Effect of Extraction Methods on Chemical and Physical Properties of *Aloe Vera* (*Aloe Barbadensis* Miller) Polysaccharides Fraction: Liguid Gel and Powders

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ABSTRACT— ***The aim of this study was to evaluate the influence of extraction methods on chemical and physical properties of Aloe vera polysaccharides. The study was conducted on two commercial products: Aloe vera powder and an extract liquid of whole leaf. The kinetics of hydrolysis is carried out on the Aloe vera products. Hot, cold extraction with water and boiled ethanol extraction were carried out to obtain polysaccharide fractions (A1, A2, A3, and A4). The molecular weights of each fraction were determined. Proteins, galacturonic acid and sugars were quantified. Results showed that approximately, 25% of sugars were present in Aloe vera powder. The best extraction method were cold extraction (pH 5.3, 25°C, 4h) which showed the higher extraction yields (69.4±0.1%) in polysaccharide (Poly) A, than other extraction methods. Interestly, results showed a decrease of molecular weights, molecular number, and protein contents from 150 to 30 kDa, from 97 to 29 kDa and from 4.9±0.1 to 0.00% respectively with polysaccharides fractionment methods. Moreover, the total sugar content increases in polysaccharide fraction: 29.2±0.1%, 76.6±0.1% and 93.4±0.4% for Poly A, A1 and A2 respectively. The highest sugar content were observed in Poly A3 ≈ 97.8±1.5% probably glucomannan, with 77.3±6.5% of mannose, 18.7±2.8% of glucose. The data suggest that the fractionment methods could lead to product the purified polysaccharide which could be use for nutritional, biological and medicinal properties.***

Keywords— *Aloe vera*, extraction methods, acemannan, protein, polysaccharides, sugar

1. INTRODUCTION

The genus *Aloe* is a succulent, belongs to the liliaceae family. There are more than 400 species of *Aloe* including *Aloe arborescens*, *Aloe succotrina*, *Aloe saponaria*, *Aloe feros*, *Aloe chinensis*, and *Aloe vera* etc. Moreovers, more than 200 substances have been isolated from the *Aloe vera* also called *Aloe barbadensis Miller* **[1, 2, 3]**. Historical evidence points, Africa was the center of *Aloe vera* origin. It is a native chiefly to the warm dry areas of South Africa **[4]**. It is also present mainly in the desert regions of Asia and America, especially in the West Indies and South America. Research efforts are now geared towards evaluating the chemical composition and nutritive value of *Aloe vera* and other tropical medicinal plants. Previous studies have shown the presence of several bioactive compounds that originated from primary and secondary metabolic pathways in *Aloe vera* plants, and these have been widely used in the commercial formulations of gels and juices **[2, 5, 6]**. Some of the compound include enzymes (lipases, and proteases), monopolysaccharides (glucomannans), amino acids, vitamins (A, B12, C and D), anthraquinones, saponins, lignin and steroids **[7]**.

The nutritional value of *Aloe vera* has been demonstrated. Study of **[8]** showed that *Aloe barbadensis* was rich in carbohydrate (73.07%). The protein and fat content were low, 2.73 and 0.27% respectively. Earlier investigations indicated 5280 and 10670 ppm of sodium and potassium content respectively **[9]**. **[10]** demonstrated that to achieve

maximum nutrient composition, the uptake of *Aloe vera* which known as the external source of nitrogen, phosphorus and potassium will be necessary.

Moreover, the tendency of *Aloe barbadensis* to regulate the osmotic balance of the body fluid as well as body pH had been showed. Some of the biological activities such as the antiseptic (saponins and anthraquinones), anti-tumor, anti-inflammatory, antioxidant (vitamins) and immune regulator effects of glucomannans have been demonstrated **[1, 2, 5, 11]**. Glucomannan so colled acemannan is the main functional component of the gel, and is formed by a long chain of acetylated mannose intercepted with glucose, in which the mannose holds branches of galactose **[12]**. It contains 97% water and 0.7% solids. However, significant variations were seen in pulp polysaccharide species in these early studies. The gel of *Aloe vera* contain 98.5% water, many polysaccharides and pectic substances have been found **[13, 14]**. These substances differt according the plant and several factors. In addition, phytochemical studies have shown the presence of several bioactive compound that originated from *Aloe vera* **[15, 12]**. In active form, glucomannan would be responsible for medicinal properties, chronic care and wounds. However, less work has been done of the conditions to isolate polysaccharides from *Aloe vera*. Moreover, seasonal variations, geographic condition, culture, different methods of extraction could lead to changes chemical composition and nutritional value of *Aloe vera* polysaccharides. The aim of this study was to isolate the polysaccharide of *Aloe vera* and evaluate the effect of these extraction methods on chemical and physical properties.

