

Physical-Chemical and non-linear rheological properties of aqueous solutions of agave fructans

Propiedades fisicoquímicas y reología no lineal de soluciones acuosas de fructanos de agave

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Abstract: Fructans are storage carbohydrates with β -fructofuranosyl linkages that are present in several plant families. Those studied in this work were obtained using a technology based on water extraction from mature hearts of *Agave tequilana Weber* var. azul. It is known that agave fructan molecules are branched while commercial inulin (from *Cichorium intybus*) is a linear molecule, which could provide different properties and technological applications. In this study, agave fructans resulted on a mixture of different size molecules with an average molecular weight of 3,182 Da and an average polymerization degree of 18. Aqueous solutions of agave fructans were prepared (5-90 Wt. %) and subjected to a battery of tests such as pH and density at 30, 40, 50 and 60°C; additionally, conductivity and particle size (*QLS*) were analyzed at room temperature. Results suggested that fructans can probably form aggregates at concentrations higher than 28 wt %. Non linear rheological measurements were carried out at 30, 40, 50 and 60°C, previous stabilization of the samples at measurement temperature, showing that solutions have a Newtonian behavior except those at 80 and 90 Wt. % where shear thickening and thinning behaviors were observed. Finally, the rheological results supported the formation of aggregates in concentrations from 30 up to 70 wt %, through a steady shear rate measurements carried out at 30, 40, 50 and 60°C.

Keywords: *aggregates, polysaccharides, shear viscosity, biopolymers.*

Resumen: Los fructanos son carbohidratos de reserva formados por unidades fructofuranosil con enlaces tipo β principalmente, que se encuentran presentes en diversas variedades de plantas. Los fructanos empleados en el presente estudio, fueron obtenidos mediante una extracción acuosa a partir de piñas maduras de *Agave tequilana Weber* var. azul. Se sabe que las moléculas de fructanos derivados del agave, poseen una configuración ramificada a diferencia de la configuración lineal presente en la inulina comercial (derivada de la *Cichorium intybus*), lo que les podría otorgar diferentes propiedades y aplicaciones tecnológicas. La composición de los fructanos de agave obtenidos en éste estudio, mostró una mezcla con diferentes tamaños y pesos moleculares promedio de 3,182 Da así como un grado de polimerización promedio de 18. Las soluciones acuosas de fructanos de agave fueron preparadas (de 5-90% p/p) y sometidas a una serie de pruebas como pH y densidad a 30, 40, 50 y 60 °C; adicionalmente, la conductividad y el tamaño de partícula (*QLS* por sus siglas en inglés) fueron analizadas a temperatura ambiente. Los resultados de las pruebas mencionadas anteriormente sugieren que, los fructanos derivados del agave pueden formar agregados a concentraciones superiores al 28% p/p. Por otro lado, las mediciones reológicas no lineales de las soluciones acuosas de fructanos de agave se llevaron a cabo a 30, 40, 50 y 60 °C (previa estabilización de la muestra a la temperatura de medición) mostrando que presentan comportamientos Newtonianos excepto aquellas a 80 y 90 % p/p, donde comportamientos de adelgazamiento y espesamiento al corte fueron observados. Finalmente, los resultados reológicos aportan valiosa información sobre la formación de agregados en las soluciones acuosas de fructanos de agave en el intervalo de concentraciones de 30 a 70 % p/p, por medio de mediciones de velocidad de corte en estado estacionario efectuadas a 30, 40, 50 y 60°C.

Palabras clave: *agregados, polisacáridos, viscosidad de corte, biopolímeros.*

Introduction

Fructans are storage carbohydrates with β -fructofuranosyl linkages that are present in several plant families. These molecules can be linear or branched fructose polymers, which are either β 2 \rightarrow 1 linked called “inulin” or “levans” for a β 2 \rightarrow 6 linkage or a mixture of both, having a reducing ending group [46]. The most common inulin type fructans are from chicory roots (*Cichorium intybus*). On the other hand, levan type fructans are present in grasses and cereals as well as those produced by bacteria [2]; however, this levan fructans are rarely commercially available.

In contrast with other carbohydrates, due to its type of linkages, fructans are resistant against human digestion having a beneficial health effects as stimulators of good flora in colon, mineral absorption improvement, rebalancing of metabolic activities (lipid homeostasis) and colon cancer protection [11, 12, 18, 24, 29, 38, 44].

At the same time, inulin type fructans are widely used as food ingredients because of their gelling and thickening capacity, its ability for replace fat by up to 100% [22] as well as substitute of other carbohydrates to reduce the calories of some foods such as ice creams, dairy products and baked goods [23, 39, 44].

The fructans used in this work were obtained from *Agave tequilana Weber* var. azul, which is cultivated in several regions of Mexico mainly for tequila production. Recent work has shown that the molecular structure of these fructans is a complex mixture of highly branched fructans, that includes linkages of β 2 \rightarrow 1 as well as β 2 \rightarrow 6 [33, 35]. This particular conformation could provide different properties and applications in the food industry, as well as non food applications, from those reported for commercial inulin that has a linear structure. Due to this fact, the aim of this work was to characterize through different techniques, the physico-chemical properties of aqueous agave fructans solutions and its rheological behavior.

Thus, in this work we reported by first time the physical-chemical properties of aqueous agave fructans solutions as function of temperature and concentration which were characterized by a battery of analytical techniques: size exclusion chromatography (HPSEC), high performance liquid chromatography (HPLC), densimetry, pH, conductimetry, quasi elastic-scattering (QLS) and rheometry measurements.

Experimental Section

Seven years old *Agave tequilana Weber* var. azul plants, were obtained from a local farm in the Tequila town at the State of Jalisco, Mexico. The leaves were removed from the agave plant and the resulting hearts were chipped (into small pieces of 5 to 10 cm) and pulverized in a Sprout Waldron miller (disc refiner Sprout-Waldron model 105-A, 12 inch of diameter) to produce a pulp. The blue agave pulp was then extracted with tap water at 90°C for about 40 min and filtrated as described elsewhere [50]. The fructan solution was then clarified with diatomaceous earth, passed through a bed of activated charcoal (120 cm of thickness) and demineralized by passing it through ion exchange columns (Diaion SK IB and Diaion PA-308) and finally, spray dried using a mini spray dryer Yamato, ADL31 (140°C inlet temperature, 75°C outlet temperature and flow solution of 5 L/hr).

Two sets of aqueous solutions of agave fructans were prepared weighting the appropriated amounts of fructans powder and bidistilled water (from a Millipore equipment) at 25 °C and 80 °C, using a AM-8 homogenizer (Nihon Seiki Co., Tokio, Japón) with a concentric propeller. The samples were mixed at 11,000 rpm for 5 min at 25 °C (first set) and 2 min for 80 °C (second set).

Average molecular weight and molecular weights distribution were obtained by Size Exclusion Chromatography (HPSEC) coupled to a RI detector Shodex RI-71, Showa Denko K.K., Tokyo, Japan. The universal calibration curve was obtained with pullulan standards from PSS, Mainz, Germany. The software WINGPC 4.0 from Polymer Standard Service (PSS) was used for data capture and evaluation. HPSEC was performed by injecting 100 µL of blue agave fructans solution (4.93 g·L⁻¹) into a TSK-SEC columns thermostatically controlled at 40 °C from Tosoh Bioscience, Stuttgart, Germany, coupled in line (TSK G5000PW_{xl}, G4000PW_{xl}, G3000PW_{xl}, each 300X7.8 mm and a G2500PW_{xl} guard column, 40X6 mm). 0.1M NaNO₃ solution was utilized as eluent at 0.4 ml·min⁻¹ flow rate.

