Rheological Study And Prebiotic Potential Of Cereus Triangularis Cladodes Extract

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Abstract: Cereus triangularis is a cactus belonging to the sub-family of Cactoideae. Its cladodes are used in food decoction as a traditional medicine in Madagascar. The chemical structure of polysaccharide extracted from its cladodes is a type I arabinogalactan with a high molecular weight.In this study we have investigated its physicochemical properties, the rheological properties and prebiotic property of the oligo- or polysaccharides cladodes of Cereus triangularis. The rheological properties of this galactan are characteristic of a pseudoplastic fluid with a weak gel behavior. Storage modulus (G′) and loss modulus (G″) of the polysaccharide in 0.5 M NaCl and KCl 0.5 M revealed the viscoelastic properties.Its enzymatic degradation using a fungal galactanase led to the production of oligomers and low molecular weight polysaccharides which have been successfully tested as prebiotics.

Keywords: arabinogalactan, Cereus triangularis, prebiotic, rheological.

1. Introduction

The familly of Cactaceae has been considered a potential source of an industrial hydrocolloid gum. The main constituent of this secreted fluid is polysaccharide mucilage [1], [2], [3]. In general, mucilage is a complex polymeric substance composed mainly of carbohydrates [4]. It also contains glycoproteins [5] and other substances such as tannins, alkaloids, and steroids [6]. Mucilages have rheological properties that are of great interest for a wide range of thickening agents for the food and pharmaceutical industries [7], [8], [9]. Two types of water-soluble materials can be extracted from Cactus: “cactus mucilage”and, “cactus pectin”, the two terms have been used interchangeably in the literature to refer to two distinct carbohydrate polymer fractions; an extract which gels in the presence of Ca^{2+} ions, and a non-gelling extract [10]. Several researchers have shown that while both extracts contain arabinose, galactose, xylose, rhamnose, and galacturonic acid, the main difference is that the gelling extract has a much higher proportion of galacturonic acid [1], [2], [7], [11], [12]. The non-gelling extract (cactus mucilage) is a heteropolysaccharide blend comprising mainly L-arabinose, D-galactose, L-rhamnose and D-xylose as well as galacturonic acid [1], [2], [12], [13], [14], [15]. The gelling extract (cactus pectin) is pectin. Hence, cactus mucilage is often referred to as a pectin polysaccharide [10]. The hydrocolloid characteristics of these biopolymers are theprimary motivation for their utilization in several domains [16]. The ability of mucilage to form molecular networks and retain large amounts of water makes it a potential source of hydrocolloids for the chemical and cosmetics industries. Several studies have evaluated its uses in water purification/filtration [5], [16], as an adhesive lime [$Ca(OH)_2$] [17], emulsifying agent [18], or flocculent [19], and as an enhancer of water infiltration in soils, due to its physical properties (viscosity, elasticity, texture, and emulsifier) [20]. Other mucilage applications include its use in foods as a stabilizer, flavoring agent, fat substitute [16]. Furthermore, the cactus family (Cactaceae) is one of nature’s most biologically active resources, as they possess a wealth of bioactive compounds. For example, compounds isolated from Opuntia have demonstrated various biological activities, such as thickening agents for the food and pharmaceutical industries [7], [8], [9], antioxidant potential [21], [22], [23], anti-inflammatory

properties [21], [24], [45], antiglycation activity [26], anti-viral activity [27] and apoptotic activity [28], [29]. As a result, Cactus-derived compounds have important applications in a range of products in food, pharmaceuticals and cosmetics [30] In addition to bioactive components, Cactus are a rich source of dietary fiber (40–60% dry weight) [31]. There are considerable numbers of researches investigating dietary fiber from new sources to use in food industry as a source of prebiotic [32], [33], [34]. Prebiotics were defined as "non digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health" [35]. Non-digestible polysaccharides such as galacto-oligosaccharides, fructooligo-saccharides and cyclodextrins are known to be prebiotic substances which selectively stimulate the growth and/or activity of the gastrointestinal micro-flora [36]. Thus, the increased demand for these mucilages in many sectors at the global level prompts us to seek new sources of mucilages [37]. Sustainable plant and marine natural resources of biomass may be used as less expensive alternatives for producing industrial mucilage. Among resources currently being sought for this purpose, indigenous plants from arid lands deserve special attention due to their agronomic advantages, such as the low input of water and energy needed for their commercial exploitation. Consequently, the study of rheological behavior and prebiotic, Activity of polysaccharide extracted from cladodes of Cereus triangularis (Cactaceae) is then needed. It contributes to identification of potential applications, development of new products [38], [39]. Cereus triangularis a cactus used in food decoction as a traditional medicine in the North region of Madagascar to reduce stomach ache and intestinal diseases. Hydrocolloids were sequentially extracted from its cladodes with a yield of 24% (240 mg/g based on dried cladodes powder). Structural analyses has revealed that this polysaccharide with a molecular mass of 8430,000 g/mol was mainly composed of a galactan backbone of a (1→4) linked β-D-Galp residues probably substituted at position 3 by L-arabinofuranosyl residues [38]. Therefore, this study was conducted to reveal the possible uses of mucilge of Cereus triangularis. The objectives of this study are: (i) to identify the rheological properties, (ii) to evaluate the prebiotic potential of the mucilage isolated from Cereus triangularis.

2. Materials and methods

2.1. Polysaccharide extraction

Specie of Cereus triangularis were collected at Sakaramy in the North region of Madagascar in December 2013. The cladodes were carefully washed in potable water. A steel knife were used to slice the stalks lengthwise to produce sheets with a length of 3 ± 0.5 cm and a width of 2 ± 0.5 cm and dry in the oven (JOUAN—No.78 120) at 40 °C. Dried materials were ground into powder using a high speed mechanical blender (BLENDER 800 ES). Polysaccharide was extracted by mixing the dry powder of cladodes in distilled water (1:20 (w/v)) at room temperature for 3 h. Solid matter was separated via filtration through glass filter G-1 (100–160 mm) and secondly through glass filter G-4 (10–16 mm). The water-soluble polysaccharide in the filtrate were precipitated with ethanol 96% (3 vol.) and washed with acetone. The polysaccharide was collected by filtration under-vacuum, dried at 40 °C overnight and then stored in hermetically sealed bottles at room temperature.