2. MATERIALS AND METHODS

1. Source of Raw Materials

The study was conducted on two commercial products from *Aloe vera* from Gembloux, Belgium: a gel powder (Aloe Gel SD 200X) and an extract liquid of whole leaf (38.8 FIOZ 1QT., 1.8FI.OZ). The first sample is from Will & COBV and the second from Forever Living Products. These two products are symbolized respectively by gel solid (GS) and liquid gel (GL).

2. Kinetics hydrolysis of simple sugars

Kinetics hydrolysis of *Aloe vera* polysaccharide is been studied: concentration of trifluoroacetic acid (2M), temperature (110°C) Hydrolysis time (1 to 8 h). The optimal hydrolysis conditions of the *Aloe vera* powder have been determinated.

3. Extraction and preparation of polysaccharide

The extraction methods of the polysaccharide (A) have been described by **[16]**. Three techniques extraction of powder and liquid gel were used to obtain the Alcohol-insoluble matter (MIA).
- Hot extraction
- Cold extraction
- Extraction in boiling ethanol

The yields and chemical characteristics of the each MIA were determined.

3.1. Extraction of polysaccharides A

3.1.1. Hot extraction of gel solid (GS)

Samples (20 g) were heated to 90°C with stirring in water. The solution was filtered, centrifuged and the supernatant was concentrated. In the presence of 96% ethanol, polysaccharides were precipitated overnight and then centrifuged at 6000 rpm for 30 min at 15°C. The residue was recovered and the supernatant was centrifuged at 10,000 rpm for 30 min at 10°C. The second residue was associated with the first and homogenized in distilled water before lyophilized and ground to obtain the fraction A or MIA.

3.1.2. Hot extraction of liquid gel (GL)

Samples (100 mL) were heated at 90°C with stirring in water. The solution was filtered and the residue was lyophilized, ground and dried. The supernatant was concentrated to 500 mL by rotavapor. In the presence of 96% ethanol, the polysaccharides were precipitated. The remainder of the protocol is identical to that described in paragraph 3.1.1.

3.1.3. Cold extraction of gel solid

Samples (20 g) were homogenized with stirring in water for 30 minutes. The solution was centrifuged at 6000 rpm for 45 min at 15°C and the supernatant was filtered. In the presence of 96% ethanol, the polysaccharides were precipitated. The remainder of the protocol is identical to that described in paragraph 3.1.1

3.1.4. Extraction in boiling ethanol

Samples (20 g) were heated at boiling ethanol with stirring for 30 minutes. The solution was filtered on the nylon filter paper (11 µm) and the residue was dried in an oven at 40°C for 6 h and is subsequently ground to obtain the polysaccharide A or MIA.

3.2. Fractionation of alcohol insoluble matter (MIA)

3.2.1. Extraction of polysaccharides A1

Polysaccharides A (8 g) were dissolved with stirring in water. Ammonium hydroxide (NH_4OH) solution was added to bring the pH to 8.5. The calcium chloride (60 ml) was added dropwise with stirring at room temperature. The residue calcium pectate was removed and the supernatant was concentrated with rotavapor. After dialysis, the retentate was centrifuged to eliminate the new precipitates formed. The two pectate residues were combined and lyophilized. The supernatant was centrifuged with 96% ethanol. The tubes was kept overnight at 8°C and the polysaccharides were precipitated. After centrifugation, the two residues were combined, lyophilized and ground to obtained polysaccharide A1.

