

Identification and Quantification of Monosaccharides in *Aloe vera* Gel by Acid and Enzymatic Hydrolysis and Wet Heat Treatment

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Abstract

Aloe vera (*Aloe barbadensis* Miller) possesses curative and therapeutic properties attributed to the polysaccharides found in its tissue. This work aimed to study hydrolyzed (chemical and enzymatical) *Aloe vera* gel extracts using ultrafiltration membranes with a pore size of 0.22 micron (μm). Nine chemical treatments were achieved with H₂SO₄ and three wet heat treatments were carried out at a pressure of 1.2 lb/in² for 15 minutes; in both cases, three different concentrations of *Aloe vera* gel juice (AGJ) were used: 1.5%, 2.5% and 3.5% w/w. The concentrations of H₂SO₄ were 0.10%, 0.25% and 0.40% w/w. Chemical experiments are performed over a factorial 3² design and results were analyzed using SPSS software (version 17, SPSS Inc.), finding the one labeled T7 (15 ml of AGJ 3.5% with 15 ml of H₂SO₄ 0.10% added) the best of them, as it led to 0.0446 mg/ml of liberated glucose. Among the three wet heat treatments, the one labeled TC3 (15 ml of AGJ 3.5% with 15 ml of H₂O added) was the best-performing one, as it led to 0.292 mg/ml of liberated glucose. Furthermore, an enzymatic hydrolysis was carried out using Novozymes' Pectinex® AR and Viscozyme®. Hydrolysis with both enzymes yield to better results than acid hydrolysis: in the treatment with Pectinex® AR, 3.282 mg/ml of liberated glucose were obtained and 3.302 mg/ml in the treatment with Viscozyme®. The hydrolyzed substances obtained by acid and enzymatic hydrolysis, as well as by wet heat treatment, were subsequently analyzed by thin layer chromatography (TLC), using glucose, galactose and arabinose 1000 ppm solutions as reference patterns. Among the treatments by H₂SO₄, the one labeled T4 obtained an R_f value of 50, the same as on the galactose reference pattern.

Keywords

Carbohydrates, Enzyme, Mucilage, Reducing Sugars, Retention Factor

1. Introduction

There exist over 350 different species of Aloe, but only a few are really of commercial interest for therapeutic uses. The most popular among them are *Aloe vera* L., also known as Barbados Aloe, and *Aloe ferox* Miller, whose common name Cap Aloe is very extended [1] [2]. *Aloe vera* possesses medicinal and therapeutic properties attributed to the high polysaccharide concentration found in its leaf inner tissue [3] [4]; they represent near 20% of total solids in the mucilaginous parenchyma tissue. It has been demonstrated that approximately 20 glycoproteins associated to these polysaccharides are responsible for *Aloe vera* L. gel pharmacological activity. Many of the medicinal effects of aloe leaf extracts, such as cell proliferation stimulation and hypoglycemic and immunostimulant properties, among others [5], have been attributed to the polysaccharides found in the inner leaf mucilaginous tissue [6] [7]. Polysaccharides, and specially the mucilaginous ones, constitute the active principle of *Aloe vera* gel and its biological activity should be assigned to them. The most important polysaccharides identified in aloe leaves are acemannan and aloeride, a high molecular weight polysaccharide consisting on several glucose, galactose, mannose and arabinose units [8]. A hydrolysis process is necessary to identify and isolate these polysaccharides, in order to break them down into smaller monosaccharide units which would be useful to determine exactly the polysaccharide composition. This polysaccharide structural rupture can be achieved by enzymatic hydrolysis as well as by heating the aloe pulp with acid added.

The aim of this work is to evaluate the different hydrolysis effects on a sample of *Aloe vera* Miller gel and identify and quantify the main monosaccharides present in this substance, as the acemannan components.

2. Materials and Methods

2.1. Prime Matter

The viscous clear liquid or “mucilage” extracted from fresh cut aloe leaves was the prime matter for this study. The *Aloe vera* plantation was located in the municipality of Umán, State of Yucatán, 35 km far from the city of Mérida, in eastern Mexico.

2.2. *Aloe vera* Gel Extraction

We followed the next steps to extract the aloe juice: we carefully washed the aloe leaves with soap in order to remove residual pollution and they were subsequently rinsed and immersed in a 200 ppm solution of Citricdin Plus for 20 minutes. Then, we cut the leaves edges, 15 cm to the apex and 5 cm to the basis.