2.2. Determination of Molar Mass of Polysaccharide

Molar mass distributions, weight average molar mass (Mw), number average molar mass were determined at 25 °C by steric exclusion chromatography (SEC), coupled to a multi-angle light scattering detector (SEC MALLS) and a refractometer differential. Multiple-angle light scattering (MALS) measurements were performed using a spectrophotometer mini DAWN. A $NaNO_3$ solution (0.1 mol L^{-1}), pH 4.5 was used as the carrier after filtration through 0.02 µm filter unit (Millipore SAS, France). This solvent was carefully degassed using a DGU-20A3 unit (Shimadzu RID-10A, Japan) and eluted at 0.5 mL min^{-1} flow rate (LC-20AD; Shimadzu). One hundred microliters of each sample, filtered at 0.45 µm, was injected using an automatic injector (SIL-20A; Shimadzu). The fractionation was obtained using an OHPAK SB 80-G guard column (6 mm x 50 mm) followed by two gel-packed polyhydroxymethyl metacrylate (OHPAK SB 803 HQ and 806 HQ) columns (8× 300 mm) in series (Shodex Showa Denko K.K., Japan). The MALS photometer, a DAWN-EOS from Wyatt Technology Corp., Santa Barbara (CA), was equipped with a K5 cell and a Ga–As laser (λ=690 nm). All collected data were analyzed using the Astra V-4.50 software package with the Zimm plot (order 1) technique for molar mass estimation.

2.3. Rheological measurements

The rheological properties were measured using a controlled stress rheometer AR-2000 (TA Instruments, New Castle, DE, USA). All the rheological studies were conducted at 25 °C using cone-plate geometry: 40 mm diameter and 2° cone. The volume of the sample was 10 ml. The double gap cylinder was thermostated with a circulating bath. Polysaccharides solutions 1 to 10% (w/v) were prepared in KCl or NaCl (0.5 M) stirred for 2 h at room temperature. The shear flow behavior was assessed over shear rates of 0.1–1,000 s^{-1}. Oscillatory sweeps were conducted between 0.05 and 100 Hz in the determined linear viscoelastic conditions.

2.4. Measurement of intrinsic viscosity

The intrinsic viscosity technique is one of the most employed methodologies with the aim of obtaining thermodynamic parameters of mixtures [41]. The intrinsic viscosity [η] of a polymer estimates its contribution to the apparent viscosity of a solution and is a convenient way to measure its change of conformation in response to changes in environmental conditions.

It is defined by Eq. (1):

$$[\eta]=\lim \eta_{réd}= \lim_{c\to 0} \frac{\eta-\eta_0}{c\eta_0}$$

The specific viscosity is related to the intrinsic viscosity by Huggins Eq. (2):

$$\eta_{sp}/C= [\eta]+ k'[\eta]^2C+\dots$$

Intrinsic viscosity is calculated by determining ηsp/C and extrapolating to infinite dilution. The constant $K_H$is termed the Huggins constant and is dependent to interactions polymer– polymer and polymer–solvent.

The Mark–Houwink–Sakurada Eq. (3) links [η] and the absolute weight-average molar mass Mw:

$$[\eta] = k. M_w^a$$

Where k and a (conformational information) are constant parameters for a well-defined polysaccharide–solvent pair. A capillary Ubbelohde viscometer (CINEVISCO, SEMATECH) was used to determine intrinsic viscosities of polysaccharides using several concentrations of polymer (between 0.1 and 0.2 $g.L^{-1}$) in $NaNO_3$solution at 0.3 M. At these concentrations, the behaviors of the solutions are Newtonian even at very high shear rate (around 800 s^{-1}) in the capillary viscometer (data not shown).

2.5. Enzymatic depolymerization

2.5.1. Enzymes

In this study, two types of enzymes were used to degrade the polysaccharide of Cereus triangularis

- an endo-β- (1,4) -galactanase (E-EGALN) of Aspergillus niger (Megazyme International Ireland) with a specific activity of 400 $U.mg^{-1}$ (40 °C, pH 4 on potato galactan) .
- an endo-β-(1,4)-galactanase (E-GALCT) of Clostridium thermocellum (Megazyme International Ireland) with specific activity $U.mg^{-1}$ (40 °C., pH 4.5 on potato galactan). The unit of galactanase activity (U) is defined as the amount of enzyme necessary to release one µmole of galactose per minute.

2.5.2. Substrates

To validate the enzymatic activities of the enzymes, two substrates were used: potato β-(1,4)-D-galactan (P-GALPOT) (Lot 120501b) (Megazyme International Ireland) composed of Gal:Ara:Rha:GalUA with the ratio 87:3:4:6 and β-(1,4)-D-galactan (P-PGAPT) (Lot 80503c) (Megazyme International) Ireland) composed of Gal: Ara: Rha: GalUA with the ratio 82:6:3:9.

2.5.3.Enzymatic hydrolysis

Substrate 0.2 g was dissolved in 19.8 mL of sodium acetate trihydrate buffer (100 mM, pH 4) with stirring (600 rpm) for 2 h at 40 °C. After this dissolution, 200 µL of enzyme solution (130 U) was added and the mixture is incubated at 40 °C. Samples are taken regularly every hour between 0 and 8 hours of incubation. To inactivate enzymes, each sample taken was incubated for 5 min at 100 °C and then, it was iced at -20 °C. To evaluate the efficiency of enzymatic hydrolysis, the degree of polymerization (DP) of the products resulting from the enzymatic hydrolysis was measured using high-performance anion exchange chromatography (HPAEC) on a Carbopac PA-1 analytical column (4mm × 250mm). Detection was performed with a pulsed amperometric ED50 detector (Dionex Corp., Sunnyvale, CA). Twenty-five microliters of sample were injected.

