

STUDY OF THE RHEOLOGICAL AND PHYSICOCHEMICAL PROPERTIES OF FRUCTAN FRACTIONS OF *Agave tequilana* cv. cenizo

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ABSTRACT

The physico-mechanical properties of fructan fractions in different agave species without Tequila Denomination of Origin (DOT) need to be studied to identify and expand industrial applications. The rheological and physicochemical characterization of three fractions of *Agave tequilana* cv. cenizo fructans without DOT, with different degree of polymerization (DP) was carried out to propose their use as an additive in the food industry. Fractions were obtained by apparent DP enrichment (DPa) by stepwise ultrafiltration with 10, 5 and 1 kilodalton (kDa) membranes and classified on the basis of the enriched fraction evidenced by HPLC profiling with amperometric detector. The ultrafiltrate of the membrane with 10 kDa, called High Degree of Polymerization (HDP), showed enrichment of fructans with high DPa ≥ 30 , the one with 5 kDa called Intermediate Degree of Polymerization (IDP), presents enrichment in DPa between 10 to 30, these fractions dried by spray, showed a modal particle size distribution (D [4,3] $< 20 \mu\text{m}$). In the ultrafiltrate of the 1 kDa membrane, called Low Polymerization Grade (LDP), fructo-oligosaccharides (FOS) and fructans with maximum DPa of 15 were enriched; this sample was concentrated to 72 °Brix. The glass transition (Tg) showed a DPa-dependent increase (LDP = 152.17 °C, IDP = 216.12 °C and HDP = 227.5 °C). Viscosity was dependent on the degree of polymerization, exhibiting thermo-mechanically stable flow behavior. The reconstituted powders exhibited Newtonian and flow-stable behavior in a concentration range of 2-50 %, temperature of 5-45 °C and pH of 2-9. The macro and micro structural properties identified in the respective fractions of agave fructans, diversify possible applications with potential interest as encapsulating material, stabilizer and prebiotic sweetener, among others that agave fructans have in the food and nutraceutical industry.

Key words: *Agave tequilana* cv. cenizo, agave fructan fractions, degrees of polymerization, rheology, glass transition.

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INTRODUCTION

Agave fructans are a complex mixture of five groups of structures with β (2–1) and/or β (2–6) bonds, among which mainly graminans and agavins predominate (Mancilla-Margalli and López, 2006). Due to their functional properties, agave fructans in their native form have attracted national and international interest mainly from the food and pharmaceutical industry (Juárez-Trujillo *et al.*, 2021; Nieto-Mazzocco *et al.*, 2022). Several studies have demonstrated its prebiotic effect, stimulating the growth of lactic acid bacteria (Martínez-Gamiño *et al.*, 2022) due to its fermentation capacity in the colon, producing short-chain fatty acids (mainly acetate, butyrate and propionate), which by acidifying the gastrointestinal tract reduce the proliferation of pathogenic microorganisms (Sáyago-Ayerdi *et al.*, 2020). Furthermore, its technological applications have been reported as a stabilizing agent and wall material in various formulations (Juárez-Trujillo *et al.*, 2021; Hernández-Granados *et al.*, 2022). However, the concentration of simple sugars in native fructans gives them high hygroscopicity and low thermal stability (Ignot-Gutierrez *et al.*, 2020). Therefore, to reduce the concentration of simple sugars, Ortiz-Basurto *et al.*, (2017) proposed the enrichment of specific polymerization degrees (DP) of agave fructan fractions by a stepwise ultrafiltration (UDF) process; where the obtained fractions showed better technological properties in relation to the native fructan mixture. Currently, it has been demonstrated that UDF-enriched fractions with high DP are an interesting, novel and effective alternative as wall material for functional compounds and probiotics due to their photoprotective effect and physico-mechanical properties (Ceja-Medina *et al.*, 2020; Martínez-Martínez *et al.*, 2022), this promotes a greater interest in their incorporation for different food matrices with improved techno-functional and sensory properties (Ceja-Medina *et al.*, 2020). However, due to the high demand for *Agave tequilana* Weber cv. azul to produce DOT beverages, it is necessary to study other agave varieties without DOT that present suitable fructan distribution profiles to obtain fractions enriched with different DPs. In this regard, Aldrete-Herrera *et al.* (2019) reported the fructan distribution profiles of five wild agave varieties without DOT (*Agave* spp., *A. atrovirens*, *A. salmiana* spp. crassipina, *A. tequilana* cv. cenizo and *A. salmiana* cv. chino), observing that DP dispersal is closely related to variety, plant age and growing zone. In turn, García-Gamboa *et al.* (2020) showed that all the varieties studied by Aldrete-Herrera *et al.* (2019), showed a prebiotic effect on the growth of probiotic lactic acid bacteria (*Lactobacillus* spp. and *Bacillus* spp), where particularly, *Agave tequilana* cv. cenizo fractions presented the best response in an *ex-vivo* intestinal tract simulator. Additionally, the high and intermediate DP fructan fractions inhibited the growth of pathogenic bacteria such as *Clostridium* sp. and *Salmonella* sp. Therefore, in this study, the characterization of the rheological and physicochemical properties of high, intermediate and low DP enriched fractions of *Agave tequilana* cv. cenizo fructans was carried out in order to identify their possible applications in the food and nutraceutical industry.