Finally, the bitter exudate was drained by keeping the leaves in a vertical position for 30 minutes. Another 1 cm large portion of the leaf basis was removed after that, in order to discard the residual exudate. The outer green rind was removed immediately after, by cutting first the serrated margins of the leaf 1 cm to the edge and then peeling off the cuticle. The inner leaf gel juice was extracted then, by means of an Osterizer blender, cleared by passing it through a gauze filter and stored at 0°C for further use.

2.3. Filtration Process

For filtering the *Aloe vera* gel juice we passed it first through a Whatman® Grade 1 filter paper and secondly through a 0.22 microns pore size Millipore membrane. Both processes were achieved with the aid of a vacuum pump.

2.4. Acid Hydrolysis

Nine acid hydrolysis and heat treatments were carried out combining three different *Aloe vera* gel juice (AGJ) % w/w concentrations with three other values for sulfuric acid % w/w concentration. The following values were chosen for AGJ concentrations: 1.5%, 2.5% and 3.5%. And for the sulfuric acid concentrations: 0.10%, 0.25% and 0.40% w/w. The nine hydrolysis processes were labeled T1 to T9 (**Table 1**). After the hydrolysis treatment, the assays were neutralized at pH 7 ± 0.34, using NaOH 0.08 N and each sample was brought up to a total volume of 50 ml and frozen. Besides, previously frozen samples were lyophilized. Dry matter obtained from each lyophilization was dissolved and homogenized in 2 ml of distilled water and was centrifuged at 12,000 rpm for 30 minutes at room temperature (24°C).

2.5. Hydrolysis by Wet Heat Treatment at 1.2 lb_f/in² for 15 Minutes

Three solutions of AGJ were prepared at concentrations of 1.5%, 2.5% and 3.5%. Each one was brought to a total volume of 50 ml using distilled water.

Three treatments were prepared subsequently (labeled TC1, TC2 y TC3), with the following concentration distributions: 15 ml of AGJ 1.5% plus 15 ml of distilled water for TC1; 15 ml of AGJ 2.5% plus 15 ml of distilled water for TC2 and 15 ml of AGJ 3.5% plus 15 ml of distilled water for TC3. Once the samples homogenized, hydrolysis by a wet heat treatment was achieved at 1.2 lb_f/in² for 15 minutes. Pressure was then lowered to 0 lb_f/in² and flasks were immersed in an ice bath in order to stop the chemical reaction. Samples were frozen and lyophilized until further use.

2.6. Enzymatic Hydrolysis Using Commercial Enzymes (*Pectinex*® AR y *Viscozyme*®)

Two commercial enzymes produced by Novozymes were used: Pectinex® AR and Viscozyme®.

Table 1. Acid hydrolysis treatments.

Treatment	AGJ concentration (%)	sulfuric acid concentration (% w/w)
T1	1.5	0.10
T2	1.5	0.25
T3	1.5	0.40
T4	2.5	0.10
T5	2.5	0.25
T6	2.5	0.40
T7	3.5	0.10
T8	3.5	0.25
T9	3.5	0.40

Concerning Viscozyme®, it is worthy to mention that the furnisher specifications and parameters for its utilization were carefully followed.

Concerning Pectinex® AR, an enzyme substrate of 3 ml of AGJ with 3 ml of citrate buffer 0.05 M pH 5 (corresponding ratio 2:1) was deposited in test tubes and homogenized; pH was measured and the test tubes were then put in double boiler incubation at $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$; once this temperature was reached, 4 μl of the Pectinex® AR enzyme were added and hydrolysis was stopped after a reaction time of 30 minutes by putting the test tubes in an ice bath for 10 minutes.

2.7. Determination of Total Sugar Content

The *Aloe vera* gel juice obtained after filtration was then treated by the phenol-sulfuric acid method [9] to determine total carbohydrates in the samples.

2.8. Determination of Reducing Sugars

Reducing sugars present in AGJ after each hydrolysis treatment were assayed using the 3,5-dinitrosalicylic acid (DNS) method [10].

2.9. Determination of Monosaccharides in the *Aloe vera* Gel Using Thin Layer Chromatography (TLC)

Merck glass TLC plate, silica gel coated with fluorescent indicator F₂₅₄, size 20 × 20 cm were used; they were cut into four pieces, size 8.5 × 10 cm, leaving 1 cm margin at the top and bottom edges, the later being used to mark the row where solution containing the sample was applied to the plate and the former, to mark the solvent front or limit to be reached by eluent after running. The spots of solution containing the aloe gel were applied on the row at a distance of 1.2 cm from one to another.