2.6. Digestibility

Study of in vitro digestibility of the polysaccharide extracted from C.triangularis cladodes was carried out in simulated conditions of artificial human gastric juice at 37°C by calculating their degree of hydrolysis when subjected to artificial human gastric juice [42]. FOS and inulin were used as controls, because, they resist enzymatic digestion in the upper gastrointestinal tract, they reach the colon virtually intact where they undergo bacterial fermentation [43]. The sample was prepared with distilled water at a concentration of 1% (w/v) and incubated at 37 ± 1 °C for 2 h. The sample (1 mL) was mixed with 5 ml artificial human gastric juice (HCl buffer) containing (in g/L): NaCl 8; KCl 0.2; Na_2HPO_4. $2H_2O$ 8.25; $NaHPO_4$ 14.35; $CaCl_2.2H_2O$ 0.1; et $MgCl_2.6H_2O$ 0.18, adjusted to pH 1 and pH 5 with the addition of 5 M HCl [41]-[42].The mixture was incubated at 37°C for 6 h. Samples were drawn at 0, 0.5, 1, 2, 4 and 6 h. Reducing sugar and total sugar content in sample was determined by Phenol-sulphuric acid method [45]. Percentage hydrolysis of sample was calculated according to equation below based on reducing sugar liberated and total sugar content of the sample [42]. % hydrolysis = reducing sugar released/total sugar content − initial reducing sugar content × 100

2.7. Test prebiotic

The medium components Man-Rogosa-Sharpe (MRS)at pH 6.3 were dissolved in deionized water and autoclaved for 20 min at 120 °C. The composition of this medium included, among others, Peptone animal (Fluka) 10 g/L, meat extract (Fluka) 8g/L, yeast extract (Fluka) 4 g/L, K_2HPO_4 2g/L, $CH_3COONa.3H_2O$ 5 g/L, Triammonium citrate 2 g/L, $MgSO_4.7H_2O$ 0.2 g/L, $MnSO_4$. H_2O 0.05 g/L and 1 mL of Tween 80. The carbonaceous substrates were either the extracts to be tested (oligofructoses (FOS F97), inulin, or arabinogalactan and oligosaccharides of Cereus triangularis), or glucose 2 % (w/v). The various carbon substrates were filtered at 0.22 µm and tested in the presence and absence of ascorbic acid 0.2 % (w/v) used as reducing agent. The culture of Lactobacillus rhamnosus was prepared from 1 mL of culture in exponential phase on MRS medium used to inoculate 10 mL of MRS medium. These 10 mL of culture were incubated for 48 to 72 hours at 37°C. Microbial growth was followed by measuring its absorbance at 600 nm. This culture is used for prebiotic tests after dilution in the sugar-free MRS medium at an Optical Density (O.D) of 0.1. Twenty µL of this culture is used to inoculate 180 µL of MRS medium containing sugars to be tested in microplate wells. The mixture is then supplemented with 55µL of mineral oil (Silicone) to prevent the evaporation of the medium. The bacterial growth is followed by measuring the absorbance at 600 nm using a bioscreen microplate reader (Perklin-Elmer) over a period of 32 h. The tests are carried out in triplicate.

3. Results

3.1. Molecular mass and viscosity intrinsic

For the polysaccharide of Cereus triangularis, the molecular weights (Mw), the number average molecular weights (Mn) and the intrinsic viscosity were determined by high-performance steric exclusion chromatography coupled with the diffusion of multi-angle laser light (SEC / MALLS) and to in-line viscosimetric detection. In this work, the analyzes were carried out on two types of polysaccharide solutions (unfiltered solution called Solution 1 « S1 » and filtered solution through a Millipore filter (0.45 µm) called Solution 2 « S2 ») to be able to measurethe disparity. The term disparity is the measure of the heterogeneity of sizes within a preparation of any polymer and can refer to either molecular mass or degree of polymerization [46]. Firstly, for the unfiltered solution, results showed that the weight-average molar mass (Mw) and number-average molar mass (Mn) of this polysaccharide were, respectively, 8.43 x 10^6 $gmol^{-1}$ and 6.96 x 10^6 $gmol^{-1}$ (Table 1). The polydispersity index was 1.21. This result indicates the presence of a homogeneous polysaccharide structure of high molecular mass. As shown

in the literature [47], the absence of heterogeneity in Mw measurements (Figure 1) indicated the very low presence of pectic polysaccharides in the mucilage extracted from the C. triangularis cladodes.

Table 1: Weight-average molar mass (Mw) and number-average molar mass (Mn) of Cereus triangularis polysaccharide

	F2	F1	S1
[η] (mL/g)	455	876	-
Mw (g/mol)	1.27×10^6	2.81×10^6	8.43×10^6
Mn (g/mol)	1.62×10^5	1.26×10^6	6.96×10^6
Ip	7.8	2.2	1.21
Rh (nm)	37	67	-
Rg (nm)	-	120	-
Rg/Rh	-	1.74	-

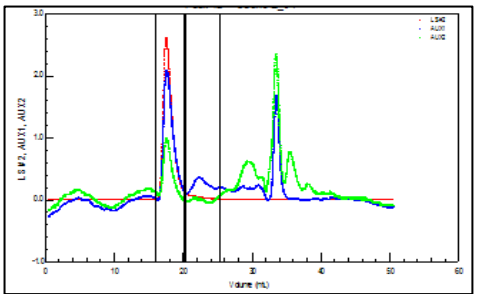


Figure 1*: [S1] HPSEC-MALLS analysis of C. triangularis cladodes (unfiltered solution). With LS: laser detection, AUX1: refractometric detection and AUX2: UV detection.*

Secondly, the analysis is carried out after filtration of the polysaccharide solution on a 0.45 µm filter in order to remove any aggregateand to measure the intrinsic viscosity (Figure 2).