MATERIALS AND METHODS

Materials

The fractions of high, intermediate and low DP fructans were obtained from samples donated by Nutriagaves de México S.A. de C.V. from *Agave tequilana* cv. cenizo harvested in the state of Jalisco (20°34'00" N 103°40'35" W) at three different maturity ranges (2 to 4, 6 to 8 and 10 to 12 years). Three agaves were collected from each range, considering each one as an experimental unit. Fructans were extracted separately from each experimental unit according to the methodology reported by Aldrete-Herrera *et al.* (2019). The fractions were separated in a laboratory system equipped with a stirred tank cell with regenerated cellulose membranes (Millipore®) of 10, 5 and 1 kDa under operating conditions of 2 bar, 200 rpm and 25 °C. The stepwise process was achieved by applying the membranes in consecutive stages, the retained in each stage was concentrated to a volumetric reduction factor of two, and immediately, diafiltered in the same cell, twice with deionized water in a 1:1 ratio (v/v), which were denominated as ultrafiltrated fractions. The double UDF retained fractions of high degree of polymerization (HDP) and intermediate degree of polymerization (IDP) were stabilized in an LPG5 rotary disc spray dryer (CIMA Industries, China) to obtain powders, as reported by Ceja-Medina *et al.* (2020) at 100 °C inlet temperature and 80 °C outlet temperature and a feed flow rate of 1.11 L·h⁻¹. Finally, the low degree polymerization fraction (LDP) was concentrated to 72 °Brix in a Rv 8 rotary evaporator (Buchi, Staufen, Germany) at 60 °C.

Dispersion of the degree of polymerization of agave fructan fractions

The apparent degree of polymerization (DP_a) of the agave fructan fractions was determined by comparison of the elution times between the samples and a standard of chicory inulin I2255, nystose, kestose, sucrose, glucose and fructose (Sigma Aldrich, MO, USA) adjusted to 80 min, similar to that reported by Aldrete-Herrera *et al.* (2019). Each of the samples and the respective standards were dissolved in Milli-Q water (1.25 µS) at a concentration of 2 mg·mL⁻¹, centrifuged 10 min at 10000 rpm, 10 °C. The supernatants were filtered with a 0.45 µm nylon membrane (Millipore®, MA, USA) before being injected into the high-resolution anion exchange chromatograph fitted with an ICS-5000 amperometric pulse detector (DIONEX-Thermoscientific, MA, USA). A Carbopac PA-100 column (45 × 250 mm) with a PA-100 precolumn (45 mm × 50 mm) with linear and stepwise gradients with two eluents E1) NaOH 100 mM and E2) CH₃COONa 600 mM dissolved in E1 at 35 °C, mL·min⁻¹. Eluent E1 allowed 100 % adjustment, while E2 was programmed with respect to time as follows: 0 min, 2 %; 5 min, 8 %; 30 min, 35 %; with staggered increments of 5 % every 5 min, until min 60, between 65-70 min it was maintained at 100 % and at min 80, it was reduced to 2 % to finish for 10 min at the same initial concentration. Chromatograms were reported in nC vs time. With the Chromeleon® processor, the areas under the curve of mono- and disaccharides, FOS DP_a 3-9, DP_a 10-30 and DP_a >30 were integrated up to the final

elution peak corresponding to a DP_{maximum} of 70 according to inulin standard. The sum of all represents 100 % in each sample.