We used three 1000 ppm solutions of glucose, galactose and arabinose as reference patterns for TLC and the AGJ and AGJ hydrolyzed samples mentioned above.

2.10. Statistical Analysis of Data

An experimental 3^2 design was used to treat chemical hydrolysis, the AGJ and the sulfuric acid being the independent variables and the reducing sugars determination being the response variable.

3. Results

3.1. TLC Reference Patterns

The obtained values for the retention factor (Rf) in the reference patterns (glucose, galactose and arabinose) were averaged and compared with those reported by Zweig and Sherma [11] finding clear similarities.

3.2. Quantification of Sugars Present in AGJ

Total and reducing sugars were quantified in the aloe extract obtained after filtration with the 0.22 μm pore size membrane. Among the total sugars present in AGJ (4.233 mg/ml of glucose), more than 50% are reducing sugars (3.088 mg/ml of glucose).

Concerning the identification of monosaccharides present in AGJ using TLC, the results can be appreciated in **Figure 1**.

The AGJ samples were compared to samples of paper filtered *Aloe vera* gel juice (PFJ) and it was found that four marks coincided, with an Rf value of 61.2 for two of them and Rf = 77.5 for the other two; similar retention factors suggest that the two samples could contain the same substance, the one obtained by filtration with a 0.22 μm pore size membrane (AGJ) and the paper filtered one (PFJ) [12].

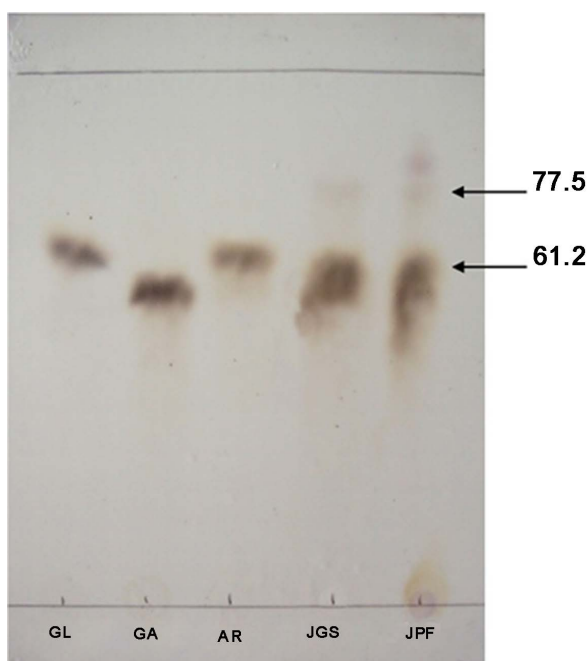


Figure 1. TLC for non-treated AGJ.

3.3. Quantification of Sugars in H₂SO₄ Hydrolyzed AGJ

The results obtained from the nine treatments of AGJ by H₂SO₄ hydrolysis were analyzed under ANOVA setting and using the SPSS software (version 17, SPSS Inc.). The aim of this analysis was to evaluate the reducing sugars concentration (mg/ml of glucose) present in each treatment; the reducing sugars (RedSu) were taken as the dependent variable in the analysis. Furthermore, we also tried to discern the most significant treatments and to notice if there exists an interactive effect due to the combined action of the independent variables (acid and gel); this is caused by the 3² experimental design, as the three sulfuric acid concentration levels are confronted to the three AGJ concentration levels.

A comparison between the different values of mean square, by a Type III sum of squares, was achieved using SPSS software in order to determine if any significance exists between treatments. And, according to this analysis, a significance of 100% was found in every treatment.

Owing to the fact that all the treatments presented a significance of 100%, a descriptive analysis was carried out for each independent variable (acid or gel) with respect to the dependent variable reducing sugars (RedSu). Details of this analysis, based on Tukey's method, can be observed in **Figure 2**, where treatments by acid hydrolysis are represented. The best results were obtained for those treatments corresponding to a 0.10% sulfuric acid concentration, *i.e.*, the ones labeled T1, T4 and T7. The best among the three of them is T7, which corresponds to a volume of 15 ml of AGJ 3.5% solution and a 0.10% sulfuric acid concentration.