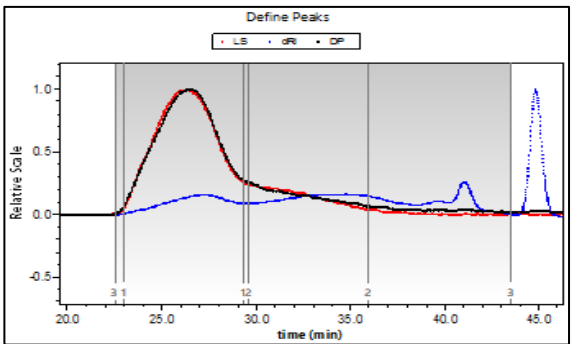


Figure 2: [S2] Chromatogram of arabinogalactan extracted from C. triangularis cladodes (solution filtered over 0.45 µm). With LS: light scattering and dRI: refractometric detection.

Thus, size-exclusion chromatography (SEC) was used to separate the chains of varying lengths, or the chains of varying molecular weights. Taking into account the volume of elution recovered, two fractions called F1and F2 were obtained. F1 and F2 respectively correspond to the fraction of polysaccharide eluted between 11.5 ml -14.8 ml and between 11.5 ml and 18 ml. For all fractions, the refractive index and the light scattering of the chains are measured, allowing determining molecular weight (Mn, Mw).Results obtained are presented in table 1. As shown in Figure 3 and Figure 4, F1 (the first fraction) corresponding to the minor peak which appeared at elution volumes between 11.5 mL-14.8 mL (Figure 3), represented about 20 % of total mass. F2 (whole sample) corresponded to the major peak which appeared at elution volumes between 11.5 mL à 18 mL (Figure 4), represented about 68 % of the total mass.

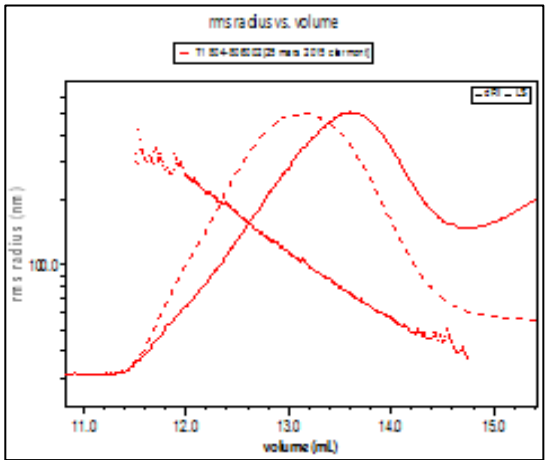


Figure 3: F1 (the first fraction) corresponding to the minor peak which appeared at elution volumes betwen 11.5 mL-14.8 mL

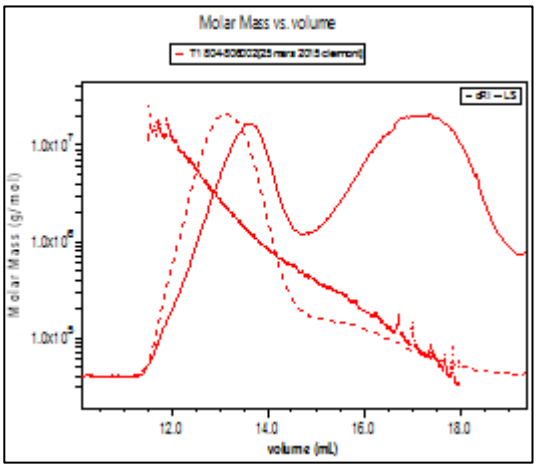


Figure 4: F2 (whole sample) corresponded to the major peak which appeared at elution volumes between 11.5 mL-18 mL.

Thus, the RI profile indicated that the filtered solution (S2) of Cereus triangularis polysaccharide sample consisted in two mail molecular species. The high molar mass of the mucilage extracted from the Cereus triangularis cladodes is also in agreement with those of the mucilages extracted from the genus Opuntia and estimated between 2.3×10^4 and 13×10^6 g/mol [7], [12], [48], [49]. In most cases, the difference observed in these molar masses could be due to: the age of the cladodes; extraction / purification methods; and the possible contamination of these hydrocolloids with other natural compounds (proteins, fibers, etc.). As a rule, a homogeneous high Mw is observed for pure polysaccharides, without any protein contamination. The polydispersity index was lower those of cladode of O ficus-indica (1.4) [49] but higher to that of red wine AGs (1.1) [50]. On the average, Mw and Mn of the first fraction was respectively 2 810 000 g mol^{-1} and 1 260 000 g mol^{-1} , the polydispersity index was 2.2. On the other side, Mw and Mn

of whole sample was respectively 1 270 000 g mol^{-1} and 162 000 g mol^{-1}, the polydispersity index was 7.8, indicating that polysaccharide of Cereus triangularis consists of molecular chains of varying lengths, or varying molecular weights. These molecular weights are varied from 1.27 x 10^6 g mol^{-1} to over 8,43 x 10^6 g mol^{-1}. The intrinsic viscosity of two fractions were 455 ±15 mL/g for the whole sample and 876 ± 18 mL/g for the first fraction. Comparing with literature, the intrinsic viscosity of AGs extracted from Cereus triangularis cladodes higher to that of endosperm GAs (10.8 mL/g) [51] and red wine GAs (10.1 mL/g) [50]. By using a representation of log ηsp versus log C [52], the obtained straight lines present intersections which correspond to the transition from the dilute to semi-dilute regime (C*), therefore the beginning of chain entanglement (Figure 5). In 0.3 M $NaNO_3$ solution, the critical concentration C* was 22 g/L à 25°C. This result was in agreement with the AGP extracted from coffee beans [53] having a molar mass of 3.78 × 10^6 g/mol.