3.2.2. Extraction of polysaccharides A2

The polysaccharide A1 (500 mg) was dissolved with stirring in water. The remainder protocol is identical to that described in paragraph 3.2.1

3.2.3. Extraction of polysaccharides A3 and A4

The polysaccharide A2 (400 mg) was dissolved in water. The freshly prepared Fehling liquor solution (20 mL) was added dropwise with stirring. A thick pale blue precipitate appears and was recovered by centrifugation. The resulting residue was washed three times with ice-water and was centrifuged. All the supernatants obtained were combined, concentrated with rotavapor to obtain the polysaccharide fraction A4. The residue which contains the copper complex precipitates at pH 8.4 was decomposed by a mixture of hydrochloric acid / ethanol (5% (v / v), 50 mL) and centrifuged. The solution was subsequently washed twice with ethanol (96%) and centrifuged. The three supernatants were recovered, evaporated to dryness. The residues were dissolved in water and homogenate to obtain the polysaccharide A3.

4. Characterization of polysaccharides fraction

• Extraction yield: The extraction yields were expressed in (%) and were calculated on the basis of the dry matter.

Extraction yield (%) = M mia * 100/ (Me*Ms)

M mia= alcohol-insoluble matter (g); Me = mass of sample (g); Ms = dry matter

• Moisture content: Moisture was determined by drying the sample at 105°C for 24 h following the method proposed by **[17]**. Samples were then cooled in desiccators and weighed. The moisture % was calculated based on the difference in sample weight before and after dryness.

• Protein content: Protein was determined by determination of total nitrogen according to the Kjeldahl method **[18]**. Under the action of NAOH and after sulfuric mineralization in the presence of catalyst (CuSO4), ammoniac formed was neutralized. The ammonia in the sample solution was then distilled into the boric acid until it changed completely to bluish green. The distillate was then titrated with 0.1 N HCl solutions until it became colorless. The percent total nitrogen and crude protein were calculated using a conversion factor of 6.25.

• Molecular weight: The samples were analyzed by size exclusion chromatography coupled to a triple detection system. This triple detection system (Light Scattering, Viscosimetry, RI) was constituted by: Refractive Index Detector (RI:

waters 2410 refractive index detector), light diffraction detector (LS) and a viscosimetric detector (visc: Viscotek Trisec dual detector model 270). Polysaccharide fraction (200 mg) was gradually added with stirring in a Schott bottle with 100 ml of distilled water. The solution was filtered under vacuum using a 0.45 µm nylon filter and then 20 ml of this filtrate was taken to determine the dry matter. About 100 µl of the filtered solution were taken using a syringe and poured into a vial. The sample was then sealed and then injected into HPSEC on a TSKPWXL column with a flow rate of 0.7 mL / min. The eluent was constituted by 0.05 M $NaNO_3$ and 0.02% NaN_3.

• Galacturonic Acid analysis: After enzymatic hydrolysis of the polysaccharide fractions (A, A1, A2, A3, A4), galacturonic acid were performed using the HPAEC-PAD (Dionex). HPAEC-PAD was composed of an autosampler AS50, a GP50 gradient pump and an electrochemical detector ED40. It is equipped with an anion exchange column Carbopac PA10 (250 × 4 mm) and a precolumn Carbopac PA100 (40 × 4 mm).

• Analysis of carbohydrate by gas chromatography (GC): The monosaccharides of Aloe vera powders were determined as their alditol-acetate derivatives by gas-liquid chromatography (GLC) analysis after hydrolysis of polysaccharides with 1M H_2SO_4 sulphuric acid (100°C/2h) according to the method described by **[19], [20]**. Optimum hydrolysis time is dependent on a balance between the rate of release of hydrolysable polysaccharides and the degradation of monosaccharides that occurs during prolonged treatment under experimental conditions. The sugars in the hydrolysate (0.4 ml) were reduced to their corresponding alditols by adding 2 ml of DMSO containing 2% $NaBH_4$. Reduction was performed for 90 min at 40°C. The excess of sodium borohydride was then destroyed by adding 0.6 ml glacial acetic acid. Acetylation was then performed with acetic anhydride (4 ml, 10 min at room temperature) in the presence of 1-methylimidazole (0.4 ml) as a catalyst. Acetylation was stopped with 10 ml deionized water and the acetylated alditols were partitioned between dichloromethane (4.0 ml) and water. After the phase separation, the lower one was removed with a pasteur pipette and putted (1 ml) in a septum-cap vial.