TLC results for the nine treatments are shown in **Figure 3**. **Table 2** represents R_f values for the pattern samples and for the H₂SO₄ hydrolysis treatments.

The averaged value of R_f in reference patterns (glucose, galactose and arabinose) was calculated for each one of the three TLC plates where hydrolyzed samples were applied. This is due to the fact that it was not possible to compare nine hydrolyzed treatments to the reference patterns in a unique chromatography. As a result, we have that the R_f value of hydrolyzed treatment labeled number 4 corresponds to galactose.

3.4. Quantification of Sugars in Hydrolyzed AGJ by Wet Heat Treatment at 1.2 lb_f/in² for 15 Minutes

It was found that the best among the wet heat treatments of hydrolyzed AGJ was the one labeled TC3 (15 ml of AGJ 3.5% and 15 ml of H₂O added) for the amount of reducing sugars (mg/ml of glucose) under the pressure and time conditions described above (see **Figure 4**).

Comparing the R_f values on the glucose, galactose and arabinose reference patterns to the ones on the wet-heat-treated hydrolyzed aloe gel juice (TC1, TC2 and TC3), no matches were found (see **Figure 5**). Something similar was found when comparing the R_f values on reference patterns to those on the non-treated *Aloe vera* gel juice samples (AGJ and PFJ). Nonetheless, if one compares the R_f

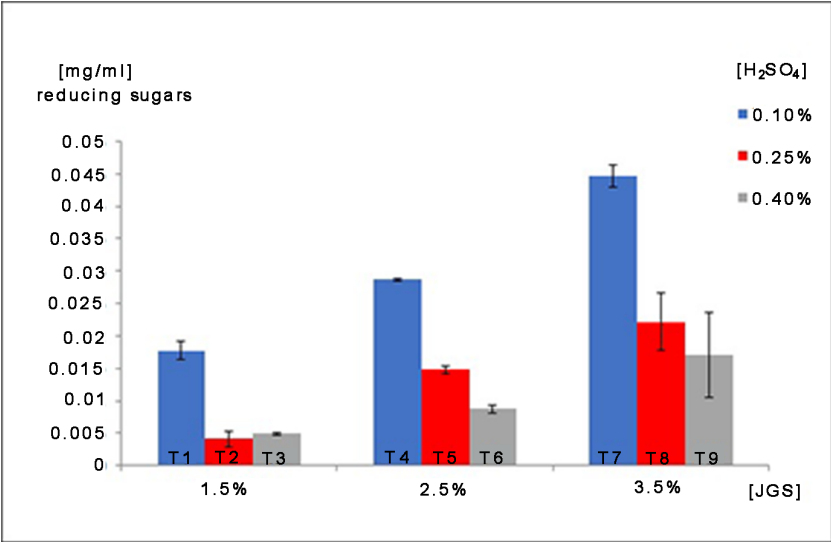


Figure 2. Acid hydrolysis treatments with H₂SO₄.

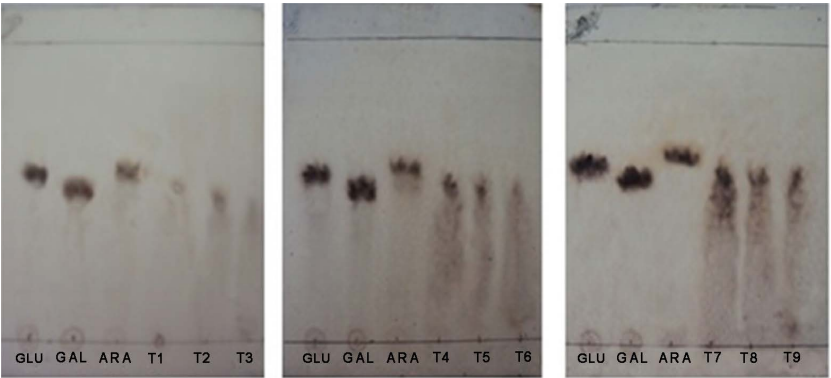


Figure 3. TLC for H₂SO₄ hydrolyzed samples.

Table 2. R_f values for the pattern samples and for the H₂SO₄ hydrolysis treatments.