The average polymerization degree ($DP_{average}$) was obtained by HPLC using a Sephadex SP0810 column with water as eluent (1 mL·min⁻¹ flow rate at 80°C) coupled to a Differential Refractometer R401 as refractive index detector from Waters Millipore. The standards solutions registered retention times as follows: glucose 9.43min; fructose 12.15 min; and sucrose 8.15 min. 100 mL of fructan solution (13.83 g·L⁻¹) was prepared by dissolving the fructans powder in distilled water (Millipore equipment) and a Sodium Citrate buffer solution, KEM Mexico, reagent grade at pH 4.7. 200 µL of fructanase enzyme from Fructozyme L ®, Novozymes, Denmark, were added to the solution at 50 °C for 4 h with stirring. Finally the solution was heated at 90 °C for 3 min to denaturalize the enzyme and then stocked it at -20 °C for subsequent analysis. The degree of polymerization of the sucrose, glucose, and fructose content was calculated as follows:

$$dp_{enzym} = \frac{(gFruc + gGluc)}{gGluc} \quad (1)$$

where, gFruc and gGluc are the grams of fructose and glucose after enzymatic hydrolysis.

Density was measured in triplicate with an Anton Paar density and sound velocity analyser DSA 5000 at 5, 10, 20, 30, 40, 50, 60 y 70 wt.%. The measurement temperatures (30, 40, 50 y 60°C) were controlled by a peltier method having a $\pm 1 \times 10^{-3}$ °K of variation and the densities repeatability were 1×10^{-6} g/cm³. The apparatus was calibrated before each series of measurements. The apparent molar volumes were obtained from densities measurements, and calculated according to [27]:

$$V\phi = \frac{10^3(\rho_o - \rho)}{m\rho\rho_o} + \frac{M}{\rho}, \quad (2)$$

being ρ the density of the fructan solutions, ρ_o the density of the solvent, M and m are fructans molecular weight and the molality of the solution, respectively.

Measurements of pH and conductivity were performed in a pH and conductivity meter from Denver Instruments 220 and an immersion cell with cell constant = 1 cm⁻¹. The cell was calibrated for conductivity measurements, with bidistilled water (conductivity = 3 µs/cm) and KCl solutions (74.1 and 7.9 µs/cm). A water bath with temperature regulator was used to stabilize the samples (previously prepared) for 5 min at

each temperature (30, 40, 50 y 60°C) and concentration (5, 10, 20, 30, 40, 50, 60 y 70 wt.%) before they were tested.

Particle size of agave fructans solutions were measured with a Malvern 4700C quasielastic light-scattering (*QLS*) apparatus equipped with a laser power source of Helium-Neon of 40 miliwatts from Siemens and a controller set PCS7 from Malvern Instruments. Intensity correlation data were analyzed by the method of cummulants to provide the average decay rate, $\langle \Gamma \rangle (=q^2D)$, where q is the magnitude of the scattering vector and D is the diffusion coefficient. The measured diffusion coefficients were represented in terms of apparent diameters by means of Stokes-Einstein equation [7]:

$$R_H = \frac{k_B T}{6\pi\eta D_1} \quad (3)$$

where k_B is the Boltzmann constant, T the absolute temperature, and η the viscosity of the solvent. The equipment was calibrated with a polystyrene standart solution (Duke Science Corp. Nanosphere size standards, NIST traceable polystyrene polymer) of known particle size. The laser angle was settled on 90° and a opening of 400 Å. The measurements were performed at 25 °C.

Non-linear viscoelastic behavior experiments were performed in a TA Instruments Ares-22 deformation controlled rheometer. Cone-and-plate geometry of 0.1 radian and 50 mm in diameter was used for $C_{FRUCTANS} = 80-90$ wt.% solutions. A double wall Couette type geometry of 29.512 and 32 mm of inner and outer cylinder diameter respectively, and 27.941 and 34 mm of inner and outer cuvette diameter respectively was used for 5-70 wt.% solutions. An environmental control unit was placed around the cone-and-plate fixture and over the Couette geometry to prevent water evaporation. Temperature was controlled within 0.1 °C during measurements. Measurements temperatures were carried out at 30, 40, 50 and 60°C.

All the experiments were made by duplicate, except for the particle size determination which was measured by triplicate, the average of the two values is reported here for all the parameters tested. Temperatures and concentrations were selected according to a previous study on levan molecules [2]. Results were subjected to an analysis of variance (one way or multifactor analysis, depending of each case) to compare mean values using a statgraphics plus 4.0 software. The significance level was $P < 0.05$.

Results and Discussion

Determination of molecular weight (M_w) and polymerization degree (dp)

[Figure 1](#) shows the HPSEC chromatogram for agave fructans. A trimodal distribution was found related with different molecular weight polymer fructans between 180 Da and 4,000 Da, corresponding to dp average values from 1 (a single monosaccharide molecule of fructose) to 22, showing that our sample is formed by a mixture of fructan molecules with different dp 's. An average molecular weight of 3,182 Da was obtained for agave fructans derived from HPSEC analysis taking into account the molecular distribution of the three peaks, the average polymerization degree was $dp_{average} = 18$. [Figure 2A](#), shows the HPLC chromatogram for the four main carbohydrates in the analyzed material, which peaks are at 5.75, 7.87, 9.43 and 12.21 min for fructans, sucrose, glucose and fructose respectively. Results from HPLC analysis shows that agave fructans sample has a 87-90 wt.% of fructans, and a 10 to 13 wt.% of mono and disaccharides content, which are distributed as follows: 48% as fructose, 35% of sucrose and glucose in a 15% w/w. After enzymatic hydrolysis, the whole agave fructans content was hydrolyzed to glucose and fructose ([Figure 2B](#))

as well as a small quantity of sucrose that was unable to be quantified due to equipment limitations. The average dp from agave fructans sample was obtained through HPLC analysis applying Eq. (1), resulting in a $dp_{average} = 16$. The differences between HPLC and HPSEC results are discussed below.

Previous results have shown that the average dp of agave fructans (*Agave tequilana* Weber var. azul) extracted with 80% ethanol method was from 3 to 29 and a molecular weight range of 527- 4739 Da [33, 35], which could be considered similar results to those obtained in our study (dp range from 1 to 22 and molecular weight range from 180 to 4,000 Da derived from HPSEC analysis) using a hot water method. We believed that at laboratory scale the 80% ethanol method decreases the amount of mono and disaccharides and increases the amount of molecules from a higher dp ($dp > 25$) due to the polarity of ethanol. At the same time, HPLC results shows a $dp_{average} = 16$, which varies from the one obtained by HPSEC ($dp_{average} = 18$). These results could be due to the performance differences in the techniques, as the HPSEC could be less accurate than HPLC due to the lack of a purified agave fructan standard; in our case, we use a pullulan standard which could have different elution times than agave fructans. In contrast with the HPSEC, the enzyme (inulinase) used in our study for the HPLC analysis, has the ability to hydrolyze β 2 \rightarrow 1 as well as β 2 \rightarrow 6 linkages, which means that after a certain amount of time the agave fructans sample was converted into quantified mono or disaccharides, in our case, sucrose, fructose and glucose, having as a consequence less mistake range.

Visual observations

Aqueous solutions of agave fructans (AFAS) for $C_{FRUCTANS} < 30$ wt.% exhibit a low viscosity similar to the water at the whole temperature range studied (30-60°C). At $C_{FRUCTANS} > 30$ wt.%, the viscosity increases with increasing concentration up to $C_{FRUCTANS} > 70$ wt.% where the system forms a highly viscous fluid that exhibits an apparent yield stress. Above $C_{FRUCTANS} = 70$ wt. % the system showed even higher viscosity that made difficult sample manipulation. Nevertheless, fructan solutions from 5, 10, 20, 30, 40 50, 60, 70, 80 y 90% did not show a gel like behavior at the conditions tested here. It is worth to mention that solutions at 90% were extremely viscous, making possible rheological measurements only.