2-deoxy-D-glucose was employed as internal standard and standards of different carbohydrates (L (+)-rhamnose, D(–)-arabinose, D(+)-xylose, D(+)-mannose, D(+)-glucose and D(+)-galactose from Fluka Chemie (Buchs, Switzerland)) were used. The analyses were accomplished using a Hewlett–Packard Agilent 6890 series gas chromatograph equipped with a HP1 column (30 m×0.32 mm, film thickness 0.25 µm). Derivatized extracts (1.0 µl) in dichloromethane were injected on-column. Helium was used as the carrier gas with a flow of 1.6 ml/min. The injection temperature was 290°C and the temperature program was: 1 min at 120°C, linear increase in 4 min to 220°C and finally in 35 min to 290°C and this temperature was then maintained for 4 min. Compounds were detected using a flame ionization detector at 320°C.

5. Statistical Analysis

Results were expressed as mean ± standard deviation from triplicate measurements. The non-parametric test of Duncan was used to analyze the difference between the means at 5% risk, using Statistica 7.1 software ANOVA analysis of variance method.

3. RESULTS AND DISCUSSION

1. Results

1.1. Hydrolysis kinetics of sugar

The hydrolysis kinetics of the neutral sugar is presented in **Table 1**. The results showed that the maximum level of sugars were produced after 3 hours of hydrolysis to reach 28.2±0.01% (TFA 2M, 110°C). Beyond this value the decrease of sugars content has been noticed. Moreover, the maximum concentrations were obtained after 3 hours for mannose (9.9±0.30%), glucose (17.9±1.50%), and galactose (0.6±0.01%). The maximum content of rhamnose, arabinose and xylose was observed after six hours and decrease with increase hydrolysis time.

Table 1: Sugar content (%) of the *Aloe vera* powder extracted with TFA 2M at 110°C.

	1 h	2 h	3 h	4 h	6 h	7h	8h
Rhamnose	0.1±0.00	0.1±0.00	0.2±0.01	0.1±0.01	0.2±0.01	-	-
Arabinose	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.00	0.2±0.01	0.1±0.01	-
xylose	0.1±0.01	0.1±0.01	0.1±0.01	0.2±0.01	0.2±0.01	0.1±0.01	-
Mannose	9.1±0.05	9.2±0.30	9.9±0.30	9.6±0.10	9.3±0.10	8.8±0.10	5.5±0.01
Glucose	14.2±0.02	15.0±0.05	17.9±1.50	15.7±0.05	14.4±0.07	13.5±0.05	10.4±0.02
Galactose	0.4±0.01	0.5±0.010	0.6±0.01	0.4±0.0	0.4±0.0	0.2±0.01	0.2±0.01
Total (%)	23.9±0.01	24.9±0.01	28.2±0.01	25.0±0.01	24.3±0.01	22.6±0.02	16.1±0.10

(-): trace; h: hours

1.2. Extraction yield

Extraction yield (R) of Poly A (GS and GL), Poly A1 and Poly A2 of *Aloe vera* were presented in **Table 2**. Results showed that cold extraction yield (≈69.4±0.1% at pH 5.3, 25°C) is higher than hot and alcohol extraction yield 46.3±0.2% and 50.3±0.4% respectively. Due to this high extraction yield, the cold extraction method is used for extraction of Poly A1 and Poly A2. After dialysis, the extraction yield increase with polysaccharides fractionment methods to reach 75.8±0.2% and 92.2±0.5% for Poly A1 and Poly A2. The lowest extraction yield ≈ 3.0 ± 0.01% was observed with liquid gel.

Table 2: Extraction yield of polysaccharides A, A1 and A2 of samples (GS) and (GL) at different extraction conditions (pH, temperature and time)

Sample	Extraction condition (pH, T°C and T)	Extraction yield
GS ≈ Poly A	(pH= 5.3 ; 90°C, 4 h)	46.3±0.2%
GS ≈ Poly A	(pH= 5.3 ; 25°C, 4h)	69.4±0.1%
GS ≈ Poly A	(Ethanol 78°C, 30 min)	50.3±0.4%
GL ≈ Poly A	(pH=5.3 ; 90°C, 4 h)	3.0±0.01%
Poly A1 (MIA)	(pH=7.4 ; 25°C, 30 min)	75.8±0.2%
Poly A2 (MIA)	(pH=7.4 ; 25°C, 30 min)	92.2±0.5%

GS : Gel Solid ; GL : Liquid Gel ; Poly A : polysaccharide A ; Poly A1 : polysaccharide A1 ; Poly A2 : polysaccharide A2 ; T°C : temperature ; T : time ; MIA : Alcohol-insoluble matter

1.3. Characterization of polysaccharide fraction

1.3.1. Physico-chemical composition

The results showed that *Aloe vera* (GS, GL and poly A) were higher dry matter content than 90% (**Table 3**). Concerning the proteins content, result showed the significant different ($p<0.05$) between polysaccharide from GL and GS. On the other hand, not significant different was observed between protein content on Poly A from GS and GL whatever the extraction methods (cold, hot, alcohol). After fractionment methods, proteins content decrease in Poly A1 (0.2±0.01%) and were in trace in Poly A2, A3 and A4.