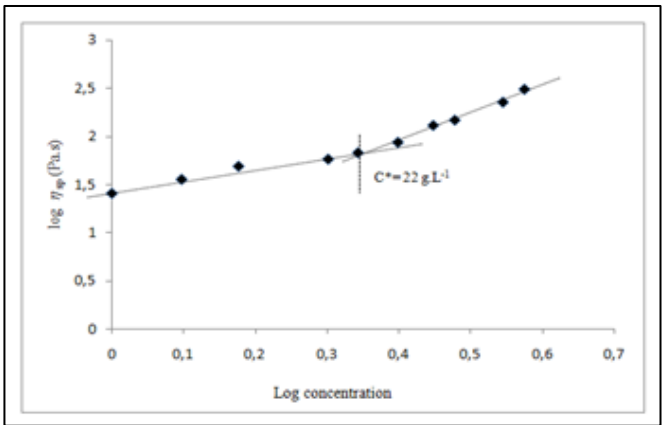


Figure 5: Determination of the critical concentration C* recovery

This C* value is 4 times smaller than that of Arabic gum for which C* was 88 g/L for a molar mass of 5.34 × 10^5 g /mol [54]. These results can be explained by the high molar masses of GAs extracted from Cereus triangularis cladodes and coffee seeds [53]. Indeed, as mentioned in the literature, the higher the molecular mass of the polysaccharides, the more rigid is the conformation and the critical concentration of coating was lower [53], [55].

3.2. Hydrodynamic Properties in Semi-dilute Solutions

The rheological properties of the polysaccharide extracted from Cereus triangularis were investi-gated in semi-dilute regime at various concentrations in H_2O and in NaCl 0.5 M. The study was carried out with polymer concentrations (between 1 and 5 % and shear rate swept from 10^{-3} to 10^2 s^{-1} (Figure 6).

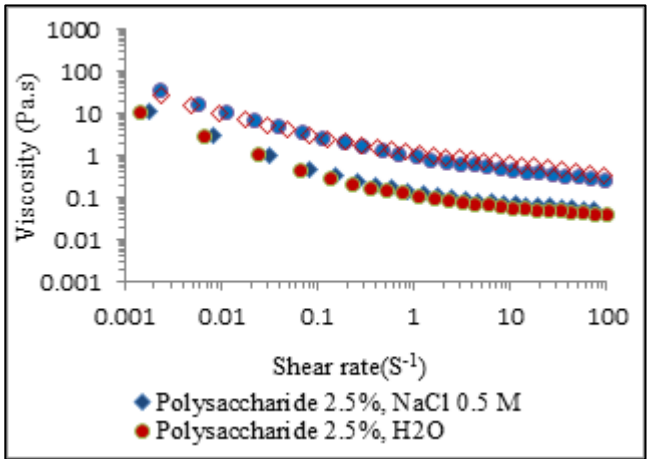
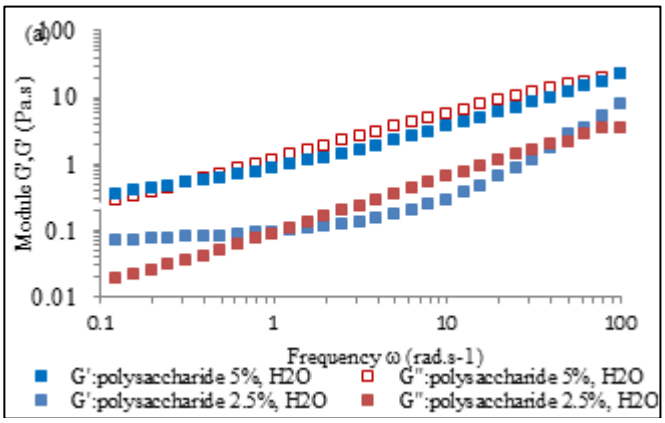


Figure 6 : Influence of ionic strength on the steady-sheart viscosity of a 2.5 % and 5 % (w/v) arabinogalactan solution, at 25 °C.

For all solutions, the viscosity of the polysaccharide extracted from Cereus triangularis contains two distinct behaviors: The rheofluidifying behavior (shear thinning) and newtonian behavior. The first behavior is observed at low shear rate and its characterized by the linear decrease in viscosity with increasing shear rate. In contrast, the rheofluidifying behavior is found in high shear rate and is characterized by the independence of the viscosity with shear rate. In this case, the polysaccharide solution had a behavior of shear-thinning fluids attributed to the disorientation and disentanglement of the macromolecular chains under influence of shear rate. In rheology, shear thinning is the non-newtonian behavior of fluids whose viscosity decreases under shear strain. It is sometimes considered synonymous for pseudoplastic behaviour[56]-[57] and is usually defined as excluding time-dependent effects, such as thixotropy [58]. Solutions of polysaccharide which displayed the higher viscosities (2.5 % and 5 %) were used for the dynamic oscillatory measurements. Storage modulus (G′) and loss modulus (G″) of the polysaccharide from C. triangularis in 0.5 M NaCl and KCl 0.5 M were quantified in the range of 0.1–100 $rad.s^{-1}$ at 3 Pa (Figure 7).



