has also been discovered in pectin extracts from various fruits and vegetables [12]. This distribution suggests the presence of an arabinogalactan-type polysaccharide in this fraction. The mucilage of the prickly pear peel contains the same type of polysaccharide [10].

The Schols and Voragen pectin classification [13], which is partly based on the Rha/GalA ratio, distinguishes rhamnogalacturonan I (RG-I) from HG by its Rha/GalA ratio ranging from 0.05 to 1. The WSP fraction ratio of 0.7% indicates that RG-I polysaccharide predominates. This ratio is higher than that of *Argania spinosa* fruit pectins (AFP) which stands at 0.2% [14].

Treatment with EDTA resulted in the fractionation of the polysaccharide CSP. This fraction, as shown in **Table 1,** contains 2.07% (w/w) neutral sugars. Galacturonic acid (1.2%), as a monosaccharide characteristic of pectins, was found dominant. Fruits are the main sources of many compounds, including pectin [15]. A decrease in uronic acids and an increase in glucose are observed in residue III after hydrolysis with TFA (**Table 2**). However, a high acidic sugar content was detected after the pectin extraction step [10]. Thus, after hydrolysis with H_2SO_4 of Residue III, glucose is the most abundant neutral sugar (9.2%). These results are similar to those of blueberries (*Vaccinium myrtillus* L.) [16], murta AIS [17] and P4-F3 (7P2) fraction [1].

Table 2 : Composition of neutral sugars and uronic acids of Residue III.

Compound (%)	After hydrolysis with H_2SO_4*	After hydrolysis with TFA 2N*
NS	11.76	9.81
UA	-	0.075
Rha	0.24	-
Ara	0.165	1.455
Gal	1.32	3.225
Glu	9.21	2.34
Man	0.51	0.615
Xyl	0.315	2.175
GalA	-	0.075

NS= neutral sugars, UA= uronic acids, - = Not detected.
* HPAEC-PAD was used to acquire the data.

3.2. ^{1}H NMR spectroscopic analysis

Nuclear magnetic resonance is a very precise physicochemical method that can provide further structural information. (Figure 2) shows the ^{1}H NMR spectrum of the WSP moiety. Peak distribution was performed according to the method of Jones and Mulloy [18] for the structural study of polysaccharides [10] and for the structural determination of the pectic fractions of the *Opuntia ficus-indica* peel. The methyl signal of rhamnose appeared as a singlet at (δ=1.26 ppm) and other signals at δ=2.15 ppm corresponding to the methoxy group were recorded in the high fields. The signals of the skeletal protons were identified towards the midpoint of the spectrum (3.3 ppm < δ<4.5 ppm).

A group of peaks resonating in the region of the anomeric protons can be distinguished in the region of the Horizontal Outer Defect (HOD) (4.5-5.0 ppm) and a second one towards the weak fields (5.0-5.5 ppm). This finding allows us to conclude the existence of the *β* anomeric and the α anomeric protons , respectively. Plant arabinogalactans are made up of a primary *β*-D-linked galactopyranosyl chain (1→ 4) substituted by an α-*L*-araban chain, which itself carries several branching points. Arabinogalactans are generally linked to the rhamnose residue of type 1 rhamnogalacturonans.

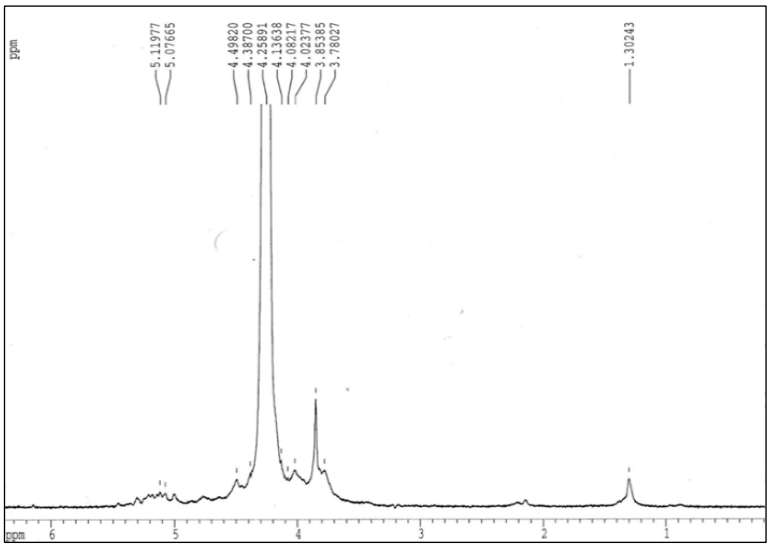

Figure 2: [1]H NMR spectrum of the WSP fraction.

The spectrum of [1]H-NMR (400.13 MHz) of the CSP fraction (Figure 3) shows three intense anomeric protons at 4.49 ppm, 4.82 ppm and 5.22 ppm corresponding respectively to the H-1 of α-arabinosyl, the H-1 of α-galacturonyl and the H-1 probably of a *β*-glucosyl or *β*-galactosyl or *β*-xylosyl. In the range 3.67-4.12 ppm were detected the protons of the sugar skeleton. The existence of a signal at 2.14 ppm corresponding to the protons of methoxy group and the presence of a methyl singlet of rhamnose at 1.31 ppm can be observed.

