Algerian Journal of Environmental Science and Technology
August edition. Vol.4. Nº2. (2018)
ISSN : 2437-1114
www.aljest.webs.com

Valorization of Algerian leguminous: extraction and characterization of galactomannans

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ARTICLE INFO

Article History :

Received : ***02/05/2018***
Accepted : ***12/07/2018***

***Key Words**:*

Polysaccharides ;
Characterization ;
Galactomannanes ;
Ceratonia siliqua ;
Gleditsia triacanthos.

ABSTRACT/RESUME

Abstract : *This study has an environmental objective based on the valorization of the seeds of legumes in order to obtain a clean nature, its seeds are considered as a good source of galactomannans which can replace other synthetic polysaccharides.They consists on the evaluation of physicochemical properties of galactomanans isolated from different seeds parts of two leguminous species widely known in Algeria: Gleditsia triacanthos and Ceratonia siliqua (carob), which represent an important source of polyscharids. The extraction gave an appreciable yield assessed at 12.97% of Gleditsia triacanthos and 11.45% of Ceratonia siliqua; values witch encourage their valorization. The physicochemical composition of the extracted galactomanans showed comparable characteristics in term of humidity; pH; °Brix and ashes. While; rheologic properties revealed that galactomanans solutions have a pseudo plastic nature.*

In the other hand; the functional properties analysis showed that the galactomanans obtained are soluble in water; the water absorption capacity was higher for Gleditsia triacanthos than Ceratonia siliqua with 20.62 and 5.98 respectively. Infrared analysis demonstrate that the same chemical structures of galactomanans were extracted from the two spices. NMR analysis allowed us to estimate the galactose / mannose ratio of the two extracts. This ratiois 1.25/1 and 3.63/1 of Gleditsia triacanthos and Ceratonia siliqua respectively.

I. Introduction

Gleditsia triacanthos and Ceratonia siliqua (Caroub), belonging to the legume family (Fabaceae), are very abundant in Algeria. In addition to their ornamental and landscape value, all the components of these trees (foliage, fruit, wood, bark, roots) are useful and can have high added value in several industrial sectors and the valorization of its seeds can guarantee the cleanliness of the environment.. These trees adapt well to extreme conditions, especially to drought and salinity [1].Fadel, Fattouch [2] have shown that Carob extracts (leaves, pulp and seed) are effective natural antifungals. Essential oils, extracted from Ceratonia siliqua, has shown antimicrobial activity against Listeria [3].Carob is used in the food industry as an antioxidant due to its polyphenol-rich composition [2]. The leaves of Gleditsia triacanthos have demostrated an antioxidant activity and are rich in polyphenols [4].

The use of these two legumes in animal feed has also been studied. Studies have been reported on carob flour [5] and Gleditsia pods [6].

Gleditsiatria canthos and *Ceratonia siliqua*are also considered as sources of polysaccharides.

Indeed, Ceratonia siliquais mainly exploited for the production of caroub bean gum E410 found in the endosperm of Caroubier seeds [7]. On the other hand, according to Sciarini, Maldonado [8] and Bourbon, Pinheiro [9], the presence of galactomannans in the seeds of Gleditsia triacanthos was first reported by Mazzini and Cerezo [10]. Galactomannan a water-soluble and neutral polysaccharides which have a main chain of mannan bearing galactose units [11, 12]. These polysaccharides can be distinguished by their molecular weight, their mannose/galactose ratio (M/G), and the distribution of galactose residues [11, 13, 14]. These polysaccharides have the ability to form high-viscosity solutions at low concentrations [9]. These stable solutions have different physicochemical properties and can be used in various forms for human consumption [15].

The non-toxicity of galactomannans allows their use in various industries. in fact, they are used in the textile, pharmaceutical and biomedical products, cosmetics as well as in the food industry [15].

Due to their techno-functional properties, these hydrocolloids are widely used in dairy and bakery products, sauces, beverages, frozen jam and other processed food products in order to improve their texture characteristics and increase their shelf life [16]. Galactomannans play an important role in the rheological properties of food products and are used as stabilizer, thickener and gelling agent [13, 16, 17].

Due to the importance of galactomannans in various industries and for possible local exploitation, the content and composition of these polysacharides were investigated in the Algerian Gleditsia triacanthos and Ceratonia siliqua seeds extracts. To the best of our knowledge, galactomannans extracted from such local seeds have never been characterized.