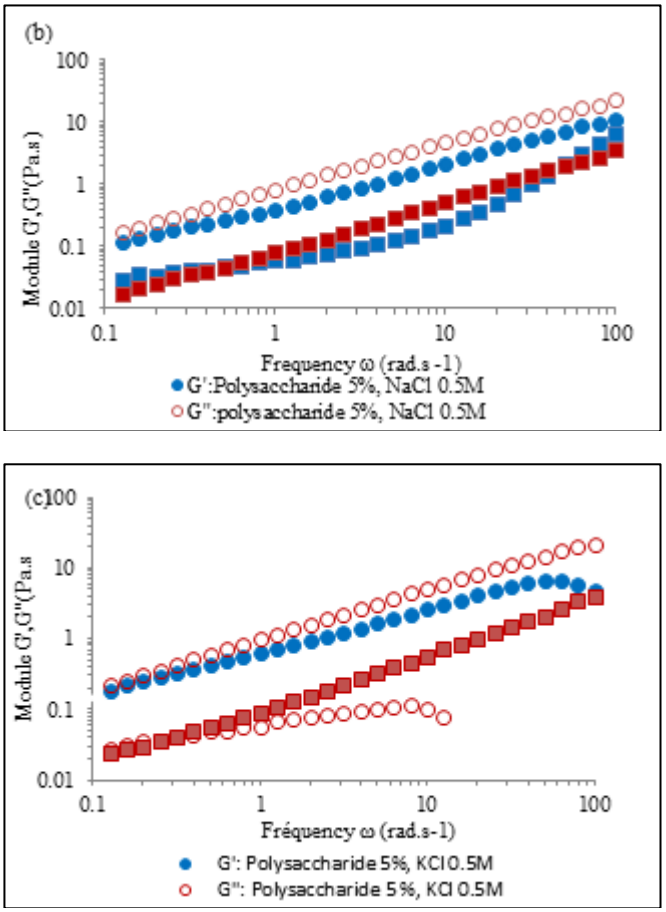


Figure 7: Complex modules G' and G''(Pa) versus strain (%) of aqueous solutions (A) and salt solutions (NaCl 0.5 M (B), KCl 0.5 M (C)) of polysaccharide 2.5 % and 5 %.

The variation of the storage G' and loss G'' moduli with frequency of oscillation for Cereus polysaccharide solutions is presented in figure (7a), (7b) and (7c). In H_20 and in NaCl 0.5 M, at 2.5 %, G''was less than G' at low frequency but became higher at high frequency indicating that the concentration is above coil overlap concentration. In H_2O, in NaCl and in KCl, for polymer concentration 5 %, G'' was greater than G' at all angular frequencies investigated. This is typical behaviour of viscoelastic fluid with low gel properties. This rheological behavior therefore suggests that the polysaccharide extracted from the cladodes of Cereus triangularis is not a gelling agent but a viscosifying and thickening agent. In general, this rheological behavior is similar to the results obtained for all the polysaccharides extracted from the cactus cladodes [11], [59].

3.3. Result digestibility

Development of prebiotics has focused on the non-digestibility of oligosaccharides to ensure they reach the colon and preferably persist throughout the large intestine such that benefits are apparent distally [36], [60]. In this study, the polysaccharide from Cereus triangularis was found to be resistant towards artificial human gastric juice as compared to FOS and inulin. Surprisingly, the maximum digestibility percentages of polysaccharide from Cereus at the lowest pH tested (pH 1) for 6 h is 4.08% as compraed to FOS (99.51%) and inulin (100%). Thus, the non-digestibility of arabinogalactan was found to be more than 95% at pH 1. The hydrolysis kinetics of Cereus triangularis AG, FOS and inulin by artificial gastric juice were presented in figure 8, 9and 10.

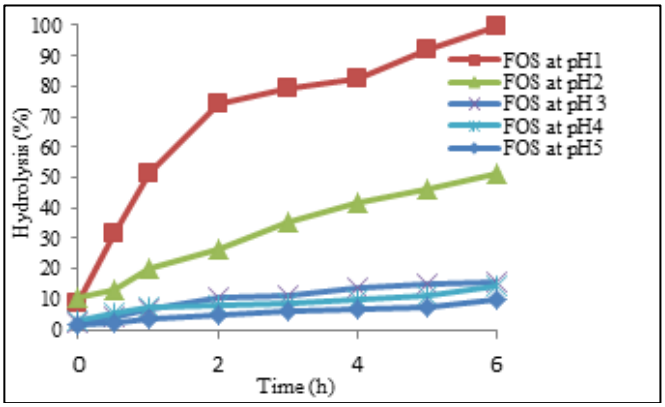


Figure 8: Degree of hydrolysis for FOSin human gastric juice.

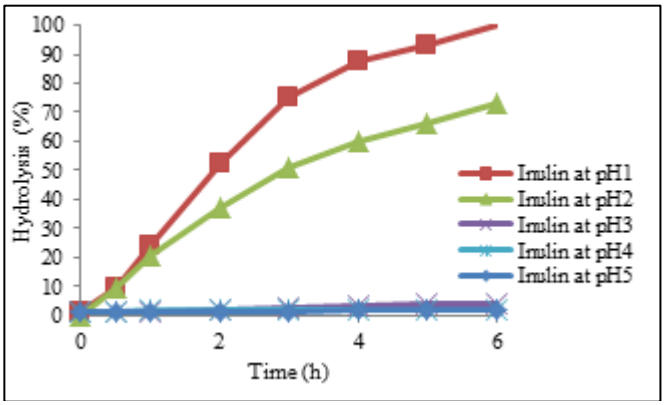


Figure 9: Degree of hydrolysis for inulin in human gastric juice.

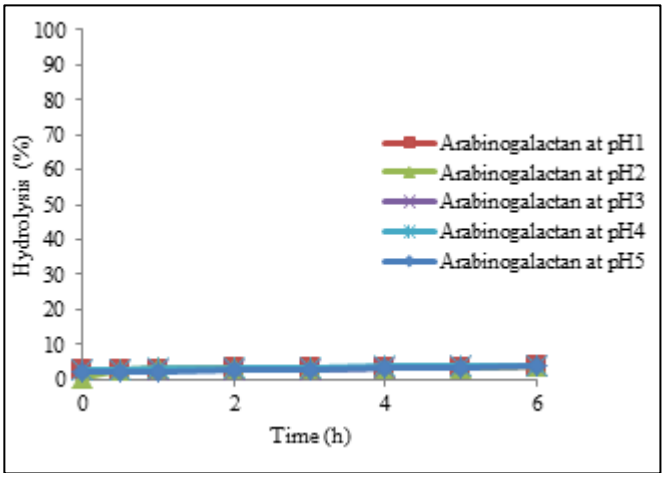


Figure 10: Degree of hydrolysis AG of C. triangularis in human gastric juice.