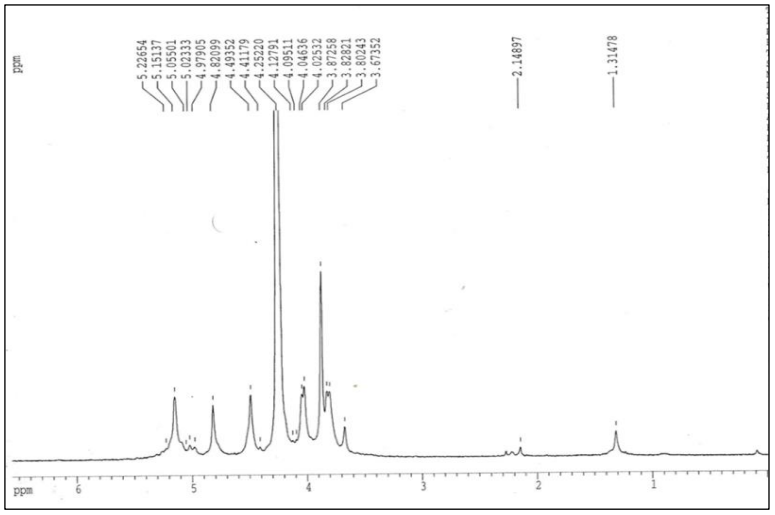

Figure 3 :[1]H NMR spectrum of the CSP fraction.

However, [18] indicated that peak signals at 1.2 ppm, 1. 35 ppm, 2.1 ppm, 3. 25 ppm, 3. 35 ppm, 4. 65 ppm, 4.95 ppm and 5.15 ppm may be the result of contamination by remnants of plant cell walls. In this study, the spectrum of ^{1}H NMR for the ASP fraction (Figure 4) shows similarities with the spectrum of ^{1}H NMR for the ASP2 fraction, extracted from the prickly pear peel. [10] was able to clarify the main structure of the ASP2 polysaccharide using methylation, ^{1}H NMR and C^{13} NMR techniques. It is a rhamnogalacturonan with a side chain containing β-D-galactosyl bound residues (1→4).

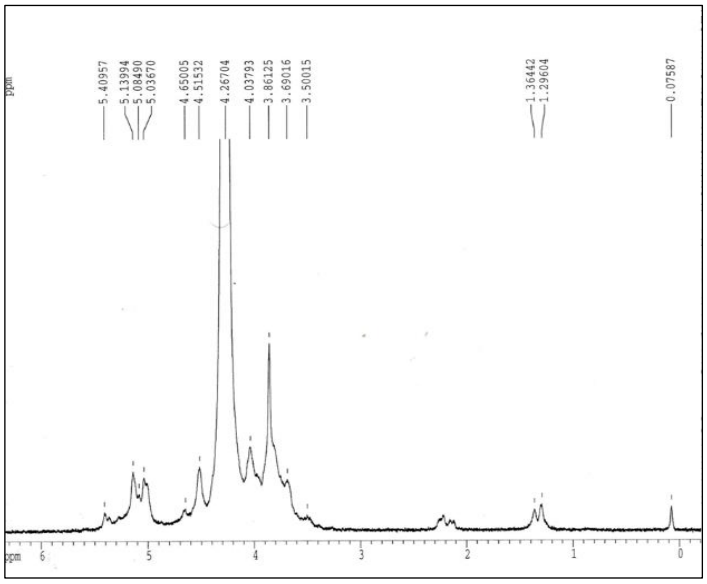

Figure 4 :^{1}H NMR spectrum of the ASP fraction.

4. Conclusion

Myrtle fruit (*Myrtus communis* L.) has been the subject of recent research and is gaining in importance in several sectors, including the food industry. Several plant matrices have been investigated, but in the case of the myrtle fruits , these constituents have never been studied in detail. Thus, this article aimed to determine the chemical composition of the pectic fractions of myrtle.

Extraction and analysis protocols by HPAEC-PAD, ^{1}H NMR led to the fractionation and identification of pectic fractions of the fruit of (*Myrtus communis* L.). The results showed that the residue D is composed of 9.8% (w/w) neutral sugars, 0.07% (w/w) galacturonic acid, and 11.7% (w/w) neutral sugars after hydrolysis with TFA (2N) and H_2SO_4 (2N), respectively. Structural identification using ^{1}H NMR spectroscopy showed that CSP and ASP are pectins.

Pectic compounds are of interest to the agricultural and food sectors for two major reasons: they play a role in the evolution of plant products and are used in industry for their gel-forming properties. These reasons take into account for the substantial quantity of our work carried out on pectic substances and pectolytic enzymes. Further studies will be performed to have a deeper comprehension of these polysaccharides' conformation and structure.