Physicochemical and rheological properties of HDP and IDP powder fractions

Physicochemical analysis

The physicochemical characterization was carried out by determining pH (method 920.43), total soluble solids (method 967.21) and moisture content (method 964.22) according to the methodology described by AOAC (1990).

Bulk density

In a 250 mL graduated cylinder, 100 g of uncompacted powder was placed; this mass was divided by the measured volume and the results were reported in $\text{g}\cdot\text{cm}^{-3}$.

Compressibility

Compressibility analysis was performed according to the methodology described by Papadakis *et al.* (2006). 100 g of the powdered fructan fraction were placed in a 250 mL graduated cylinder; subsequently, the cylinder was tapped 100 times in a vertical direction on the workbench at a height of 10 cm. The compressibility percent was determined according to equation 1.

$$\text{Compressibility (\%)} = \frac{\text{Unsettled apparent volume (mL)}}{\text{Total volume (mL)}} \times 100 \quad (1)$$

Hausner Index

The Hausner index was reported as the ratio of the compressed volume to the total volume recorded in the compressibility test.

Particle Size Distribution (DTP)

DTP was determined in a Master Sizer 3000 analyzer (Malvern Instruments Ltd, Malvern, UK) by laser beam diffraction, equipped with an Aero S attachment for dry powder and fine particles. Particle size was evaluated as the mean surface diameter [D 3.2], mean volume diameter [D 4.3], and arithmetic mean length [Dx]. The refractive index established in the analyzer was 1.382 and absorbance of 0.1 (Medina-Torres *et al.*, 2019).

Scanning Electron Microscopy (SEM)

The morphology of HDP and IDP agave fructan powder fractions were observed in SEM JSM6300 (JEOL USA, Japan). The powder samples were placed on a copper base, fixed with a conductive tape and coated with gold to obtain surface micrographs. Samples were observed at 250 to 5000 x (Medina-Torres *et al.*, 2016).

Differential Scanning Calorimetry (DSC)

The glass transition temperature (T_g) of HDP, IDP and LDP was determined by DSC on a DSC 250 (TA, instrument, DE, USA), using an indium standard (melting temperature: 156.6 °C). 2.5 mg of sample was placed in a hermetically sealed aluminum capsule. Thermal events were recorded in a range from 0 to 300 °C with a heating rate of 10 °C·min⁻¹ in a nitrogen atmosphere (Ramos-Hernández *et al.*, 2018).

Rheological properties of powdered fructan fractions reconstituted in an aqueous medium.

Aqueous suspensions

Prior to rheological analysis, the powdered fructan fractions were resuspended and homogenized in MQ water to obtain solutions at different concentrations [2, 5, 7, 10, 20, 30, 40 and 50 % (w/v)], the process was carried out by constant stirring in a magnetic stirrer (CLS6795420 KIT Sigma) at 250 rpm for 15 min at 25 °C.

Rheological analysis

In the rheological analysis, different concentrations of fructan fractions (2, 5, 7, 10, 20, 30, 40 and 50, % w/v), at different temperatures (5, 10, 25, 37 and 45 °C) and pHs (2, 3, 5, 6 and 9), were analyzed in a controlled-stress hybrid rheometer DHR-1 (TA Instruments, DE, USA) employing a concentric cylinder rheometric (21.96 mm outer diameter and 20.38 mm inner diameter, 59.50 mm high, and 500 μm gap). The estimation of viscosity in flow at single shear was performed in duplicate, in a shear range from 0.1 to 300 s⁻¹. The data were analyzed with TA Instruments TRIOS software (version 5.2.1).