Result also showed that the molecular weight (Mw) of GS and GL were 150 kDa and 145 kDa respectively (**Table 3**). When polysaccharides were fractioned, the molecular weights decrease from 150 kDa 30 kDa with the fractionment method. Moreover, the same tendance for the molecular number (Mn) was observed. This number decrease from 97 kDa to 29 kDa. The molecular sizes (Rg) were lower in Poly A1, Poly A2, Poly A3 and Poly A4 comparatively to GL and GS, Poly A from GS and Poly A from GL. The polydispersity index value (Ip) varies between 1.0 and 1.6.

Table 3: Chemical and physical characterization of different polysaccharide fractions of *Aloe vera*.

	GL	GS	PolyA GS (pH=5.3 ; 90°C, 4 h)	PolyA GS (pH= 5.3 ; 25°C, 4h)	PolyA GL (pH=5.3 ; 90°C, 4 h)	Poly A (Etha 78°C, 30 min)	Poly A1	Poly A2	Poly A3	Poly A4
Dry matter (%)	4.6±0.1[c]	93.1±0.1[a]	93.4±0.8[a]	92.3±0.6[a]	94.0±0.4[a]	94.20±0.3[a]	86.8±0,7[b]	87.7±0.4[b]	0.44±0.01[d]	0.12±0.01[e]
Protein (%)	3.8±0.1 [b]	4.9±0.1[a]	2.6±0,3[c]	2.5±0,2[c]	2.5±0,3[c]	2.4±0.01 [c]	0.2±0.001[d]	-	-	-
Mw (kDa)	145	150	134	128	124	112	74	65	30	45
Mn (kDa)	93	97	89	87	82	81	69	59	29	39
Rg (nm)	22.1	22.8	21.6	21.4	21.8	21.4	12.3	13.1	-	ND
Ip	1.6	1.9	1.5	1.5	1.4	1.4	1.1	1.2	1.1	1.0

(-) : trace ; ND : not determinated ; GS : Gel Solid ; GL : Liquid Gel ; Poly A : polysaccharide A ; Poly A1 : polysaccharide A1 ; Poly A2 : polysaccharide A2 ; Poly A3 : polysaccharide A3 ; Poly A4 : polysaccharide A4 ; MIA : Alcohol-insoluble matter, molecular weight (Mw), molecular number (Mn), molecular size (Rg), polydispersity Index (Ip)

Analysis by size exclusion chromatography coupled of triple detection system showed the chromatogram of polysaccharide A2 (**Figure 1**). Result showed 2 peaks for the refractive index detector (RI): the first peak show the molecules of high molecular weight and the second show the molecules of low molecular weight. The light scattering detector (LS) and viscosimetry (visc) detector were showed one peak at the same number 6.35-7.73 in X-axis which indicates the molecule of high weight.