Glucose	Galactose	Arabinose	T1	T2	T3	T4	T5	T6	T7	T8	T9
											64.2
									60		
		57									
55											
			52.5								52.8
	50					50			50.6	50.7	
							49.3	49.3			
				47.5							
					46.6						
									42.6		
											7.14

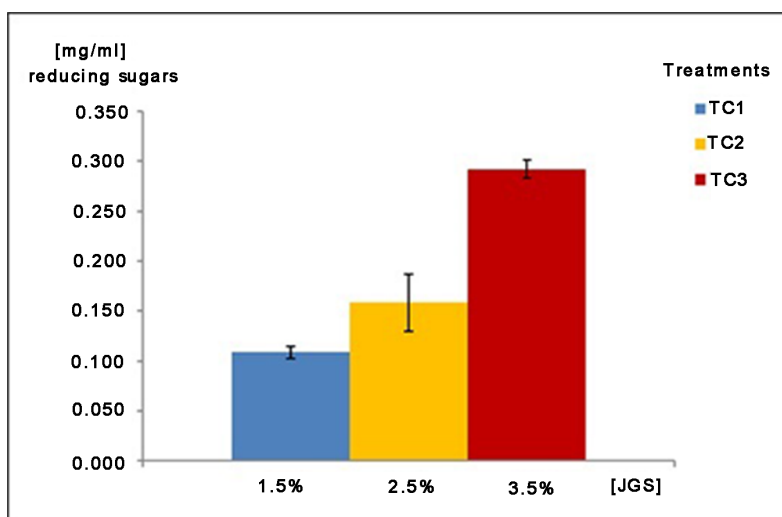


Figure 4. Reducing sugars for wet-heat-treated samples at 1.2 lb/in² for 15 minutes.

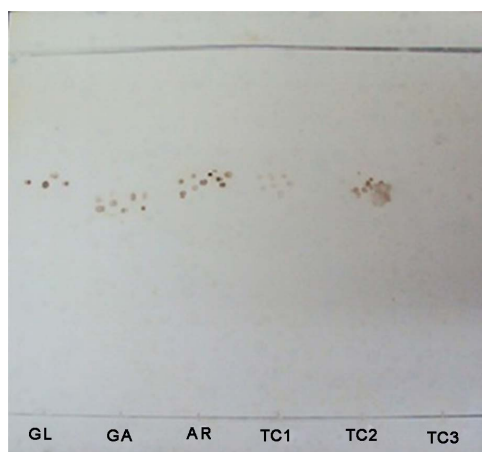


Figure 5. TLC for wet-heat-hydrolyzed samples at 1.2 lb/in² for 15 minutes.

values obtained for the three wet-heat-treated samples (TC1, TC2 y TC3), it is observed that marks on TC1 and TC2 coincide with $R_f = 59.4$. However, there does not exist such a development for TC3.

3.5. Quantification of Sugars in Hydrolyzed AGJ Using Enzymes

It is obvious that in TLC results, where enzymatic treatments were applied, (see **Figure 6**) no monosaccharide separation is observed. Despite that, when comparing the results given for both enzymes, it is found that Pectinex® AR is the best for using on TLC.

Nevertheless, the results obtained in the quantification of reducing sugars for samples treated with enzymes are larger than for samples otherwise treated (by means of acid hydrolysis or wet heat). Moreover, there was no significant difference between both enzymes, as can be seen in **Figure 7**.

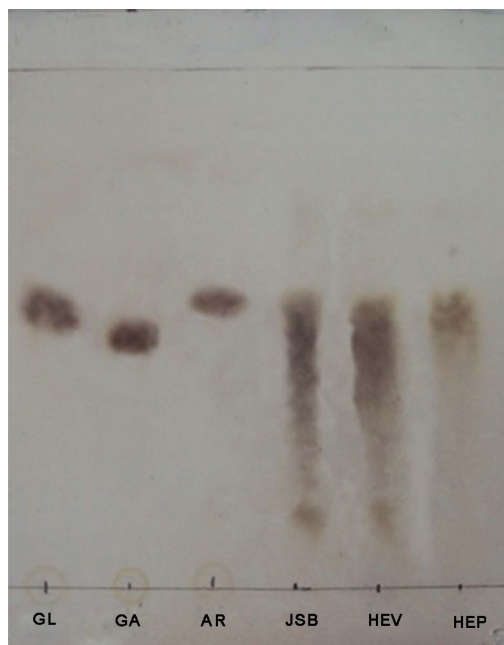


Figure 6. TLC for enzymatic-hydrolyzed samples.