Furthermore, the estimate of the activation energy at flow was calculated using the Arrhenius equation (Equation 2):

$$\eta = A \exp \left[\frac{Ea}{R(L/T - L/T_v)} \right] \quad (2)$$

Where, η is the viscosity at a set shear rate, Ea is the activation energy at flow (Kcal·mol⁻¹), R is the gas constant (1.987207 cal·mol⁻¹·K⁻¹), A , is the molecular frequency constant between T and T_0 (K).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) following the linear statistical model:

$$Y_{ij} = \mu + \tau_i + \xi_{ij} \quad (3)$$

Where, μ is the global mean that determines the scaling parameter common to all treatments, τ_i is a parameter that measures the effect of treatment i , ξ_{ij} is the error attributable to measurement Y_{ij} . The analyses were performed using the STATISTICA version 12 package (StatSoft. Inc. 1984-2014, Tulsa, OK, USA). Fisher's LSD test was used with a 95 % confidence level for comparison of means between samples. All experiments and analyses were performed in triplicate and the results were expressed with means and their respective standard error of the mean.

RESULTS AND DISCUSSION

Dispersion of the apparent degree of polymerization

The DPa dispersion profiles of the ultradiafiltered agave fructan fractions confirm the enrichment in their concentration (Figure 1). Since there are no agave fructan standards to define the DP, the comparison of the elution profiles with the chicory inulin standard, kestose, nystose, sucrose, fructose and glucose (Figure 1E and 1F), allowed defining the DPa in each of the fractions studied. The native fructan sample