As a result, can be said that polysaccharide from Cereus triangularis was less susceptible towards gastric juice, regardless of the incubation time, while hydrolysis was seen to continuously increase in the case of FOS. However, food is usually retained in the human stomach for about 2 h at the pH is about 2-3 [42], [61]. The results gathered gave a good indication that polysaccharide from Cereus triangularis can be regarded as a potential prebiotic, as it meets prebiotic characteristics, which need it to be non-digestible. It is

therefore possible to conclude that the AG meets the first criterion of non-digestibility defining a prebiotic.

3.4. Enzymatic degradability

Prebiotics are usually in the form of oligosaccharides. The most described in the literature are fructoologosaccharides (FOS), galactooligosaccharides (GOS) and xylooligosaccharides (XOS) [59]. These compounds are not widespread in plant or microbial biomasses. It is usually necessary to degrade polysaccharides to obtain them. Thus, enzyme technology has proven useful to produce various potentially prebiotic oligo- and polysaccharides from plant fiber polysaccharides [63], [64], [65]. Enzymes are highly specific and easily implemented in reactors. Indeed, the AG extracts cladodes of C. triangularis, a β-(1,4)-galactane pectic galactan (P-PGAPT) and a β- (1,4) -galactan extracted from potato (P-GALPOT) were incubated with an endo-β-(1,4) galactanase (E-EGALN) of Aspergillus niger. The objective was to validate the enzymatic degradability of this polymer by these enzymes by comparing it with that obtained with commercial galactans. Thus, kinetics were analyzed to measure the depolymerization of the polysaccharide by methods: reducing sugars assays, molecular weight changes (HPSEC). The evaluation was carried out between 0 to 8 hours of incubation. As demonstrated in figure 11, the 3 substrates are degraded by the endogalactanase used. In the incubation conditions, the reducing sugar concentration for the C. triangularis AG assay was 4.8 g/L. Taking into account that this polysaccharide contains 55.4% Galp residues, it can be considered that 86% of the glycosidic linkages between these residues have been hydrolysed.

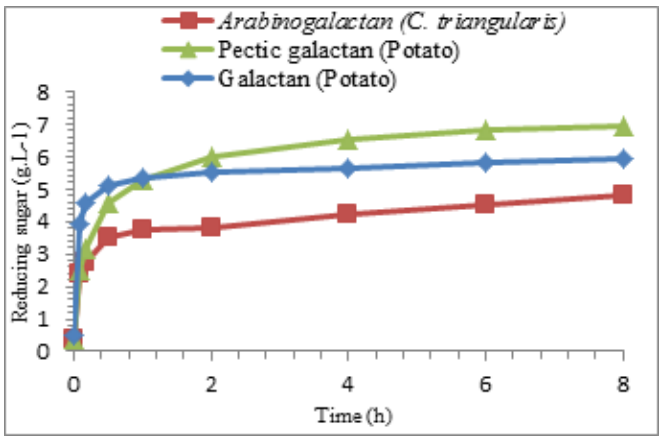


Figure 11: Enzymatic hydrolysis kinetics of AG extracted from cladodes of C. triangularis, a pectic galactan (P-PGAPT) and a galactan of potato (P-GALPOT).

The hydrolysis rates obtained with Galp-rich model galactans (P-PGAPT (82%) and P-GALPOT (87%) are comparable with percentages of degradation at 8 hours of between 69% (P-GALPOT) and 85% (P-PGAPT). In order to study the evolution of the molecular weights of oligosaccharides resulting from the enzymatic degradation of C. triangularis AG by endo-β-(1,4) -galactanase (E-EGALN), molecular weight was measured using size exclusion chromatography. Thus, galactose-galactobiose (disaccharide: β-D-Gal-(1,4)-D-Gal) were used to calibrate a PA 200 column in terms of DPs and the evolution of these two Sugars was followed throughout the hydrolysis. The results presented in table 2 confirm without ambiguity the hydrolysis of arabinogalactan from C. triangularis with the appearance of galactose and 4-β-Galactobiose. The results show rapid hydrolysis leading to an estimated polymerization degree distribution of between 2 and 15 for short degradation times (between 5 and 10 minutes) and a total degradation of the oligosaccharides produced for incubation times of more than 1 hour. It should be noted that the concentrations obtained in galactose and β-4-galactobiose confirm a non-total hydrolysis of the polysaccharide whose starting concentration was 10 g/L.