Density measurements and apparent molar volumes determination

The density (ρ) of agave fructans aqueous solutions (AFAS) was measured as function of the $C_{FRUCTANS}$ and temperature. [Figure 3](#) shows the obtained results. Density increases with increasing concentration. The linear relationship between ρ and $C_{FRUCTANS}$ could be explained with a power law with form $\rho \propto C_{FRUCTANS}^a$. For low to medium concentration (0-20 wt%), ρ has a linear behavior with an average slope of $m = 0.04085$ while for medium-to-higher concentration region, ($C_{FRUCTANS} = 30-70$ wt.%) ρ increases with increasing concentration with an average slope value of $m = 0.19872$. This change in the slope may suggest that, at this concentration value (27.2 wt.%), some aggregation phenomenon could occur and it could be related to structural changes that will be discuss below. The temperature effect at constant concentration was the expected, ρ decreases as increasing temperature [25].

In [Figure 4](#), for a low-concentration region ($C_{FRUCTANS} \leq 5$ wt.%), the apparent molar volume (V_ϕ) was concentration independent. For the range of fructans concentration between 10 and 70 wt. %, the apparent molar volume decreases as the concentration increases ([Figure 4](#)). Similarly to the density measures ([Figure 3](#)), a slope change was observed in a critical concentration value ($C_{FRUCTANS}^*$) for $C_{FRUCTANS}^* \approx 28$ wt.%. The decreasing on the apparent molar volume could be due to a bigger occupation volume of a single fructan

monomer in a diluted solution; if more molecules are added to this system (medium-to higher concentration region), the distance among them became smaller as well as the volume occupied for a single molecule. In this case, above 28 wt.% of agave fructans concentration, the partial molar volume decreases in a more pronounced slope ($m=-0.337802$) supporting the aggregate formation theory previously mentioned. The behavior of the diluted region could be explained according to Odell et al. [41] that reported that polysaccharide's molar volume in water (v_m) is maximum because charge repulsion forces the molecule into the stiff (uncurled) conformation of a rigid rod that cannot be extended further.

For medium to higher concentration region, the addition of solutes (fructans and other impurities), promotes that molecules begin to exercise stronger attraction for each other reducing the available space for molecule extension reducing its molar volume. Walter R. [49] affirms that as solvent quality deteriorates, e.g. by changes in salt or ethanol concentrations, molecular motion declines, molar volume becomes less and the solute molecules begin to exercise a strong attraction for each other. This could be also enhanced by the reduction of free water as solutes increase concentrations. The effect of water activity reduction is discussed in more detailed below in relation to pH and conductivity behaviors.

pH and conductivity (*k*)

According to [Fig. 5](#), the pH of AFAS decreases as agave fructans concentration increases. At the same time, [Figure 5](#) also shows that the AFAS average pH increases according to temperature, and even though both factors have a statistical influence over the solution's pH value ($P < 0.05$) it is easily seen that the strongest influence is provided by fructan concentration. The relationship between pH and C_{FRUCTANS} shows two behaviors the same as density and apparent molar volume. In this case, the slope change (from $m=-0.043$ to $m=-0.023$) is located at 36 wt.% of agave fructans concentration. Similarly, [Figure 6](#) shows the conductivity values for solutions prepared at 25 and 80°C, for concentrations from 5 to 60 wt.% of agave fructans. According to the statistical analysis, only the agave fructans concentration was significant ($P_{\text{value}} < 0.001$) in the conductivity value, and this is reflected in [Fig 6](#), where the conductivity value increases with concentration and reaches a maximum value of 14 $\mu\text{S}/\text{cm}$ at C_{FRUCTANS} 28 wt.% without any influence of temperature. After reaching the 28 wt.% of agave fructans concentration the conductivity value falls down to 3.1 $\mu\text{S}/\text{cm}$ at 70 wt.% of agave fructans concentration, which is very close to water conductivity value.

The behavior of pH above $C_{\text{FRUCTANS}} = 36$ wt.% in AFAS could be attributed to the presence of ions as impurity (from ion exchange step mentioned in the extraction process) in the agave fructans sample. As C_{FRUCTANS} increases, the amount of ions (impurities) increases being reflected in the agave fructans solutions pH measurement. Moreover, the reduction of free water available for solvation as more fructan molecules are present in the system may increase the concentration of hydrogen ions that is directly reflected in the decrease of pH. As polysaccharides are hydrocolloids, their physicochemical properties depend on macromolecule-water, macromolecule-macromolecule interactions and macromolecule chains with disperse entities [21]. Therefore, when there is a saturation of macromolecular-water interactions, it is expected a reduction of the free water of solvation. This phenomenon supports the increase of ions by the reduction of free water and also the possibility of more molecule-molecule fructan interactions reducing the apparent molar volume as described above. The effect of polysaccharides as water activity reducing agents is also discussed by Badui [6].

For conductivity relationship with agave fructan concentration, the maximum value (and the slope change) at $C_{\text{FRUCTANS}} = 28.9 \text{ wt.}\%$, suggests that below the 28.9 wt.% (first slope) the conductivity could be dominated by the intrinsic ions (as it was previously mentioned in pH) interaction with water, while above this critical concentration the agave fructan molecule inhibits the ion effect hindering the electron flux and, therefore, the conductivity decreases dramatically. The reduction of conductivity could also reflect that the agave fructan molecules are not charged, which is a favorable condition for molecular aggregation through hydrophobic interactions. Moreover, when comparing with pH behavior, there is a less pronounced slope at while fructan concentrations increased which could support again the reduction of free water previously mentioned. Nevertheless, we believed that more studies need to be done to clarify our hypothesis about agave fructans conductivity behavior of these specific polysaccharide molecules.

Particle size determination

Particle size in this study can provide evidence of molecular aggregation at some concentrations tested. [Fig. 7](#) shows the values of particle size for AFAS. Similarly to the previously discussed properties, there are two behaviors with increasing concentration, which is reflected in a slope change at 28 wt.% of agave fructans concentration for those solutions prepared at 25°C, and a slope change at 29.5 wt.% of agave fructans concentration in solutions prepared at 80°C. The average particle size of high temperature (80°C) AFAS is $6.20 \pm 4.75 \mu\text{m}$ which is about twice the particle size of room temperature solutions ($2.72 \pm 2.12 \mu\text{m}$) statistical analysis showed a strong dependence of both factors in the particle size of AFAS. Moreover, the particle size increased the same as concentration in both cases, having a maximum particle size of $26.70 \mu\text{m}$ at 60 wt.% of agave fructans concentration for aqueous solutions prepared at high temperature versus $6.913 \mu\text{m}$ of those prepared at room temperature at the same concentration percentage.

According to Kim et al. [\[30\]](#) in inulin type fructans, thermally induced inulin gels shows the smallest particle size (above 60°C), which in our case is the opposite, at higher temperatures there is a bigger particle size ($2.31 \mu\text{m}$ from inulin versus $6.20 \mu\text{m}$ of agave fructans). This is an evidence that agave fructans possess a different behavior from the inulin type fructans, despite of being a member of the fructan family and having β 2→1 linkages. The branched conformation with the β 2→6 linkages might affect its interaction mechanism with solvents like water. Moreover, it was found in our study that AFAS did not exhibit any gel formation as inulin does, this behavior could obey due to the agave fructans branched structure, which makes harder their alignment, having as a consequence a none gel formation due to weaker hydrogen bonds which is comparable with the behaviors of both amylopectin [\[48\]](#) and levan polysaccharides [\[2\]](#).