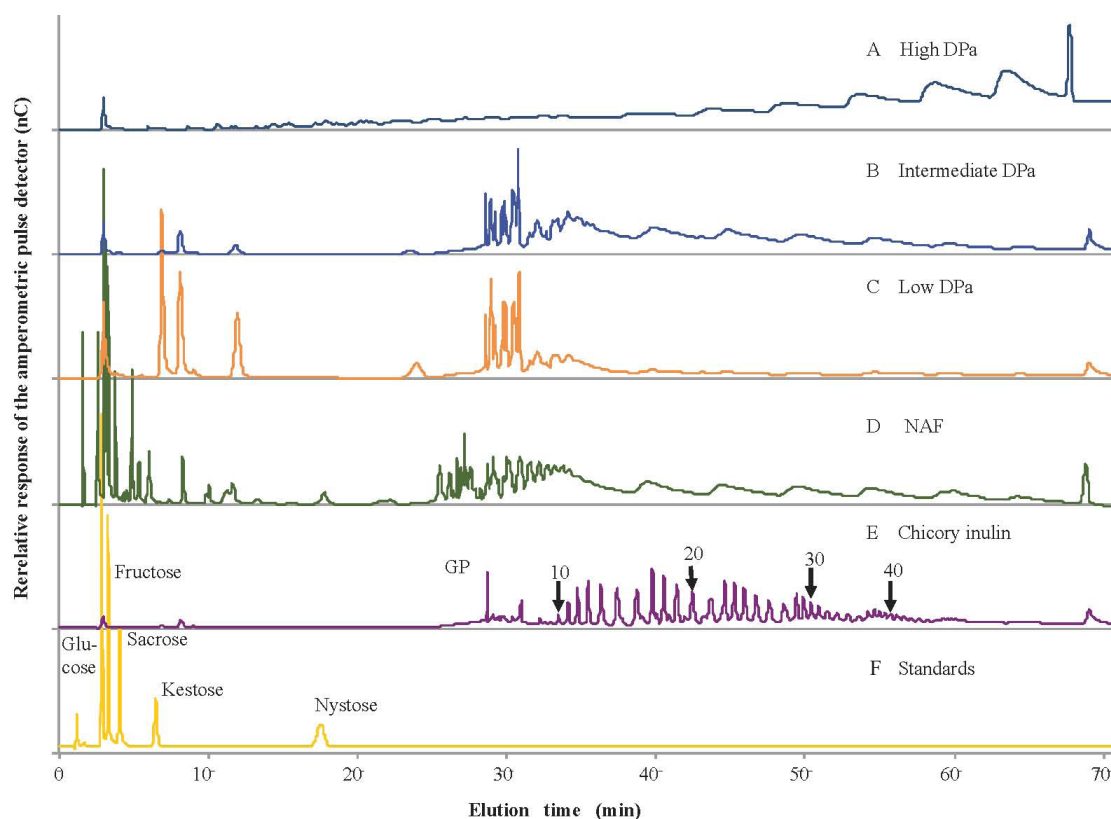


Figure 1. Anion exchange chromatographic profile showing the dispersion of the apparent degree of polymerization (DPa) of fructan fractions enriched by ultradiafiltration (A-C), NAF= native fructans of *Agave tequilana* cv. cenizo (D), compared with chicory inulin (E) and saccharides (F) standards.

(Figure 1D) contained a concentration of 16.74 ± 4.6 % of mono- and disaccharides, representing 15.71 ± 2.8 % of the total area under the curve of the sample, calculated with Chromeleon. The profile of the ultradiafiltered samples of HDP (Figure 1A) show the enrichment of fructans with DP_a greater than 30 at a concentration of 51.19 %; the accumulation in the final elution peak corresponds to DP_{a_{maximum}} of 70, coinciding with that reported by Aldrete-Herrera *et al.*, 2019. This fraction reduced its concentration of mono- and disaccharides to 3.2 % and of FOS to 9.2 %, including kestose and nystose. On the other hand, the IDP fraction (Figure 1B) presents 5.6 % FOS and an enrichment of the fructan concentration with DP between 10 and 40 is observed. Finally, the LDP fraction (Figure 1C) presented 10.8 % of DP 1 and 2 with a DP_{a_{maximum}} dispersion of 15. The structural diversity of agave fructans is indicative of the large number of fructosyltransferase enzymes that are present and active in the vacuoles of the “mezontle” of *Agave tequilana* cv. cenizo during its growth, which defines the presence of different linear and branched isomers in the agave family (Mellado-Mojica *et al.*, 2017). Although baromembrane processes separate components mainly by size difference, the structures of agave fructan isomers and with close DP_a appear to be intertwined, so that despite the two diafiltrations, it is not possible to have a specific dispersion defining the cut-off in a DP for each fraction. Additionally, the osmotic equilibrium that regulates the diffusivity of the components of the mixture to be separated in systems with porous surfaces makes it difficult to obtain highly purified fractions. This is why it is not possible to have profiles with a specific DP that distinguishes each fraction. However, the dispersion of these profiles clearly demonstrates that it is possible to obtain fractions enriched with fructans of different DP_a (Figure 1), defined by the molecular weight cut-off of the membranes selected in the stepwise ultradiafiltration process.

Physico-mechanical properties of powder fractions with high (HDP) and intermediate (IDP) Apparent degree of polymerization

The flow-mechanical properties of powders reconstituted in an acid medium determine a series of conditions for their possible application in the chemical industry, ranging from transport, storage conditions, shelf life and incorporation in different formulations (León-Martínez *et al.*, 2011). The bulk density of HDP and IDP fractions showed no significant difference between samples ($p < 0.05$), presenting values of 0.39 ± 0.003 g·cm⁻³ for HDP and 0.36 ± 0.009 g·cm⁻³ for IDP (Table 1). This parameter is dependent on particle size, shape and surface area (Pereira-Bicudo *et al.*, 2015), characteristics that are defined by the operating conditions defined in spray drying (Ceja-Medina *et al.*, 2020), where, high temperatures induce rapid evaporation of water, which forms a porous particle structure with lower droplet shrinkage (Walton, 2000). Another factor is the moisture content; a higher percentage of moisture in the powder causes the particles to agglomerate, leaving larger spaces between them, which affects the bulk density of the resulting powders (Goula and Adamopoulos, 2005). Ortiz-Basurto *et al.* (2017), reported similar bulk density values for microparticles using HDP fructan

Table 1. Physicochemical analysis of *Agave tequilana* cv. cenizo fructans in powdered form.