3.5. Test prebiotic

To evaluate the prebiotic activity, it is necessary to analyse the evolution of the bacterial population in the presence of the substrate being tested. Lactobacillus rhamnosus strain was chosen as probiotics in this study. The growth of Lactobacillus rhamnosus was monitored by measuring increase in OD 600 of the AG from Cereus triangularis polysaccharide (PolyS, 2% w/v), its oligosaccharide derivative (OligoS, 2% w/v) and of MRS supplemented or not with vitamin C (2% w/v), Glucose (2% w/v), inulin (2% w/v), FOS (2% w/v). In comparison with the negative control (MRS without substrate), It is observed that the growth of Lactobacillus rhamnosus is stimulated in the presence of carbon substrates (glucose, FOS and inulin), the probiotic strain Lactobacillus rhamnosus reaches the stationary phase around the 500 minutes of incubation. Note that, the maximum increase in OD was noticed in the presence of glucose 2%. But still, glucose cannot be considered a prebiotic because of its early absorption at the intestinal level [66]. Thus, even the effect observed in the presence of glucose is better, the growth of Lactobacillus rhamnosus observed in the presence of inulin and FOS is considered to be significant and reflects well Prebiotic character. Indeed, a prebiotic character is attributed to a substance tested as soon as this produces an increase in probiotic growth of the order of 0.1 in absorbance after 16 h of culture [66], [67], [68]. At this stage of the study we validated our commercial prebiotic controls (Inulin and FOS) and our probiotic model strain (Lactobacillus rhamnosus). Therefore, the prebiotic potential of AG from Cereus triangularis cladodes and its oligosaccharide derivative (OligoS) can be compared with the growth of commercial prebiotics (inulin and FOS). Thus, as observed in Figure 13, the L. rhamnosus strain tested showed a significant growth in MRS culture media supplemented with AG (PolyS) and oligosaccharides (OligoS). These results demonstrated the ability of these fractions (PolyS and OligoS) to be metabolized by lactobacilli, demonstrating their prebiotic potential. The strain used in this study metabolizes polysaccharides more rapidly than oligosaccharides. Similar behaviors have been reported previously using FOS and inulin as carbon sources for some species of bifidobacteria [69]. However, a number of other polysaccharide studies have reported that low molecular weight oligosaccharides or hydrolyzed oligosaccharides have better persistence in the colon, which increases their fermentation by intestinal microbial communities [70]. The obtained results (Figure 12) are comparable to those described by Gavlighi et al. [71]. Thus, Cereus triangularis AG can be considered as a potential new prebiotic source in future.

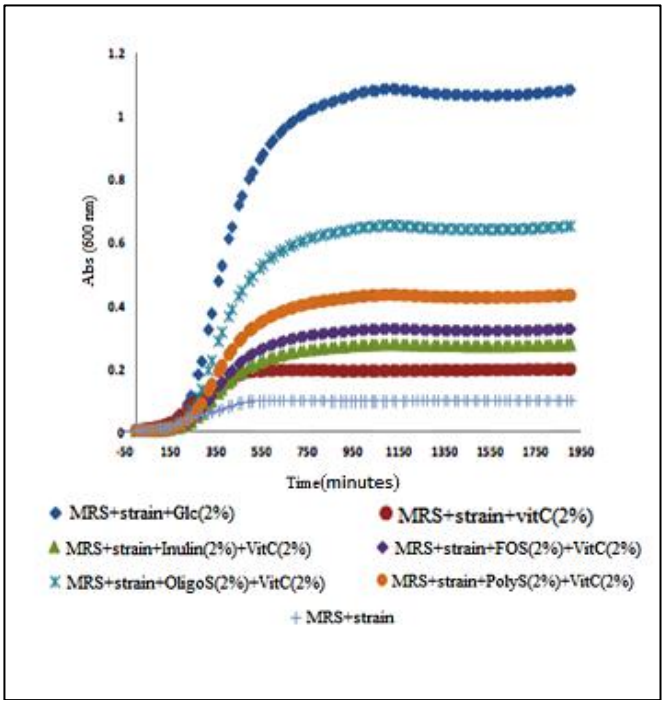


Figure 12 : *Growth curve of Lactobacillus rhamnosus on MRS supplemented or not with vitamin C (2% w/v), Glucose (2% w/v), inulin (2% w/v), FOS (2% w/v), Cereus triangularis AG (PolyS, 2% w/v) and its oligosaccharide derivative (OligoS, 2% w/v).*

4. Conclusion

Arabinogalactan isoled from Cereus triangularisis a good candidate for the production of food thickeners but also of fibres to the nutraceutic proprietes. After Characterization of arabinogalactan mucilage from Cereus triangularis cladodes, this study aims to evaluate physicochemical properties, the rheological properties, and prebiotic property of the oligo- or polysaccharides cladodes of Cereus triangularis. Characterization of this biopolymer in a diluted regime by exclusion chromatography high performance steric coupled with light scattering and refractometry differential identified its molecular weight as being between 1.270 and 8.430 kDa and its intrinsic viscosity (online viscosimetry) at 455 ± 15 mL/g. Rheological analyzes in semi-diluted medium showed that the arabinogalactan of Cereus triangularis has a rheofluidifying behavior not affected by the presence of salts (KCl or NaCl). Oscillatory analysis completed this characterization with a rheological behavior typical of a viscoelastic fluid having weak gel properties. It is therefore possible to associate this type I arabinogalactan (AG-I) with a thickener in the same way as certain natural gums such as guar and carob. In view of the important use to aqueous cladode decoctions of Cereus triangularis in traditional Malagasy medicine to relieve some digestive disorders, investigations have been undertaken to identify certain activities biological agents associated with this type I arabinogalactan. Indeed, in addition to the effect of "dressing gastric" probably associated with the consumption of this thickener, many polysaccharides are shown to be excellent prebiotics capable of significantly improving the intestinal balance of the consumer. Digestibility tests performed in an artificial gastric juice at different pH and for different polysaccharide concentrations demonstrated clearly the resistance of the arabinogalactan of Cereus triangularis at acidic pHs such as those encountered in the human stomach. This characteristic, specific of dietary fiber, is essential to ensure their integrity until arrival in the small intestine where they can be fermented by microbiota intestinal. The strategies of enzymatic degradation testees are promising and the products obtained will be able quickly to make the object of new tests prebiotic. A strain of Lactobacillus rhamnosus referenced has been chosen as model probiotic bacteria in order to study the fermentability of the oligo- and polysaccharides of Cereus triangularis and compare them with commercial prebiotics such as inulin and fructooligosaccharides (FOS). The results have shown very clearly that the use of oligo- or polysaccharides cladodes of Cereus triangularis allows the growth of Lactobacillus rhamnosus which use as carbon source in anaerobic conditions, as well as FOS or inulin. This result justifies the use of this cactus in the traditional treatment of digestive disorders. This is an encouraging result which should be explored on other probiotic strains in vitro and in animal models.

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