Rheological Measurements

Steady shear viscosity measured at 30 and 60 °C as a function of shear rate is presented in [Figure 8A](#) and [Figure 8B](#) respectively for agave fructans solutions. A complex behavior is observed as a function of C_{FRUCTAN} in both temperatures. In all cases, η_0 increases the same as agave fructans concentration and decrease with temperature for all the concentrations studied. [Figure 8A](#) shows a Newtonian behavior (viscosity independent of the shear rate) for all the concentration range studied except for the $C_{\text{FRUCTANS}} = 90 \text{ wt.}\%$ where shear thinning is observed at higher shear rates. Shear thinning behavior is characterized for a viscosity decrease with the shear rate due to structure destruction or a new structure formation. It is important to mention, that for $C_{\text{FRUCTANS}} = 90 \text{ wt.}\%$, the agave solutions are very viscous and they were measured in a cone-plate geometry. However, notice that for $C_{\text{FRUCTAN}} \leq 30 \text{ wt.}\%$ (which were measured in a double wall Couette geometry), and at shear rates smaller than 70 s^{-1} shear thinning was detected followed by shear thickening at shear rates higher than 270 s^{-1} . Arvidson et al. [\[2\]](#) found shear thinning for

aqueous levan polysaccharide solutions of 30 wt. % and measured at 20 °C. The shear thickening behavior observed at high shear rates could be attributed to hydrodynamic flow instabilities formation. It is well known that Double wall Couette type geometry presents secondary flow formation or Taylor Vortices at higher shear rates, behaving turbulent like type flow [19, 45, 47]. This phenomenon has been studied predicting the stress near to the wall with the theoretical analysis of Stuart [10, 19]. Several authors have reported hydrodynamic instabilities such as Taylor-Couette Vortices in Newtonian fluids [10] and viscoelastic fluids [1, 5]. Macias et al. [34] observed flow instabilities at shear rates higher than 250 s⁻¹ for water in Double wall Couette type geometry. The shear thinning observed at low fructans concentration ($C_{\text{FRUCTANS}} \leq 30$ wt. %) could be due to these flow instabilities, since the viscosity of agave solutions is near to the water viscosity. But more analyses should be done.

[Figure 8B](#) depicts steady shear viscosity measured at 60 °C as a function of shear rate for agave fructans solutions. Similar behavior was observed to those measurements observed at 30 °C ([Fig. 8A](#)). Newtonian behavior is observed for $C_{\text{FRUCTAN}} < 80$ wt. %. The solutions were shear thinning at higher fructan concentrations ($C_{\text{FRUCTANS}} \geq 80$ wt. %). For $C_{\text{FRUCTANS}} = 90$ wt. %, shear thinning at low shear rates (< 0.1 s⁻¹), followed by shear thickening at high shear rates (> 1 s⁻¹) was observed. The shear thinning like behavior at low shear rates (20 s⁻¹) followed by shear thickening like behavior observed for $C_{\text{FRUCTAN}} \leq 30$ wt. % at high shear rates (300 s⁻¹) could be to the flow instabilities discussed above 4.

The shear thinning behavior has been observed and studied extensively by several polysaccharides [4, 8, 14, 15, 28, 32, 36, 37]. Chronakis et al. [8] and Chen et al. [15] reported flow steady state shear rate measurements for a series of concentrations of Kappa-carrageenan polysaccharides diluted solutions. Chronakis et al. [8] showed a typical shear thinning behavior with a Newtonian plateau at low shear rates was exhibited at concentrations higher than 0.1 % w/v. The limiting Newtonian viscosity η_0 was experimentally attainable for concentrations up to 0.65 % w/v. Below 0.1 %, the flow curves were essentially Newtonian. Burkus and Temelli [8] reported the shear viscosity of β -Glucan solutions (1 wt. %) at different temperatures (0.1-75 °C). Over the whole range of investigated temperatures, the viscosity decreases with increasing the temperature. Similarly to other polysaccharides, they found a shear thinning behavior up to 55 °C. Also a Newtonian behavior was observed at 65 and 75 °C. However, all the studies are related to the rheological behavior of polysaccharides diluted solutions, but not in the semi-diluted and concentrated regime as it was done in this work.

[Figure 9](#) depicts the zero-shear viscosity (η_0) dependence with fructan concentration obtained under steady shear rate measurements at various temperatures. As can be notice, this plot reveals three distinct linear regions. η_0 exhibits two slope changes at 35 and 70 wt. % of fructans, which are related to structural changes. The first slope change coincides with that one observed in density, apparent molar volume and pH measures. For $C_{\text{FRUCTANS}} \leq 35$ wt.% where correspond to the diluted regime isolated fructan chains are present. At the first slope change ($C_{\text{FRUCTANS}}^{**} = 35$ wt. %) the chains start to overlap and the free motion of a single chain is restricted by the presence of other. Here, the branched fructan molecule is considered like ideal isolated coils with low each other molecules interactions and increasing the fructan concentration the coil overlap to form a molecularly entangled solution ($C_{\text{FRUCTANS}} = 35-60$ wt. %). Thus C^* marks the onset of significant coil overlap and interpenetration. At the second slope change ($C_{\text{FRUCTANS}}^{**} = 70$ wt. %) macromolecular chains are entangled forming a weak gel where “hyperentanglements” occurs similarity like those observed for polysaccharides with linear backbones [20, 26]. Similar behavior is well known for synthetic polymer solutions in the diluted regime [17], and is attributed to the structural transitions from diluted solutions where individual polymer molecules are present as isolated coils, to concentrated solutions

where entanglements occurs increasing the hydrodynamic volume of the individual chains [37]. Morris et al. [37] reported the concentration dependence of zero-shear viscosity (η_0) for some “random coil” polysaccharides diluted solutions: guar gum, dextran, high guluronate alginate and polystyrene in toluene (a synthetic polymer). They found that regardless the structure and polymer origin and type, all the curves has the same behavior and shape, but the C_{POLYMER}^* varies and shift. This is a strong evidence that the fructans solutions behave as random coil chains before their coil overlap concentrations is reached ($C_{\text{FRUCTANS}}^* = 35$ wt. %).

The viscosity increase is explained on the basis of a transition from non-entangled fructant chains, in which the entanglement number density augments with increasing the fructant concentration. The two slope changes are detected at medium-concentration regimen at $C_{\text{FRUCTANS}} = 35$ wt.% which are related to the overlap chains concentration (C_{FRUCTANS}^*) and coincides with the results obtained from density, apparent molar volume and pH measures. At higher concentrations at $C_{\text{FRUCTANS}} = 70$ wt.% which corresponds to the C_{FRUCTANS}^{**} , the solution behaves like a weak gel where high entanglements between fructan chains are detected. This result could not be corroborated with the densimetry, pH, conductimetry, QLS measurements due the high solution viscosity.

For low-concentration region ($C_{\text{FRUCTANS}} \leq 20$ wt.%) η_0 follows a scaling law as follow: $\eta_0 \approx C_{\text{FRUCTANS}}^{0.4}$. Similarity, in the range of C_{FRUCTANS} concentration between 30 and 60 wt.%, η_0 follows a scaling law of $\eta_0 \approx C_{\text{FRUCTANS}}^{6.1}$. $\eta_0 \approx C_{\text{FRUCTANS}}^{6.0}$ power law dependence has been observed previously in linear polysaccharides like carraggenans [16]. For solutions of linear polymers in good solvents the dependence of η_0 with concentration should be $\eta_0 \approx C_{\text{FRUCTANS}}^{4.7}$ [43]. However, as can be seen in [figure 9](#), the exponent values in the viscosity scaling law increases as the polysaccharide concentration augments. Therefore the power law value gives a screen to understand the solution structure. Kapoor et al. [28] reported for amylopectin solutions at 2-4 wt. % a scaling law of $\eta_0 \approx C_{\text{FRUCTANS}}^{1.4}$. They observed a shear thinning behavior throughout the shear rates range of 0.01-10 s^{-1} , witch was attributed to stress-induced breakdown of the network. However, they did not study at medium-to-high- concentration regimens. Similar results has been reported by Lopes da Silva and Rao [32] for pectin solutions at 1.5-3.3 wt %, where a shear thinning behavior was observed. Morris and Ross-Murphy [36] reported for high-methoxyl pectin solutions at 0.8-1.4 wt. % a scalling law of $\eta_{SP} \approx C_{\text{HM-PECTIN}}^{3.6}$. Here η_{SP} is the specific viscosity defined as $\eta_{SP} = [(\eta_0 - \eta_S) / \eta_S]$, where η_S is the solvent viscosity. This solutions where studied above the critical concentration (c^*). The slope 3.6 is near the expected value for disordered rando-coil polysaccharides [37] and consistent with the vale of 3.3 obtained for citrus pectin solutions [4, 14].