Sample	Moisture (%)	Bulk density (g·cm ⁻³)	Compressibility (%)	Hausner Index
HDP	6.05 ± 0.094 a	0.39 ± 0.003 a	58.39 ± 1.715 a	1.48 ± 0.034 a
IDP	4.18 ± 0.020 b	0.36 ± 0.009 a	53.15 ± 1.029 b	1.45 ± 0.015 a

*Letters indicate statistically significant difference ($p = 0.002$) between columns. HDP = High degree of polymerization, IDP = Intermediate degree of polymerization.

fractions and high yields of *Agave tequilana* Weber cv. azul with porous and irregular surface particles, which influenced the bulk density of the powder.

On the other hand, the Hausner index (IH) obtained for the HDP and IDP fractions was 1.2, which indicates that they are powders with low fluidity, which is related to their hygroscopicity that, although reduced compared to native fructans, still determines low fluidity in the enriched fractions, due to the presence of between 8.6 and 12.4 % FOS (Table 1). The IH and percent compressibility of powders correlates with morphology and DTP, in which case spherical and monomodal DTP ensure low interaction between particles, and avoid some of the stickiness effects (Rodríguez *et al.*, 2014), resulting in higher stability in the ultrafiltered fractions.

DTP is one of the most important physical parameters in the mechanical properties at flow. Small particles with monomodal distribution are desirable, as they have a larger contact surface, and in principle, they are mechanically stable to flow, so their incorporation into food matrices is homogeneous and they dissolve quickly (Shi *et al.*, 2013). The powdered fructan fractions showed a monomodal DTP for HDP and IDP with a D [4,3] size of 18.6 µm and 17.6 µm, respectively (Table 2). This indicates that regardless of the DP_a, most of the particles have a uniform size, this as a result of the conditions under which the fractions were stabilized in the spray drying process (Cervantes-Martínez *et al.*, 2014; Medina-Torres *et al.*, 2019).

Table 2. Particle size distribution of powdered *Agave tequilana* cv. cenizo fructans.

Sample	D [3,2]	D [4,3]	Dx (10)	Dx (50)	Dx (90)
HDP	6.33 µm	18.6 µm	6.49 µm	16.5 µm	33.7 µm
IDP	5.28 µm	17.6 µm	5.47 µm	15.8 µm	31.9 µm

HDP=High degree of polymerization, IDP=Intermediate degree of polymerization.

Morphology of powdered fructan fractions

The surface morphology of the particles of the HDP and IDP fractions (Figure 2 A, B, C, D), showed a spherical shape, non-porous and with certain cracks that could have been caused by the rapid evaporation of water within the spray drying chamber,