II. Materials and methods

II.1.Plant material

The pods of Gleditsia triacanthos and Ceratonia siliqua were harvested in Boumerdes region (northern Algeria, 50 km east of Algiers with the coordinates of 36 ° 45'37.23 "N 3 ° 28 '20 .52 "E) from September 2014 to December 2014 (Picture 1). The seeds were separated by manual crushing of the dried pods and stored in glass jars until they were used.

Picture 1. *The pods and seeds of Gleditsia triacanthos (left) and Ceratonia siliqua (right)*

II.2.Raw material preparation

Raw material was obtained following the described procedure with small modifications [7, 8, 14]. Brefly, Gleditsia triacanthos and Carob seeds were allowed to swell overnight in distilled water in a ratio of 1: 6 (W/V) in order to facilitate the manual separation of the various parts. The samples were then dried followed by grinding using a laboratory mill type Ika M20. A fine powder of each part (tegument, endosperm and embryo) was obtained.

II.3.Galactomannans extraction

According to the literature and following preliminary extraction tests, the galactomannans were located in the seed coat for the Gleditsia triacanthos seeds, where as they were located in the endosperm in the case of carob seeds.

The polysaccharides were extracted following the procedure reported by Cerqueira, Souza [18]. From an amount of powder of each constituent washed with ethanol (1/3: w/v), followed by heating at 70 ° C during 15 minutes. The soluble substances were displaced and a cake was recovered. The pellet was then immersed in distilled water (1/5: w/v) during 24 hours. Distilled water was gradually added to the aqueous solution with stirring during 60 minutes at room temperature. The solutions obtained were centrifuged during 20 minutes at 10000 rpm in order to remove the insoluble matter. The polysaccharides solubilized in the crude gum solution were precipitated and purified with absolute ethanol. A lyophilization step is carried out after purification followed by grinding in fine particles or flour. The yield of galactomannans yield was determined as the ratio of the mass of the powder of the polysaccharide present in a unit of mass of raw material (Gleditsia and Carobe powder) expressed as a percentage [18].

II.4.Physicochemical characterization of the galactomannanes

II.4.1.Chemical characterization

The chemical characterization of the obtained galactomannans was based on the following parameters: water content (NF V 05-108, 1970), ash: (NF V 05-113, 1972), pH (NF V 05-108, 1970), and the Brix degree (NF V 05 -109, 1970).

II.4.2.Physical Characterization

The physical characterization of the obtained galactomannans was based on the following parameters: color, grain size, water absorption capacity, and solubility.

II.4. 2.1.Color measurement:

This parameter was measured using a CIE-Lab colorimeter following the described procedure [14]. The values of L, a and b were measured according to the color space for the surface colors defined by the

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International Commission on Illumination (CIE), with:
L: represents lightness from 0 (black) to 100 (white).
a: 60 (green) a + 60 (red).
b: 60 (blue) a + 60 (yellow).

II.4.2.2.Granulometry measurement

The particles size measurement was carried out by dry route using a Matersizer type laser particle size analyzer from Sirocco2000A-Malvern, Instruments Ltd, equipped with a data processing software version 5.10F. The laser diffracts on the particles and the width of the air task to estimate the powder particles size.

II.4.2.3.Water Absorption Capacity (WAC)

As previousley described, the water absorption capacity was determined with a sample of 0.5 g weighed in a tube and then supplemented with distilled water until powder was completely submerged. The tubes were then centrifuged at 1600 g during 10 min. The supernatant, separated from the swollen pellet,was then weighed [8, 19].
The water absorption capacity (WAC) was calculated by the following formula:

$$WAC = [(P_{sediment} - P_{sample})/P_{sample}] * 100$$

$P_{sediment}$: mass (g) of the solution after centrifugation (pellet).

P_{sample}: mass (g) of the solution before centrifugation.

II.4.2.4.Solubility

The water solubility of the extracted galactomannanes was measured following the described procedure [8]. Brefly, 30 ml of a 1% (w / v) galactomannan solution was heated at 30°C, 60 °C, and 90 °C in a water bath with stirring during 30 min. The suspensions were then centrifuged at 800 .mu.m -1 during 15 min. Finally, supernatant (10 ml) was dried at 125 °C overnight to a constant weight.
The solubility was calculated by following formula:

$$Solubility\ (\%) = [(mf-mi)*30/10]*100$$

m_f: final mass (g) of supernatant after drying.
m_i: initial mass (g) of supernatant before drying

II.4.2.5.Viscosity (Rheological analyzes)

The rheological properties were evaluated using a rotary rheometer (AR 2000) following the described procedure with slight modifications [7]. 1% gum solution (100 ml) were incubated for 1 hour at 25, 35, 45 and 55 ° C before the viscosity reading.