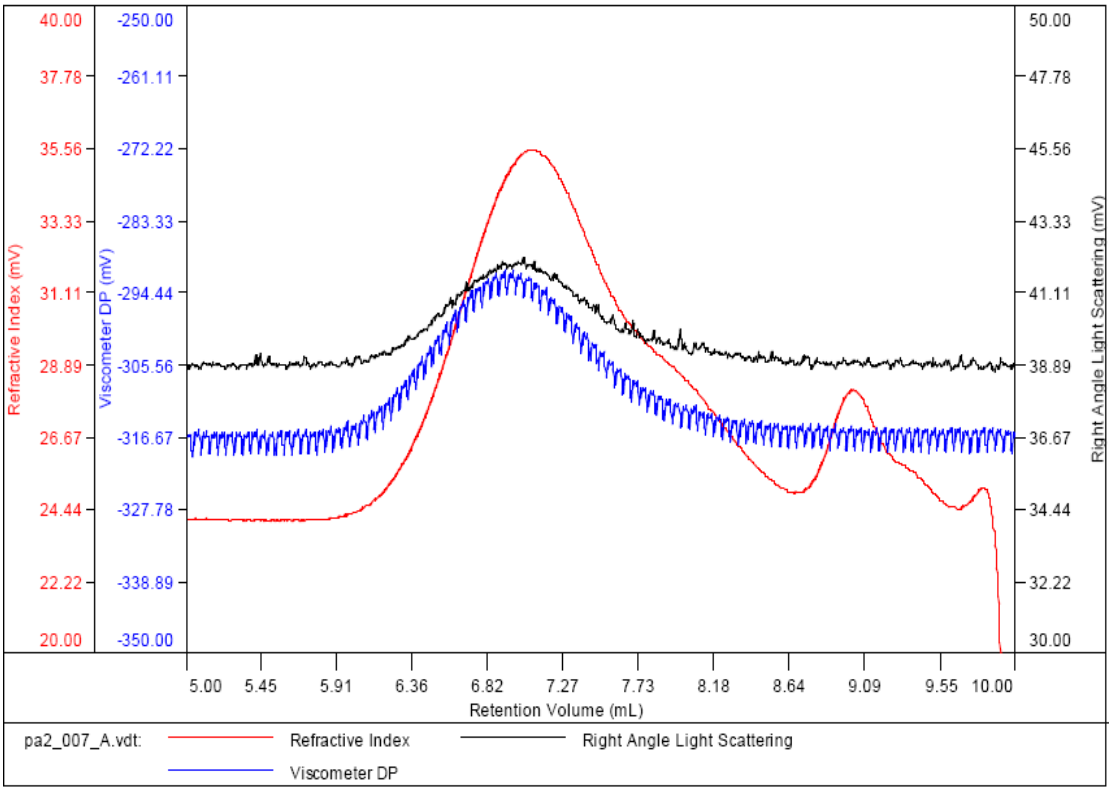

Figure 1: Chromatographic Profile Of Polysaccharide A2

1.3.2. Carbohydrate and galacturonic acid composition

Carbohydrate and galacturonic acid content with TFA (2M, 110°C, 3h) were presented in **table 4**. The results showed the higher carbohydrate content in poly A from GS after cold extraction 29.2±0.01%. This value was higher than those obtained in GS and GL whatever the extraction conditions (water and alcohol). After dialysis, polysaccharide A1, polysaccharide A2, A3 and A4 were showed 76.6%, 93.5%, 97.5% and 11.4% of total sugars respectively. The results also showed the highest level of mannose and glucose ratio in poly A3 (77.3%; 18.7%) comparatively to poly A1 (59.9%, 10.2%) and poly A2 (48.7%, 19.8%). Acid galacturonic content was in traces in all samples analyzed.

Table 4: Carbohydrate and galacturonic acid composition

Samples	Carbohydrate and galacturonic acid							
	Rhamnose (%)	Arabinose (%)	Xylose (%)	Mannose (%)	Glucose (%)	Galactose (%)	Total sugars (%)	AGA (%)
Poly A GL (pH=3.7; 90°C, 4 h)	0.0±0.0	0.2±0.0	0.1±0.1	8.7±0.3	2.4±0.2	0.8±0.0	12.2±0.1	trace
Poly A GS (pH=5.3; 90°C, 4 h)	0.0±0.0	0.4±0.1	0.3±0.0	2.4±0.5	2.0±0.3	1.1±0.0	6.2±0.02	-
Poly A GS (pH=5.3; 25°C, 4h)	0.1±0.2	0.5±0.2	0,3±0.2	10.6±3.2	15.8±4.9	1.7±0.5	29.0±0.3	
Poly A (Alcohol 78°C, 30 min)	0.0±0.0	0.2±0.0	0.0±0.0	12.4±0.6	5.8±0.1	0.8±0.1	19.20±0.01	-
Poly A1 (pH=7.4 ; 25°C, 30 min)	0.0±0.0	1.4±0.0	0.9±0.0	59.9±0.4	10.2±0.1	4.2±0.0	76.60±0.3	-
Poly A2 (pH=7.4 ; 25°C, 30 min)	0.1±0.0	6.3±0.6	4.4±0.7	48.7±4.5	19.8±1.5	14.1±1.1	93.40±0.1	-
Poly A3	0.0±0.0	0.0±0.0	0.0±0.0	77.3±6.5	18.7±2.8	1.46±0.6	97.46±1.5	-
Poly A4	0.0±0.0	2.26±0.1	1.46±0.3	6.1±1.8	0.0±0.0	1.6±0.2	11.43±1.01	-