References

[1] A.Chidouh, S. Aouadi & A. Heyraud, "Extraction fractionation and characterization of water-soluble polysaccharide fractions from myrtle (*Myrtus communis* L.)," fruit. Food Hydrocolloids, vol.35, 2014, pp. 733-739.

[2] F. Couplan, Le régal végétal: Plantes sauvages comestibles. Ed. Sang de la terre, Paris : 2009, 528 p.

[3] T. Sarl, T, La boutique en corse, les plantes adaptées aux jardins et espaces verts varois. France: régis rostein : 2007, 55 p.

[4] Alkorta, C. Garbisu, M.J. Liama & J.L. Serra, " Industrial applications of pectic enzymes: a review;" Process Biochemistry, vol. 3 no.1,1998, pp. 21-28,.

[5] K. Iwasaki, M. Inoue & Y. Matsubara, "Continuous hydrolysis of pectate by immobilized endopolygalacturonase in a continuously stirred tank reactor," Bioscience, Biotechnology, and Biochemistry, vol. 62, no. 2,1998, pp.262-272.

[6] B.J.H. Stevens & R.R. Selvendran, "Structural features of cell wall polymers of the apple", Carbohydrate Research , vol. 135, no. 1, 1984, pp. 155-166.

[7] F. Guillon, J.F. Thibault, F.M. Rombouts, A.G.J. Voragen & W. Pilnik, "Enzymatic hydrolysis of the "hairy" fragments from sugar-beet pectins," Structural investigations of the neutral sugar-side chains of sugar-beet pectins, Part 2. Carbohydrates research, vol.190, no.1, 1989, pp. 97-108.

[8] E.A. Davis, C. Derouet, C. Herve Du Penhoat & C. Morvan, "Isolation and an N.M.R. study of pectins from flax (*linum usitatissimum* L.)," Carbohydrate Research, vol. 197, 1990, pp. 205-215.

[9] M.R. Vignon & C. Garcia-Jaldon, "Structural features of the pectic polysaccharides isolated from retted hemp bast fibers," Carbohydrates Research, vol. 296, no. 1-4, 1996, pp. 249-260.

[10] Y. Habibi, Contribution à l'étude morphologique, ultrastructurale et chimique de la figue de barbarie Les polysaccharides pariétaux : caractérisation et modification chimique, Thèse de doctorat : Université Joseph Fourier – Grenoble I & Université Cadi Ayad, Semlali –Marrakech, 2004, 265p.

[11] Y. Habibi, A. Heyraud, M. Mahrouz & M.R. Vignon, "Structural features of pectic polysaccharides from the skin of *Opuntia ficus-indica* prickly pear fruits," Carbohydrate Research, vol. 339, no. 6, 2004, pp.1119-1127.

[12] F.G.J. Voragen, I.P.J. Timmers, J.P.H. Linssen, H.A. Schols & W. Pilnik, "Methods of analysis for cell-wall polysaccharides of fruits and vegetables," Zeitschriftfür Lebensmittel-Untersuchung und Forschung, vol. 177, 1983, pp.251-256.

[13] H.A. Schols & A.G.J. Voragen, " Progress in biotechnology," In: J. Visser & A.G.J. Voragen. (eds.) Pectins and pectinases , . Elsevier Science BV, Amsterdam, 1996, pp. 3-19.

[14] S. Aboughe-Angone, E. Nguerna-Ona, P. Ghosh,, P. Lerouge, T. Ishii, B. Ray & A.Deriouich, "Cell wall carbohydrates from fruit pulp of *Argania spinosa*: structural analysis of pectin and xyloglucan polysaccharides," Carbohydrate Research , vol. 343, no.1, 2008, pp.67-72.

[15] F.L. Normand, R.L. Ory & R.R. Mod, "Binding of bile acids and trace minerals by soluble hemicelluloses of rice," Food Technology, vol. 41, no. 2, 1987, pp. 86-99.

[16] H. Hilz, E.J. Bakx, H.A. Schols & A.G.J. Voragen, "Cell wall polysaccharides in black currants and blueberries-characterisation in berries, juice, and pressed cake," Carbohydrate Polymers, vol.59, no. 4, 2005, pp.477-488.

[17] E. Taboada, P. Fisher, R.. Jara, E. Zúǔiga, M. Gidekel, J.C. Cabrera, E. Pereira, A. Gutiérrez-Moraga, R. Villalonga, & G. Cabrera, "Isolation and characterisation of pectic substances from murta (Ugni molinae Turcz) fruits," Food Chemistry , vol. 123, no. 3, 2010, pp. 669-678.

[18] Jones, C., Mulloy, B.: The application of nuclear magnetic resonance in structural studies of polysaccharides. In: Jones, C., Mulloy, B., Thomas, A. H. (eds.) Methods in molecular biology, spectroscopic methods and analysis NMR,mass spectrometry and metalloprotein techniques (1993), pp. 149-167 . NJ: HumansPressInc, Totowa (1993).