At higher fructans concentration, (70-90 wt.%) η_0 follows a scaling law of $\eta_0 \approx C_{\text{FRUCTANS}}^{27}$. The calculated slope values were taken from the average of all temperature curves. Three regimes of concentration-dependence have been observed previously for polysaccharides in the diluted regime such Dika and Okra gums [40], ordered Xanthan [13], cellulose derivatives [9] and carrageenans [16]. Arvidson et al. [2] reported for levan polysaccharide, which is a branched type of fructan, four distinct regions for diluted fructant solutions ($C_{\text{LEVAN}} \leq 6$ g/dL) measured at 20 °C. However, they found a small slope values due their solutions follows the Hugging equation for polymers in diluted in range of solutions.

The inset on [figure 9](#) shows η_0 as a function of inverse temperature for the range of fructans concentrations from 5 to 70 wt. %. As was expected, η decrease with temperature for all the concentrations studied. The Arrhenius plot indicates a thermal activated process of the form $\eta(T) = \eta(T_0) \exp(E_a / RT)$,

where R is the gas constant and E_a is the activation energy and $\eta(T_0)$ is a coefficient depending on the nature of the solution. E_a is the energy barrier that must be overcome before the elementary flow process can occur. [8] The values of the activation energy calculated from the inset on [figure 9](#) are reported in [table 1](#) as a function of fructan concentration. Good agreement (R^2) with linearity was found. The activation energy of agave fructan is concentration dependant. It remains nearly constant in the diluted and semi-diluted regime ($C_{\text{FRUCTANS}} \leq 50$ wt.%). At higher fructans concentrations ($C_{\text{FRUCTANS}} 60-70$ wt.%) E_a increase tree times indicating the increase of solutions viscosity. Also, higher E_a means higher viscosity dependence on temperature changes [42].

The values of E_a for the agave fructans studied here are lower than those founded in other polysaccharides, such as glucose polymer β -Glucan [8]. They reported values of 33.2 KJ/mol for 1 wt. % at 0.1-75 °C. However, other authors had been reported values much lower for β -Glucan gums ($E_a=3.1$ KJ/mol) [3]. E_a differences with the fructans type studied here could be related to the structural differences with the β -Glucan, which it contains $\beta(1\rightarrow3)$ and $\beta(2\rightarrow6)$ linkage with glycosidic bonds, which makes it more viscous than the fructans and therefore with a higher E_a .

Conclusions

Physical-chemical and non linear rheological characterization of agave fructan aqueous solutions was firstly reported in this study. Density, partial molar volume, pH, conductivity, particle size and zero shear viscosity reflected that at increasing concentrations of agave fructans there were at least two behaviors reflected in slope changes in the relationship between every specific parameter and fructan concentration. These behaviors could be considered different regimes, where the first critical point at increasing concentrations of agave fructan was about 28 wt.% for most of the measured properties, suggesting that above this value fructan molecules begin to form aggregates as more important increases in particle size and shear viscosity were evident. This also was consistent with behavior changes in the other evaluated properties. Moreover, the zero-shear viscosity as a function of fructan concentration exhibited three distinct regions where the third region suggested another structural change in the system when molecules became closed packed.

Shear viscosity of agave fructan solutions exhibited a Newtonian behavior at 30 °C, for samples with concentrations ≤ 80 wt.%. For higher concentrations when a highly viscous solution is formed, the system exhibits a shear thinning behavior at room temperature while at higher temperatures a shear thickening is observed. Shear viscosity showed a thermal activated process as shear viscosity decreases along with temperature for all the concentrations studied according to Arrhenius model.

Results showed in this work contributed to make evident some structure related properties of agave fructans in aqueous solutions. Further investigations are needed to explain factors promoting aggregate formation, and for a deeper exploration the shear thickening and shear thinning behaviors. Additionally, linear rheology studies will complement the characterization of the viscoelastic properties of agave fructan aqueous solutions as they showed no gel formation at the conditions of this experiment.

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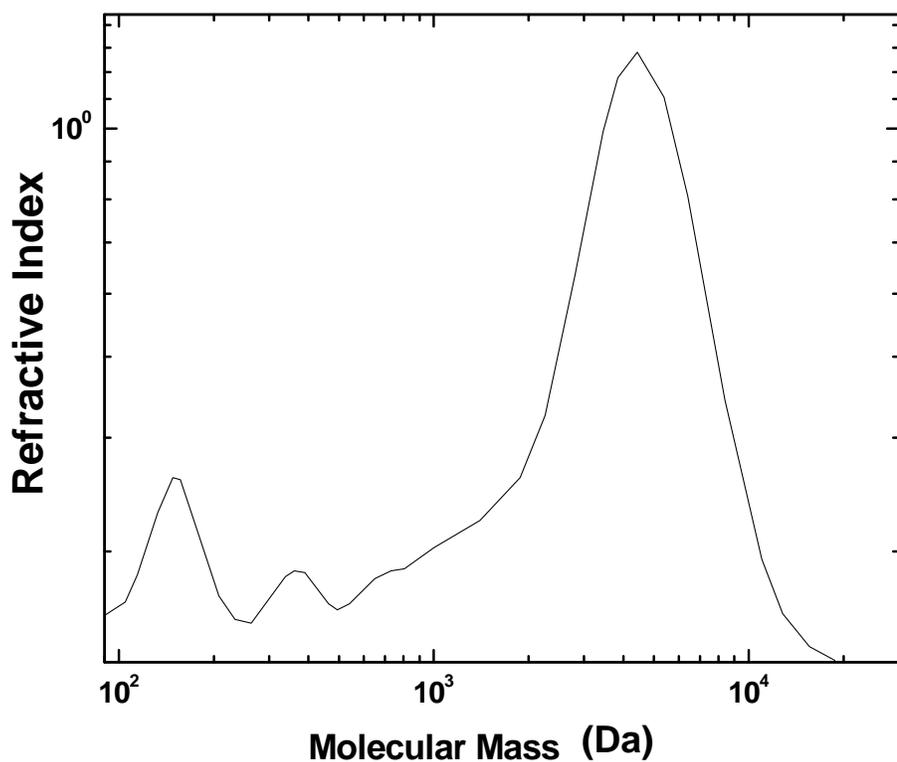