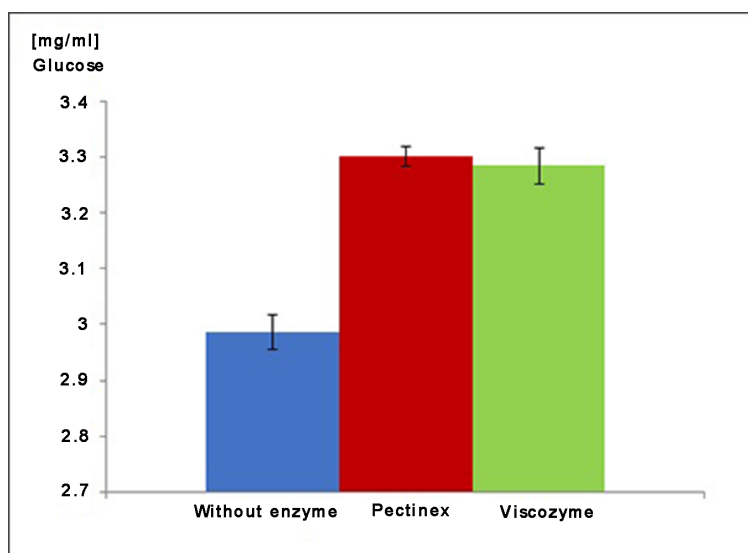


Figure 7. Reducing sugars for enzymatic-hydrolyzed samples.

3.6. Comparing Different Hydrolysis Methods in Producing Reducing Sugars

Figure 8 shows the compared results for the amount of reducing sugars (mg/ml of glucose) obtained by different hydrolysis treatments: the chemical one, with H_2SO_4 , the one using wet heat at $1.2 \text{ lb}_f/\text{in}^2$ for 15 minutes and, finally, the enzymatic one. Comparison is made with respect to total sugars and reducing sugars in non-treated AGJ samples.

Regarding the chemical hydrolysis with H_2SO_4 , the best treatment was the one labeled T7 (15 ml of AGJ 3.5% and 15 ml of a 0.10% H_2SO_4 solution), where 0.045 mg/ml of glucose were obtained together with a standard deviation of

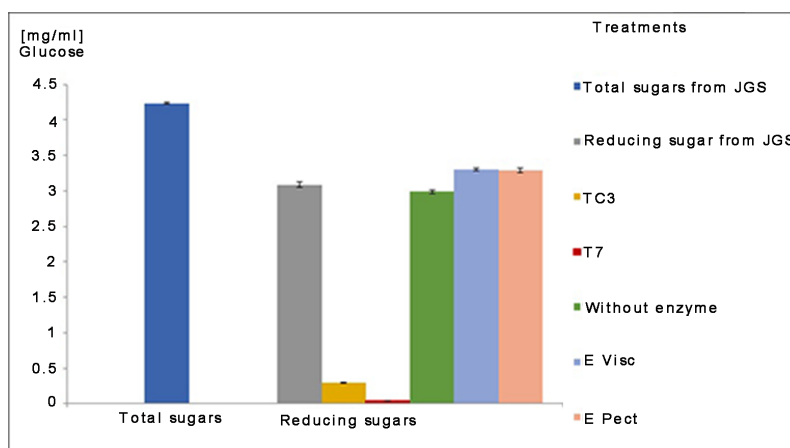


Figure 8. Best hydrolysis treatments.

± 0.0017 . For the wet heat treatments at 1.2 lb/in^2 for 15 minutes, TC3 (15 ml of AGJ 3.5% and 15 ml of H_2O) was the most efficient one, with 0.292 mg/ml of glucose and a standard deviation of ± 0.0087 .

In the case of enzymatic treatments, both of them showed high results. For the treatment using Viscozyme® (3 ml of AGJ with 3 ml of citrate buffer 0.05 M pH 5 and 4 μl of the Viscozyme® enzyme) 3.302 mg/ml of glucose were obtained and a standard deviation of ± 0.0166 . For the treatment with Pectinex® AR (3 ml of AGJ with 3 ml of citrate buffer 0.05 M pH 5 and 4 μl of the Pectinex® AR enzyme) 3.282 mg/ml of glucose were obtained together with a standard deviation of ± 0.0327 .

Among the total sugar (4.233 mg/ml of glucose) present in non-treated AGJ samples, 72.95% of them are reducing sugars (3.088 mg/ml of glucose).