II.5. Characterization of galactomannans by FTIR

Infrared (IR) absorption spectra were recorded using a Fourier Transform Infrared (FTIR) spectrophotometer type αBrukerlazer.

II.6. Determination of theMannose/Galactose ratio of the Isolated Galactomannans byNMR

^{1}H NMR spectra of the isolated galactomannans were recorded at 25 ° C using a BrukerAvanceIII® Spectrometer 400 MHz.The concentration of the gum concentration (0.1% (w/v) in D_2O)as well as the duration of analysis have been optimized in order to obtain NMR spectra with good resolution.

III. Results and discussion

III.1. Galactomannan yield

The yield of galactomannans is respectively 12.97% and 11.45% for Gleditsia triacanthos and Carob; values judged appreciable that may favor their valuations. In fact, multiple parameters influence the rate of return such as the extraction method, the nature of the used solvents, the origin and the culture conditions [19-21]. Indeed Sciarini, Maldonado [8] in their work, used three methods of extraction and recorded yield values between 11,90-34,16%

III.2.Physicochemical characterization of galactomannanes

The obtained physicochemical properties of the galactomannans are presented in **Table 1**.

Table 1. *Chemical characterization of galactomannans obtained from the seeds of carob and Gleditsiatriacanthos*

	Moisture (%)	Ash(%)	pH	°Brix(%)
*Carob*Galactomannans	12.04±0.49	0.65±0.03	7.05±0.14	0.93±0.03
Gleditsiatriacanthos Galactomannans	13±0.1	0.89±0.01	7.07±0.11	0.80±0.02

According to the results recorded, it is noted that galactomannans from both species have the same properties. The results of the water content and ash are very close to those reported in the literature (12 to 13% moisture and between 0.65-0.89% ash in the two obtained galactomannans). On gums extracted by different solvents, ,[14]recorded moisture and ash values between 8.11-11.30% and 0.84-1.02% respectively. In addition, Bourbon, Pinheiro [9] reported moisture values between 10.17-13.39% and ash levels between 0.08-0.72% respectively on industrial carob and guar gums and galactomannans extracted from Gleditsia triacanthos and Sophora japonica.

The pH values confirm the neutrality of the polysaccharides. Our results are in the range reported by Pawar and Lalitha [22], as a value between 6.5 and 7.3.

According to the obtained Brix degree values, the analyzed galactomannans seem to retain their initial structure and have not undergone any hydrolysis during the extraction step. These results are very close to those given by Kıvrak, Aşkın [14]as the values of 0.57, 0.73 and 0.93.

III.2.1. Physical properties

III.2.1. 1.Color

The obtained results **(Table 2)** confirm what is observed with the unaided eye, namely that the color of the carob galactomannan is whiter than that of G. triacanthos.

Table 2. *CIE Lab values for galactomannan color*

Sample	**L**	**a**	**B**
Carobe	84.16±0.3	0.2±0.01	3.4±0.1
Gleditsiatriacanthos	81.8±0.1	12.7±0.01	8.4±0.1

Using different methods of extracting the gums,Kıvrak, Aşkın [14] had reported very similar values to ours, as measurements of L in the range between 80.55 and 86.28.

III.2.1.2.Granulometry of galactomannan powders:

The obtained particle size distribution is shown schematically in **Figure (1).** The grain size curve of the Gleditsia powder shows that the latter has a bimodal distribution (less homogeneous) where as the Carob curve shows a uniform size distribution at a single peak.

For the powder of Gleditsia triacanthos, a dominant (90%) of the population whose particles have a diameter of 394, 438 microns, This distribution has finer sizes than the carob powder which has a dominant population (90%) of which the particles have a diameter of 567,023 µm.

It should be noted that despite the importance of granulometry in the solubilization of polysaccharides and the preparation of gums and in the light of the literature reviewed, no work in our opinion has been reported on this factor, which probably depends on the type of grinding the powder.