Figure 1. HPSEC-chromatogram of agave fructans

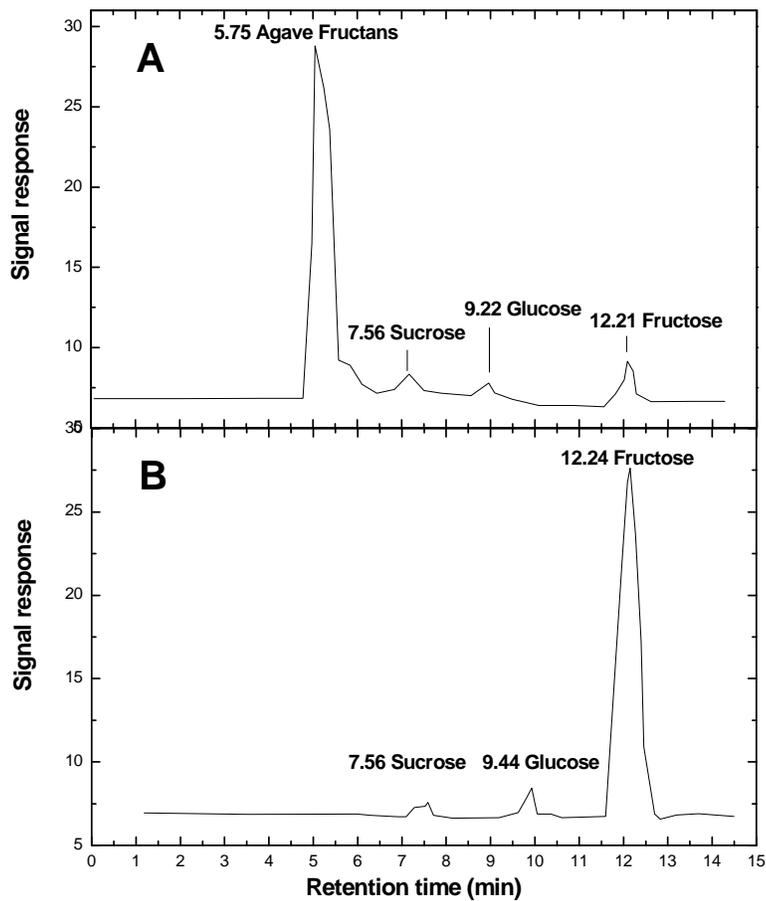


Figure 2. HPLC detector output versus elution time of agave fructans

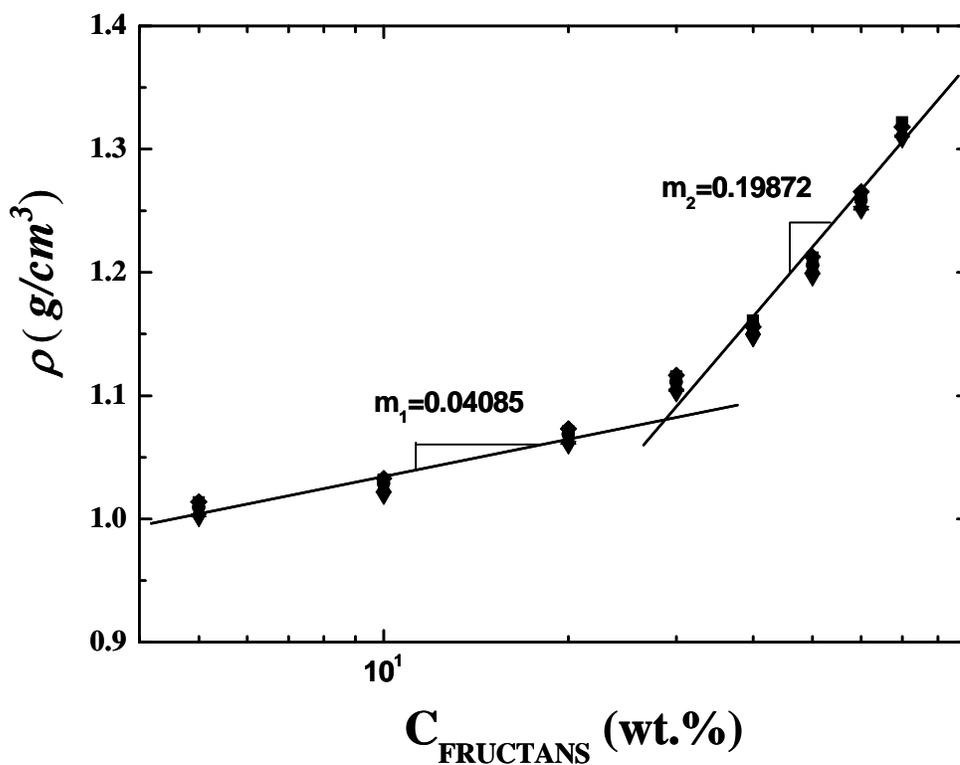


Figure 3. Density of agave fructans solutions versus concentration at different temperatures (°C): (■) 30; (●) 40; (▲) 50; (▼) 60; (◆) 30 prepared at 80 °C. The solid line is an aid to the eye.

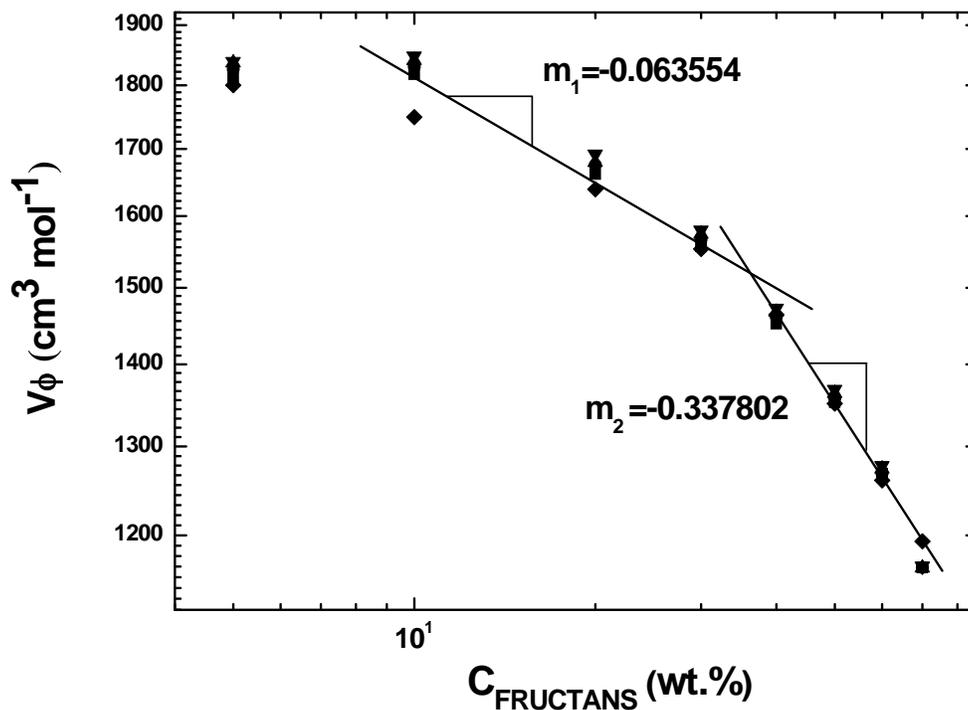


Figure 4. Apparent molar volume collapse with temperature of agave fructans solutions temperatures ($^{\circ}\text{C}$): (■) 30; (●) 40; (▲) 50; (▼) 60; (◆) 30 prepared at 80 $^{\circ}\text{C}$. The solid line is an aid to the eye.