From that 72.95% of reducing sugars one can obtain the 1.44% (0.0446 mg/ml of glucose) using treatment T7, involving an H_2SO_4 hydrolysis; the wet heat treatment (at conditions described above) produces 9.45% (0.292 mg/ml of glucose) of total reducing sugars, if one follows treatment TC3.

Concerning the enzymatic treatments, the non-treated solution (3 ml of AGJ with 3 ml of citrate buffer 0.05 M, pH 5 and corresponding ratio 2:1) produced 96.6% (2.986 mg/ml of glucose) of reducing sugars with a standard deviation of ± 0.031 ; on the other hand, solution treated with Viscozyme® produced 106.9% (3.302 mg/ml of glucose) of total reducing sugars and solution treated with Pectinex® AR produced 106.3% (3.282 mg/ml of glucose) of total reducing sugars.

3.7. TLC Analysis

Regarding chromatography results, Measurements on TLC plates using a solution of non-treated *Aloe vera* gel juice gave the following values for the reference patterns: $R_f = 50$ for glucose, $R_f = 46$ for galactose and $R_f = 55$ for arabinose; none of these values matched those found for analyzed samples. However, two patterns coincide in their R_f values among the AGJ samples, one with $R_f = 61.2$ and the other one with $R_f = 77.5$; similar retention factors suggest that the two substances could be the same, one present in *Aloe vera* gel juice obtained by fil-

tration with a 0.22 μm pore size membrane (AGJ) and the other one present in *Aloe vera* gel juice filtered with Whatman® Grade 1 filter paper (PFJ). TLC showed a larger scanning for the PFJ sample than for the AGJ sample due to the removal of some solid substances after filtering it with the 0.22 μm size pore membrane.

Worth mentioning, the average value of Rf in reference patterns (glucose, galactose and arabinose) was calculated for each TLC plate where acid hydrolyzed samples were applied. This is due to the fact that it was not possible to compare nine hydrolyzed treatments to the reference patterns in a unique chromatography.

The Rf values for the reference patterns were the following: Rf = 55 for glucose, Rf = 50 for galactose and Rf = 57 for arabinose. The Rf values for the samples analyzed are listed right away: T1 showed Rf = 52.5; T2 an Rf of 47.5; T3 an Rf of 46.6; T4 an Rf of 50; T5 an Rf of 49.3; T6 an Rf of 52.2; T7 an Rf of 50.6; T8 an Rf of 50.7 and for T9, Rf = 52.8. According to the Rf values listed above, it can be said that galactose is present in treatment T4 and it would be equally present in T5, T7 and T8 (with Rf values of 49.3, 50.6 and 50.7, respectively).

TLC plate analysis for samples treated with wet heat showed the following Rf pattern values: 58.8 for glucose, 56.3 for galactose and 61.3 for arabinose. The Rf values for samples TC1 and TC2 were identical and equal to 59.4; as it can be seen, they do not match any of the pattern's, and this means that this substance is none of the sugars taken as reference, but it can be concluded that the same substance is present in TC1 and TC2. The treatment labeled TC3 did not show any Rf value due to the fact that it was not developed in chromatography, as a result of the low concentration of the dry sample obtained after lyophilization. All the wet heat treatments needed a spot application on silica plates of over 50 μl and even though bands appeared very weakly.

TCL plate analysis for samples treated with enzymes did not show Rf values due to the lack of proper separation between compounds on the silica plate. This was caused by the wrong concentrations of enzyme and substrate in the enzyme-substrate complex used. It can also be explained by the characteristics of the enzymes used, as they were not specific for obtaining monosaccharides as glucose, galactose and arabinose.

4. Discussion

A non-treated fraction of *Aloe vera* gel juice was analyzed using the phenol-sulfuric acid and DNS methods and it was found that a little more than 50% of its total sugars are reducing sugars. AGJ total sugars deliver 4.233 mg/ml of glucose from which 3.088 mg/ml are from reducing sugars.