GS : Gel Solid ; GL : Liquid Gel ; Poly A : polysaccharide A ; Poly A1 : polysaccharide A1 ; Poly A2 : polysaccharide A2 ; Poly A3 : polysaccharide A3 ; Poly A4: polysaccharide A4; AGA : Acid galacturonic

2. Discussion

The hydrolysis kinetics showed that the maximum level of total sugars were produced after 3 h and decreased with hydrolysis duration. Moreover, the maximum concentrations of mannose, glucose and galactose were obtained after 3 h. The decreased of sugar content with hydrolysis duration could be explained by the degradation of sugars during acid hydrolysis (TFA 2M, 110°C). Given these results, it appears necessary to control hydrolysis conditions and limit the sugars degradation. To prevent this degradation after hydrolysis conditions, enzymatic method could be suggested. For pectin hydrolysis, **[21, 22]** recommended to combine the chemical and enzymatic hydrolysis under less severe conditions. These authors showed that this combination results in a complete hydrolysis of various pectin fractions to free galacturonic acid and the neutral sugars. The results also showed that the maximum hydrolysis of rhamnose, arabinose and xylose was reach after 6 h and decrease with hydrolysis duration. The long time to release those sugars could be explained either by the resistance to acid hydrolysis, or by the link between those sugar and galacturonic acid in the main chain of pectin. The low rhamnose content in this study could be explained by the rhamnogalacturonic chains present in the *Aloe vera* gel. In the previous studies, **[23]** have showed that *Aloe vera* gel was rich in polysaccharides such as pectin, cellulose, hemicellulose. These compounds could prevent the release of sugars during acid hydrolysis.

The extraction yield varies according to the extraction condition. Percentages extraction obtained for Poly A ≈ GS ≈46.3% (pH 5.3 at 90°C), poly A ≈ GS ≈ 69.4% (pH 5.3 at 25°C) and Poly A ≈ GS ≈ 50.3% (Ethanol 78°C) were not similar to those of **[24, 12]**. The difference of these results could be explained by the *Aloe vera* varieties, by the extraction conditions (pH, temperature, time) used. These parameters (pH, temperature and time) could lead to changes in the gel structures and influed their extraction yields. The highest extraction yield obtained in poly A ≈ GS ≈69.4% were higher than those found by **[12]**, who obtained 14.8% with 50 mM trans-1.2-cyclohexanediamine N'-tetraacetate (CDTA) and 9.5% sodium carbonate (Na2CO3). This high extraction yield at 25°C and pH 5.3 could be explained by the fact that the polysaccharides were precipited with calcium chlorure ($CaCl_2$, $2H_2O$) solution used at low temperature. The losses recorded during the extractions of poly A (cold, hot, alcohol) comparatively to Poly A1 et A2 could be due in particular to the dialysis performed. During this dialysis, the small molecules lower than the diameters of membrane pore were eliminated with each renewal of water. The increase extraction yield (75.8±0.2% and 92.2±0.5% for Poly A1 and Poly A2) with polysaccharide fractionment at 25°C, pH 5.3 could be due to the purification methods. Results also showed the lowest extraction yield (3%) with the liquid gel. This low extraction rate could be explained by the presence of impurities in the gel which could be eliminated with polysaccharides fraction during the filtration steps.

The *Aloe vera* gel (GL) analyzed presented a higher value for moisture content. The water quantity obtained in this study was similar to that described in previous studies **[12, 25, 26]**, between 96.5% and 98.6%. The results show that *Aloe vera* powder (GS) and Poly A presented a lower water content compared to the whole leaf whatever the extraction condition. This difference in moisture content could be explained by sample varieties.