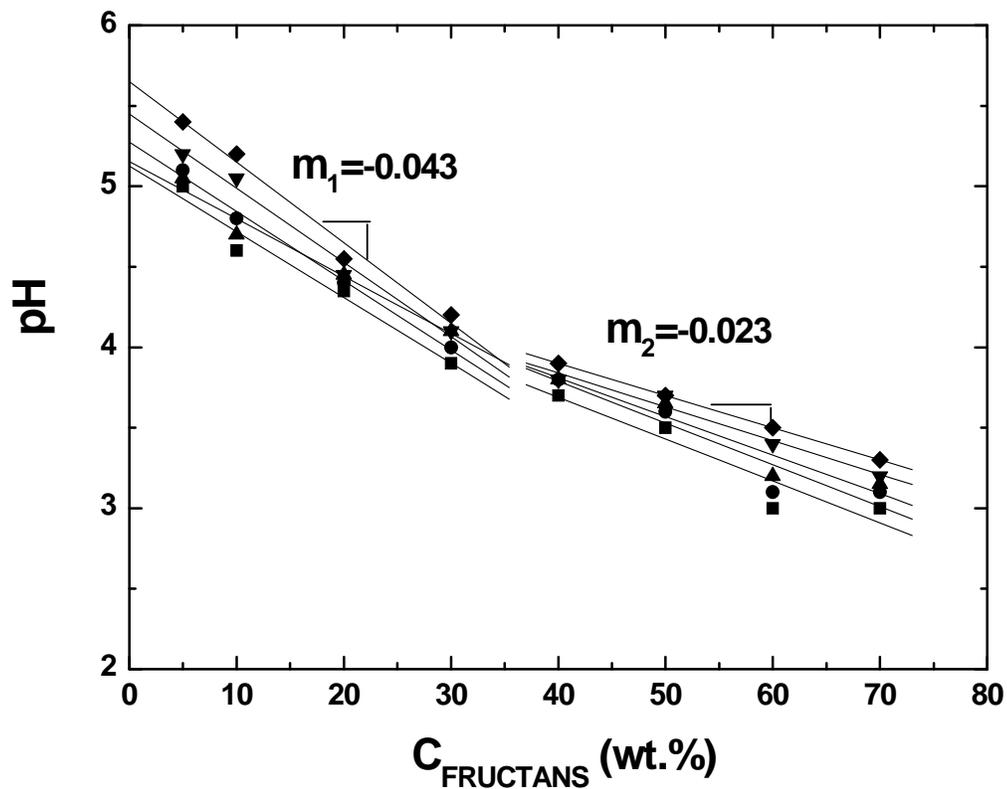


Figure 5. pH of agave fructans solutions versus concentration at different temperatures (°C): (■) 30; (●) 40; (▲) 50; (▼) 60; (◆) 30 prepared at 80 °C. The solid line represents the fitting using a linear behavior.

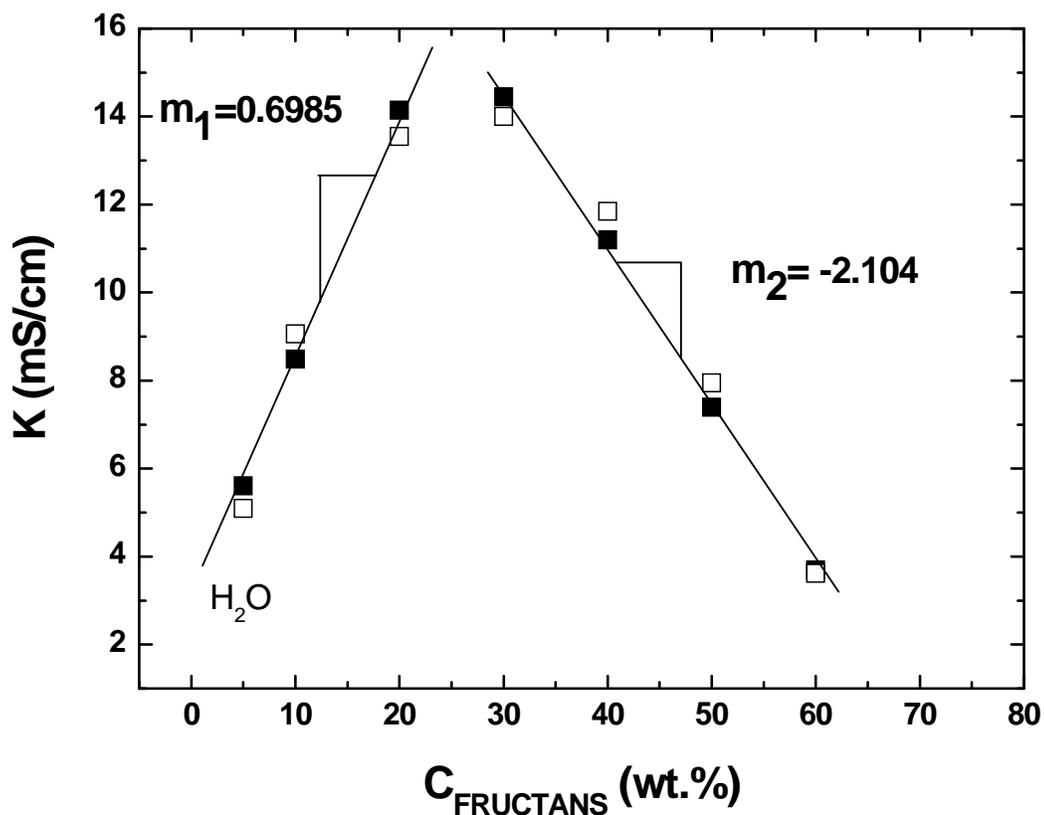


Figure 6. Electrical conductivity (K) of agave fructans solutions versus concentration at different preparation solution temperatures ($^{\circ}C$): (■) 25; (□) 80. The solid line is an aid to the eye.

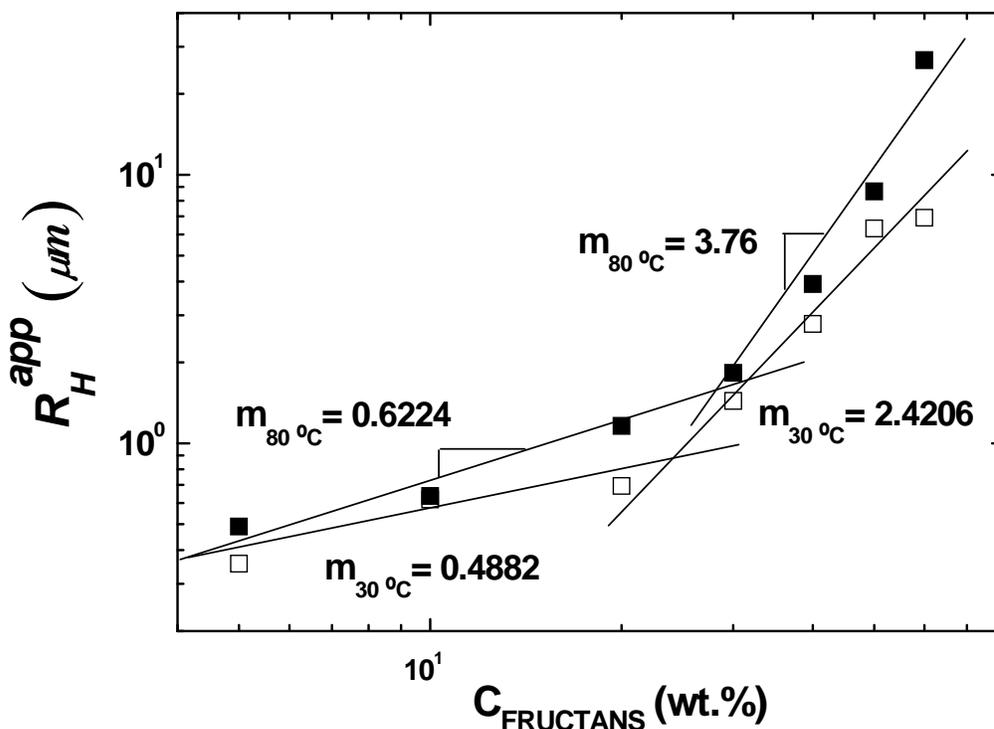


Figure 7. Apparent hydrodynamic radius of agave fructans solutions versus concentration at different preparation solution temperatures ($^\circ C$): (■) 25; (□) 80. The solid line is an aid to the eye.