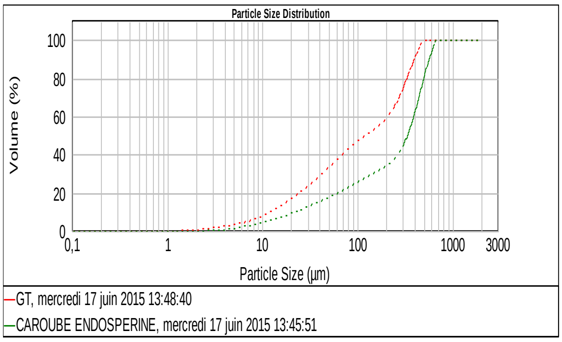

Figure1 .*Volume granulometric distribution of galactomannans isolated from two legumes - Particle size distribution by volume of galactomannans isolated from two legumes*

Algerian Journal of Environmental Science and Technology
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III.2.1.3. Rheological Property of Galactomannans :

Figures 02 and 04 show the viscosity curves of the galactomannan solutions. Similar patterns are observed in both species and coincide with those reported previously in the work of Cengiz, Dogan [19]. Moreover, the constraint curves shown in **Figure3** and **Figure 5** are typical of Rheofluidifying liquids. The decrease in apparent viscosity is followed by an increase in shear rate where the viscosity is inversely proportional to the temperature (Dakia et al., 2010).

At 55 ° C, the viscosity is higher in Gleditsia compared to that observed in Carob which requires according to Dakia, Wathelet [11], heating with strong stirring up to 80 ° C to reach its maximum viscosity. According to these authors, the difference observed is related to the number of galactose residues on the galactomannan chain.

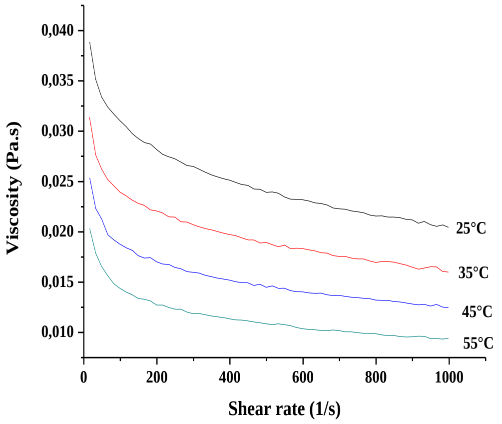

Figure 4. *The viscosity of Gleditsia galactomannans at different temperatures*

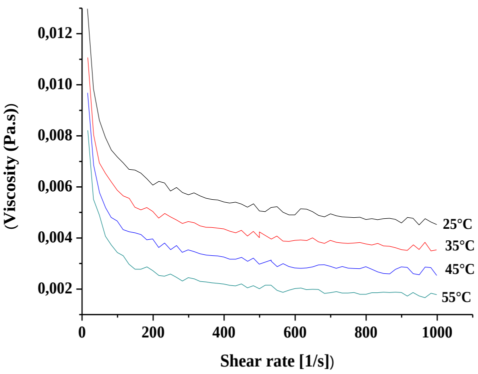

Figure 2 .*The viscosity of galactomannans of Carob at different temperatures*

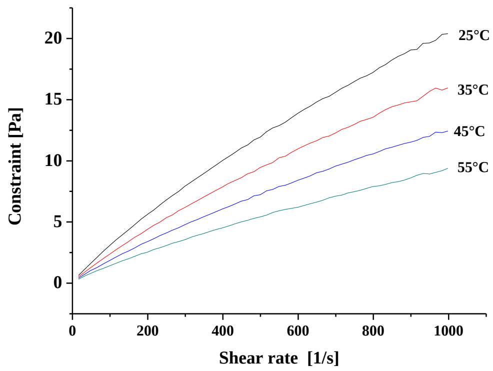

Figure 5. *The constraint of the galactomannans of Gleditsia at different temperatures*

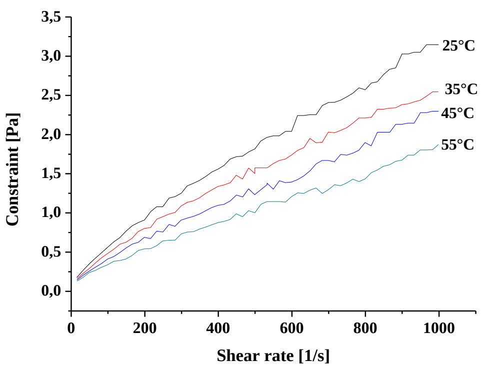

Figure 3. *The constraint of Carob galactomannans at different temperatures*

III.2.1.4. Water Absorption capacity:

The highest value was observed in galactomannans of Gleditsia triacanthos compared with that of carob **(Figure 6)**. In this sense, Sciarini, Maldonado [8]in their work on the water absorption capacity in commercial gums (xanthan and guar gum) and in galactomannans isolated from Gleditsia triacanthos by different extraction methods, recorded values between 8.19 and 27.33. He explains this variation by a higher proportion of branching in Gleditsia and a lower M / G ratio.

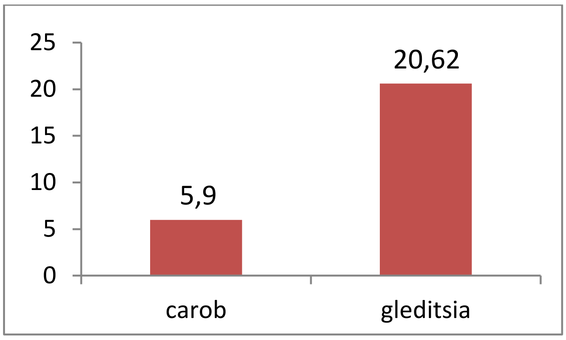

Figure 6. *Water absorption capacity of studied powders.*

III.2.1.5. Determinations of solubility:

The solubility analysis shows that at low temperature (30 ° C), the galactomannans of the studied species are partially soluble in water. The solubility evolves with the increase of the temperature and more than 50% of the initial value is observed towards 60c ° **(Figure 07).** According to López-Franco, Cervantes-Montaño [17] and Sciarini, Maldonado [8] the low solubility values of the polysaccharides are probably due to their low degree of M / G ratio, whereas the high values are observed when there is a break in the hydrogen bonds. These observations are recorded in galactomannans extracted from Gledisia which has a M / G ratio lower than that of Ceratonia. Indeed, during dissolution, macromolecules containing more galactose residues will be more easily soluble cold [11, 23],Therefore, the higher the degree of substitution, the higher the solubility of galactomannans in water.

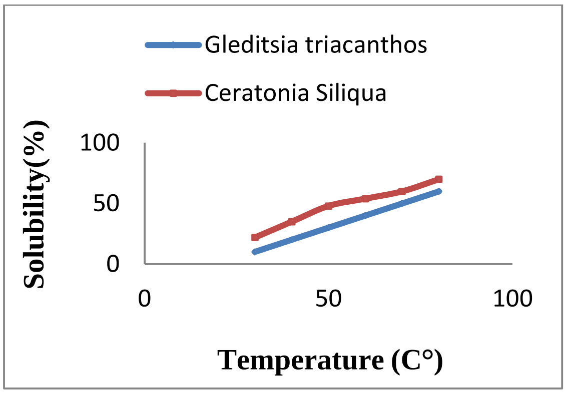

Figure 7. *Solubility of galactomannans as a function of temperature*

III.3. FT-IR analysis

Figure 08 shows the FTIR spectra of galactomannans analyzed. The spectra of Gleditsia triacanthos and Ceratonia siliqua show similar patterns characteristic of the structure of polysaccharides. The appearance of the obtained FTIR curve is similar to that reported by several authors [17, 24, 25]obtained from galactomannans from different sources.

The bands between 3100-3500cm-1 indicate the presence of the C-H and O-H polysaccharide linkages attributed to the hydroxyl function [17, 24], and the 1639cm-1 and 1645cm-1 bands correspond to bound water [24, 25]. The 1377cm-1 band is attributed to the C-H [24]laison and another to 1028-1020cm-1 associated with the vibrations of C-O and C-O-H functions of carbohydrates[15] where for example glycoside bonds linked to the composition of galactomannanes[17, 24]. The bands that lie between 811-870cm-1 are attributed to Β-D mannopyranose and αD galactopyranose [17, 24, 26].