Concerning the protein, results showed that protein contents in GL and GS was 2.04 times greater than those of Poly A whatever the extraction condition (Water, alcohol, pH, T°C and Time). The decrease in protein levels with polysaccharides frationment methods could be related to protein degradation. The protein contents obtained were lower than those found by **[27]** which vary between 6.2% to 8%. On the other hand the protein contents obtained in this result were higher than those obtained by **[28]** and **[25].** This difference of protein content obtained compared by those in littérature could be explained by the different varieties studied or by the different extraction methods. Others reasons, these discrepancies could be explained probably by the seasonal change and/or by the different geographic conditions of *Aloe vera* crops. In Polysaccharide A2, A3 and A4, the results showed that protein content were in trace amounts. The decrease in protein levels may be due to the purification process. This result was in accordance with those obtained by **[29]** who showed that proteins were undetectable in purified fractions. Utilization of *aloe vera* gel in food product and clinical properties is increased in recent years. The loss of protein content on purified fraction (Poly A3) could strengthen the clinical and medical properties of this polysaccharide so called acemannan.

The decrease in molecular weight from 150 to 30 kDa and the decrease in polysaccharide number could be explained by the aggregation phenomenon. The molecular weight obtained was lower than those of **[29]** which varied between 10 kDa to 1000 kDa. These differences in molecular weight could be explained either by the type of column used, by the calibration of standard dextrans, or by the different purification techniques of the raw material. The polydispersity index of the polysaccharides A1, A2 and A3 was respectively 1.1, 1.2 and 1.1. These values were close to 1, which reflect the purity of these samples.

The results showed that mannose and glucose were important in poly A1 (59.9%, 10.2%), Poly A2 (48.7%, 19.8 %.) and A3 (77.3%; 18.7%). The significant percentages of mannose and glucose in the different fractions could be explained by the fact that the main compound of the *Aloe vera* gel in this study was glucomannans with approximately one glucose for six mannoses for poly A1 (ratio M / G = 5.9), one glucose for three mannoses for poly A2 (M / G ratio = 2.5) and one glucose for four mannoses for poly A3 (ratio M / G = 4.1). Other important polysaccharides such as glucomannan **[24]** and fructans **[30]** have been showed in *Aloe vera* pulp. These two products are the main beneficial polysaccharides in *Aloe vera*. However, significant variations were seen in pulp polysaccharide species in previous studies. **[14, 31]**

established that acemannan contains galactose branches linked to mannose with α-(1, 6) glycosidic linkage. **[32]** found a pectic substance to be the primary polysaccharide with absence of mannan. The reasons for these discrepancies of polysaccharides component are not understood, but are probably largely due to seasonal change, or the different geographic conditions of *Aloe vera* crops, or the different varieties used and the different extraction methods. In this study, the high content of hexose in poysaccharides A3 (glucomannan) could be due to the purification steps. For the purification steps, use of calcium chloride at pH 8.5 to precipitate the calcium pectates could be the optimal condition for enriching the polysaccharide in hexoses. In addition, the loss of protein content in polysaccharides A3 could be reinforcing their medicinal and clinical properties.

In the different fractions, the xylose content could be explained by the existence of lignins and hemicelluloses (xyloglucans and xylans) in the *Aloe vera* gel. The fructose content in the gel of *Aloe vera* could lead to the different difficulties of analysis. The small proportion of rhamnose, arabinose and galactose could be explained by the pectic substances such as arabinans, galactans or arabinorhamnogalactanes in the cell wall of *Aloe vera* pulp. Galacturonic acid was constitued of pectic substance. The pectic substances were responsible for the rigidity of plant cells walls and have anti-haemorrhagic activities. Qualitatively, galacturonic acid was found in trace amounts in each polysaccharide fraction (poly A, A1, A2, A3 and A4) studied. These results were not agreed with those obtained in previous studies **[16, 13]**.

4. CONCLUSION

In summary, this paper reports that *Aloe vera* contains several polysaccharides. These polysaccharides differ according to several factors such as extraction condition, plant crop varieties and geographic position. On the basis of the results obtained, it could be concluded that cold extraction is the best of all extractions used. With an extraction yield of 69.2% and high carbohydrate content after acid hydrolysis (29.2%), this extraction method is used for the fractionation of *Aloe vera* polysaccharides. The decrease of molecular weight and the protein content have been observed in Poly A1, A2 and A3. On the other hand, the increases of monosaccharide with polysaccharide fractionment method have been show. The monosaccharides analysis of the different polysaccharides fraction showed that mannose and glucose are the main sugars of *Aloe vera*.

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