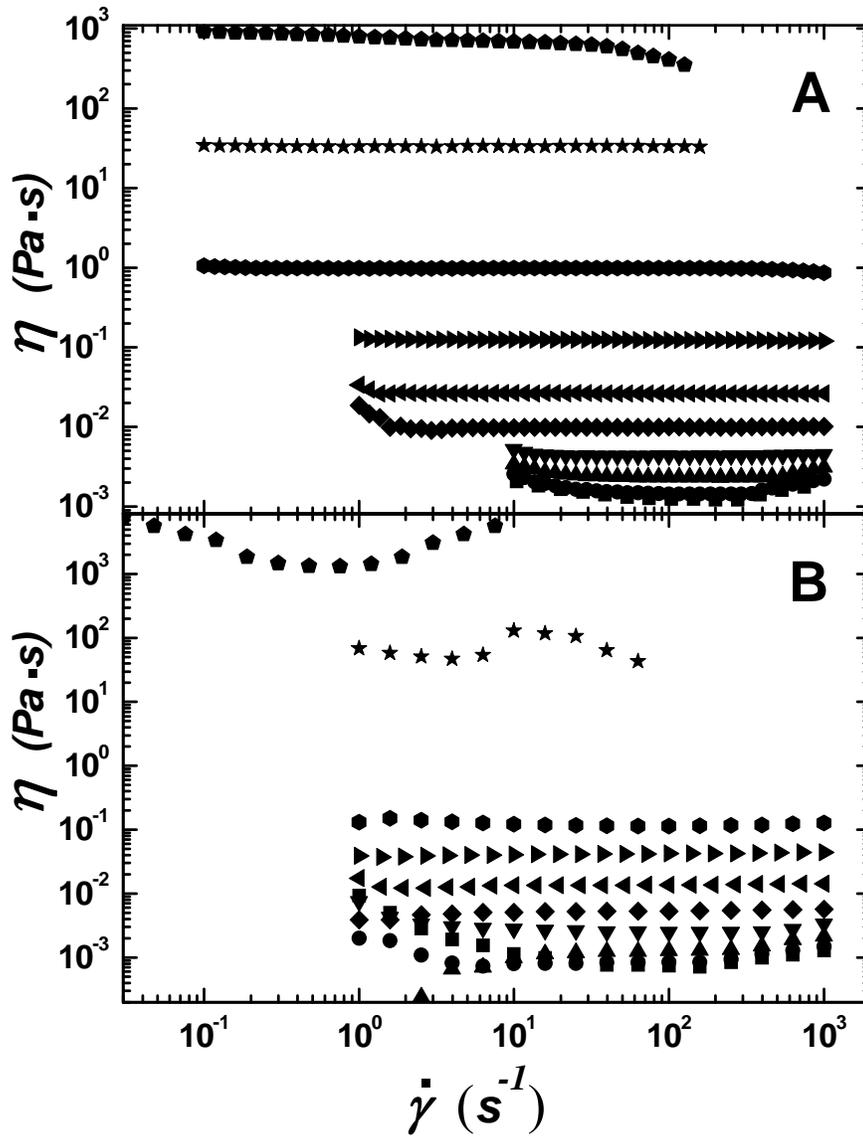


Figure 8. Shear viscosity η versus shear rate $\dot{\gamma}$ measured at (A) 30 °C and (B) 60 °C for several agave fructans concentrations (wt.%): (■) 5; (●) 10; (▲) 20; (▼) 30; (◆) 40 (◄) 50; (►) 60; (●) 70; (★) 80; (◆) 90.

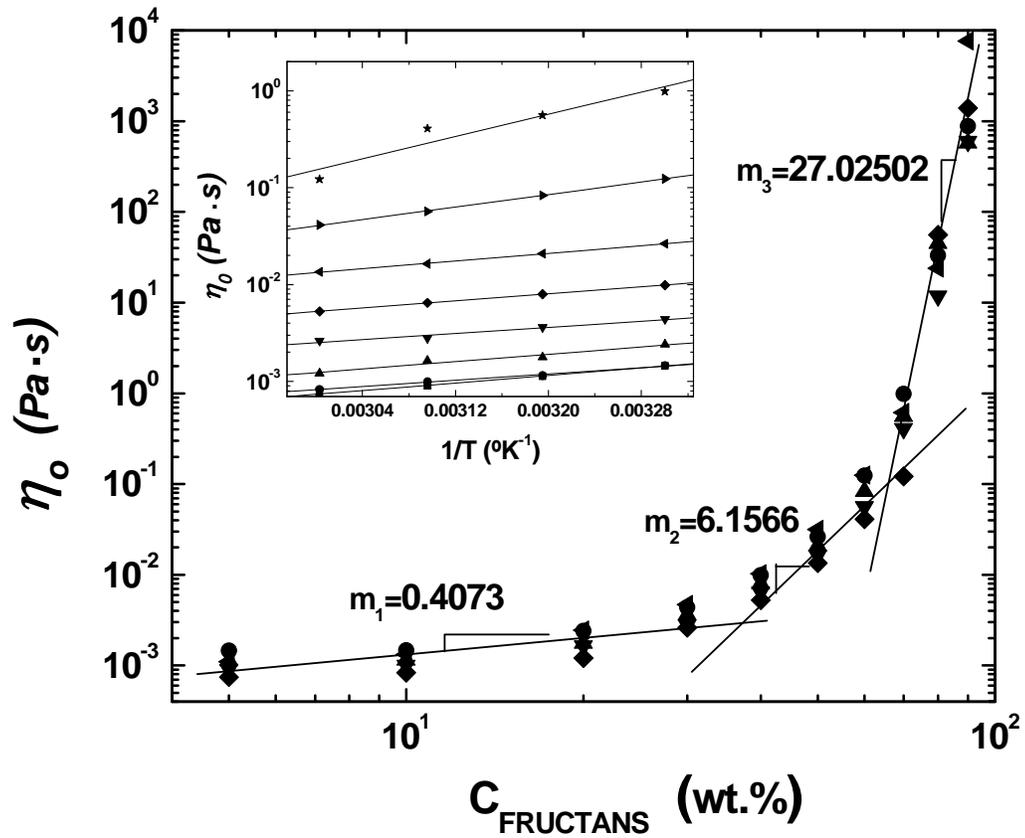


Figure 9. Zero shear viscosity η versus concentration at different temperatures ($^{\circ}\text{C}$): (\bullet) 30; (\blacktriangle) 40; (\blacktriangledown) 50; (\blacklozenge) 60; (\blacktriangleleft) 30 prepared at 80 $^{\circ}\text{C}$. The solid line is an aid to the eye. Inset: Arrhenius plot of η versus inverse temperature at different concentrations (wt.%): (\blacksquare) 5; (\bullet) 10; (\blacktriangle) 20; (\blacktriangledown) 30; (\blacklozenge) 40; (\blacktriangleleft) 50; (\blacktriangleright) 60; (\star) 70. The solid line represents the fitting using a linear behavior.

Table 1. Activation energy values for agave fructans from 5 to 80 wt. %

$C_{FRUCTANS}$ wt. %	A	Ea/RT	$Ea,$ KJ/mol	R^2
5	9.15e-7	2231	18.54	0.9987
10	3.01e-6	1871	15.55	0.9973
20	1.85e-6	2168	18.02	0.9847
30	1.13e-5	1801	14.97	0.9758
40	9.02e-6	2123	17.65	0.999
50	1.44e-5	2274	18.9	0.9985
60	5.6e-7	3723	30.95	0.9985
70	3.4e-10	6639	55.19	0.963
80	0.152	1547	12.86	0.1229