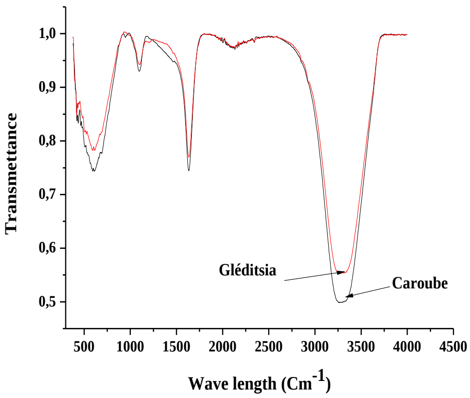

Figure8 . *IR spectra of FTIR galactomannans analyzed.*

III.4. NMR analysis:

The [1]H-NMR spectra of a solution of 0.1% galactomannans in D_2O are shown in Fig. 9 and 10. The two distinct signals in the anomeric region at δ 4.07 and 4.95were assigned to the anomerichydrogens (H-1) of β-D-mannopyranose (M) and α- D-galactopyranose (G) respectively.

The ratios of mannose to galactose (M/G) can be also obtained directly from the relative areas of the signals for H-1, the ratio were1.52 for Gleditsia triacanthos and 3.63 for carob.

The obtained M/G are in concordance of the reported ratios in the literature [13, 24]. These rations confirm the results of the rheological and physicochemical analyzes of the isolated galactomannans (solubility, water absorption capacity and viscosity), whose galactose's content controls the mechanism of the interactions that influence the macromolecular characteristics [11].

Based on the M / G ratios observed in both legumes, the NMR spectral results confirmed those obtained with solubility measurement, water absorption capacity, viscosity and infrared analysis.

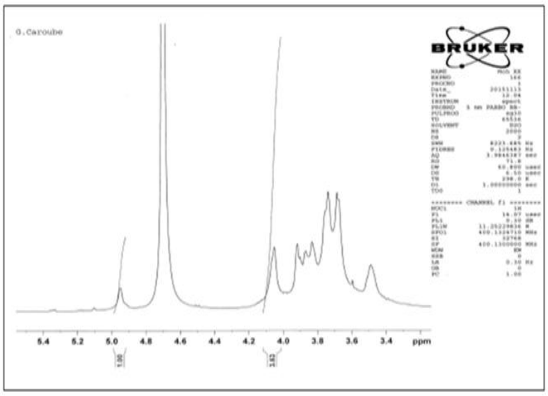

Figure 9 . *NMR Spectrum of Galactomannans Isolated from Carob Seeds*

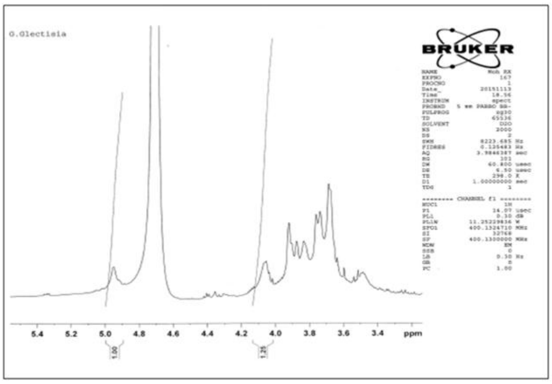

Figure 10. *NMR Spectrum of Galactomannans Isolated from GleditsiaTriacanthos.*

IV. Conclusion

The advantage of the study of Gleditsia triacanthos and Ceratonia siliqua grown in Algeria is based on the abundance of these trees that are known to be ornamental legumes and the prevention of their waste reconciles economic benefits and environmental protection.

In this work, we have tried to contribute to the characterization of galactomanames from these two plants to better understand the valorization process by exploiting their richness in polysaccharides. Indeed, because of their rheological and physicochemical properties, the two galactomannan species have similar profiles but the M / G ratio remains the critical point to determine in the structure of these polysaccharides. Moreover, the difference observed in the "microstructure" of the samples would strongly influence the properties of the galactomannan solutions.

Finally, the knowledge acquired during this study on the characteristics of the polysaccharides studied will undoubtedly allow us to better understand the influence of the structure and the composition of the solutions on the technological aptitudes of the galactomannans in order to be used as a major ingredient in various products developed by the industry.

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Please cite this Article as:

Bouhadi N.,Hachemi N.,Kahlouche A.,Djeziri M.,Touabia M., Nouani A., Valorization of Algerian leguminous: extraction and characterization of galactomannans, **Algerian *J. Env. Sc. Technology*, *4*:2 (2018) 4 -11**