This portion of AGJ was hydrolyzed to break the polysaccharide glycoside bonds with sulfuric acid and nine samples were prepared combining three concentrations of the AGJ solution (1.5%, 2.5% y 3.5%) with three other H_2SO_4 concentrations (0.10%, 0.25% y 0.40%). A wet heat treatment was also applied at a pressure of 1.2 lb_f/in^2 for 15 minutes. The results (amount of reducing sugars) obtained for each of these nine samples were submitted to a statistical analysis

using the SPSS software. This software used Tukey's test to compare the dependent variables (acid and aloe gel) to the independent or response variable (reducing sugars) and it reveals that the best sulfuric acid treatments are those where the acid concentration was 0.10%, *i.e.*, T1 (15 ml of AGJ 1.5% and 15 ml of H₂SO₄ 0.10%), delivering 0.0177 mg/ml of glucose and having a standard deviation of ± 0.0013 , T4 (15 ml of AGJ 2.5% and 15 ml of H₂SO₄ 0.10%) delivering 0.0287 mg/ml of glucose with a standard deviation of ± 0.0001 and, finally, T7 (15 ml of AGJ 3.5% and 15 ml of H₂SO₄ 0.10%) delivering 0.045 mg/ml of glucose with a standard deviation of ± 0.001 . T7 merged as the best-performing one among these three treatments.

Having studied mango extract, Mejía [13] declares that reducing sugars tend to diminish their concentration for sulfuric acid concentrations over 0.50%. This is due to the fact that sulfuric acid degrades the fermentable sugars.

Pérez [14] has previously studied chemical hydrolysis on dried solid residual *Aloe vera* pulp using diluted sulfuric acid, and he detailed that the largest concentrations of reducing sugars were obtained for low acid concentrations.

Otherwise, reducing sugar production was considerably higher for the wet heat treatment at 1.2 lb_f/in² for 15 minutes than for the acid hydrolysis process. This is due to the fact that the wet heat treatment is manifestly a strong treatment for AGJ. Results for reducing sugars showed that the best treatment was the one labeled TC3 (15 ml of AGJ 3.5% and 15 ml of H₂O) delivering 0.292 mg/ml of glucose with a standard deviation of ± 0.0087 , while treatment TC2 (15 ml of AGJ 2.5% and 15 ml of H₂O) delivered 0.158 mg/ml of glucose with a standard deviation of ± 0.0289 and treatment TC1 (15 ml of AGJ 0.5% and 15 ml of H₂O) delivered 0.109 mg/ml of glucose with a standard deviation of ± 0.0057 .

Enzyme treatments gave much better results for reducing sugars than chemical treatments (H₂SO₄ hydrolysis and wet heat treatment at a specific pressure and time). Novozymes commercial enzymes were used and 3.302 mg/ml of glucose were obtained with Viscozyme® while 3.282 mg/ml of glucose with Pectinex® AR, compared to the total amount of reducing sugars present in non-treated AGJ, corresponding to 3.0882 mg/ml of glucose. The best results were then obtained with Viscozyme®, though the difference in reducing sugars production was only of 0.18 mg/ml higher than the production with Pectinex® AR.

In a previous study [14] [15], also carried out enzymatic hydrolysis on solid residual *Aloe vera* pulp using commercial enzymes as Celluclast®, Termamil® 2X, Pectinex® AR and Viscozyme®, reporting the best results in reducing sugar production for the last two enzymes. However, he notices that the reducing sugar levels attained with Viscozyme® at a temperature of 50°C could be till 2.64 times more hydrolyzed than those attained with Pectinex® AR at 40°C. Moreover, it is reported that the pH at which treatments were performed, with one enzyme first and then with the other, were different.

5. Conclusions

It is possible to quantify and identify the monosaccharides present in the main

polysaccharides in *Aloe vera* gel using a chemical hydrolysis with H₂SO₄. The best responding treatment is the one labeled T7 with 1.44% (0.0446 mg/ml of glucose) of reducing sugars.

Nevertheless, this does not mean that acid hydrolysis is the best method, as 9.45% (0.292 mg/ml of glucose) of reducing sugars were obtained for sample TC3 (wet heat treatment at 1.2 lb_f/in² for 15 minutes).

In spite of that, the Viscozyme® enzymatic treatment was much better than the previous ones for delivering 106.9% (3.302 mg/ml of glucose) of reducing sugars.

There was no presence of monosaccharides similar to the reference ones, as no matches were found between R_f values on wet-heat-treated samples and non-treated AGJ samples.

It was not possible to determine an R_f value for enzymatic hydrolyzed samples owing to an inadequate separation of compounds on the TLC plate.

In TLC identification of monosaccharides, better results were obtained for chemical hydrolyzed samples, as for five of them (T4, T5, T7, T8, T9) the polysaccharides present in AGJ were hydrolyzed up to monosaccharides, like galactose.

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