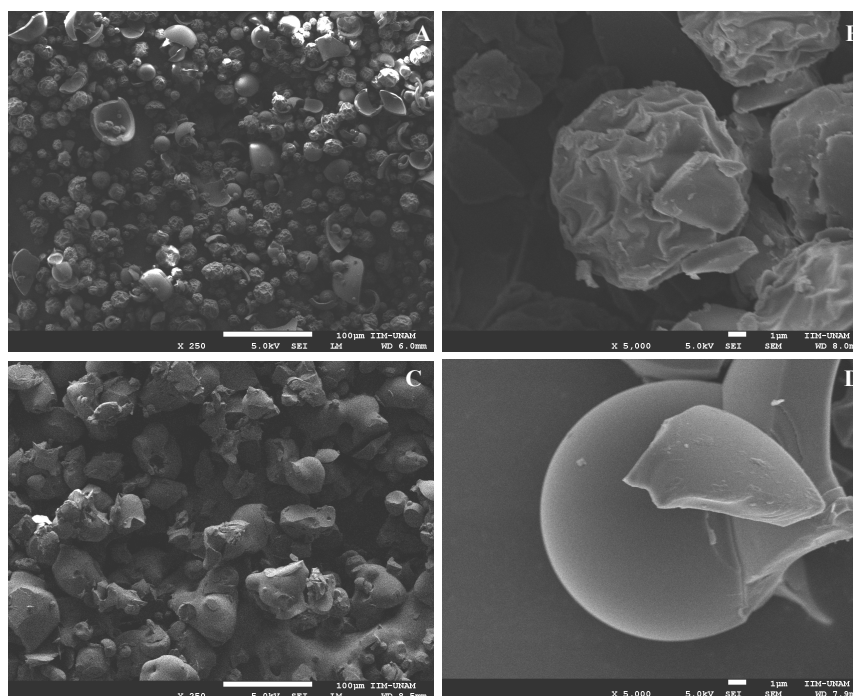


Figure 2. Scanning electron micrograph of powdered *Agave tequilana* cv. cenizo fructans of (a) HDP = High degree of polymerization (250x); (b) HDP (5000x); (c) IDP = Intermediate degree of polymerization (250x); (d) IDP (5000x).

followed by a rapid cooling of the particle during the final part of the drying process (Macías-Cortés *et al.*, 2020; Medina-Torres *et al.*, 2019).

Furthermore, some of the spherical particles showed hollow centers; this was possibly caused by vapor accumulation and differences in vapor pressure inside (Anandharamakrishnan and Ishwarya, 2015).

The above results indicate that fructan fractions have great potential as an encapsulating agent, as they could be used as a wall material. Similar results were reported on the use of agave fructan fractions for the encapsulation of bioactive compounds and probiotic microorganisms by Ceja-Medina *et al.* (2020).

Differential Scanning Calorimetry (DSC)

Thermograms of the agave fructan fractions (Figure 3) showed a shift in the glass transition temperature (T_g), largely dependent on DPa. The T_g for each fraction was 152.17; 216.12 and 227.5 °C, for the fractions enriched in LDP, IDP and HDP, respectively.

These results are in the range of 150 to 230 °C, similar to those obtained by Cruz-Salas *et al.* (2019) who reported a T_g for fructan fractions from *Agave tequilana* Weber cv. azul of 174-232 °C, and Ramos-Hernández *et al.* (2018) a T_g of 205.48-257.7 °C; the variability of T_g temperatures reported in the scientific literature, is mainly due

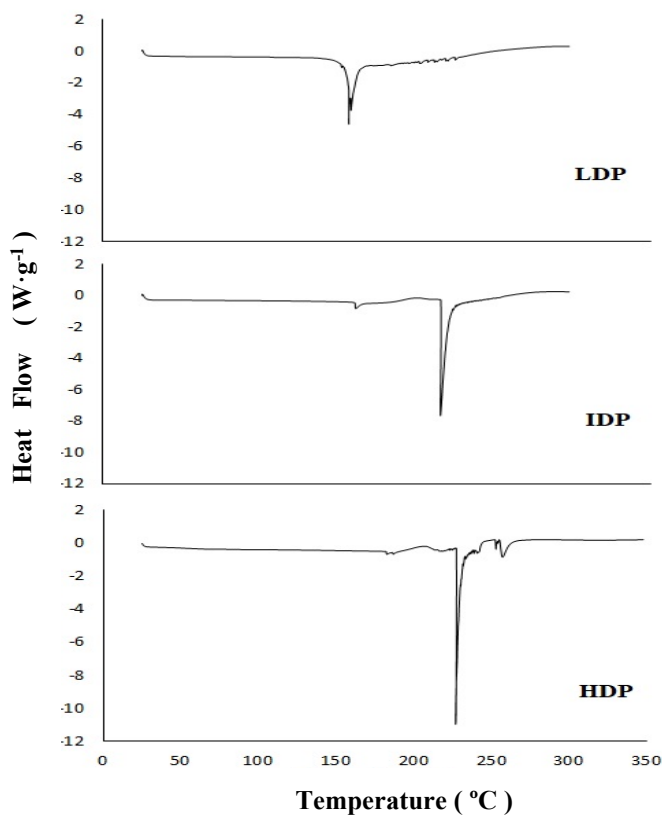


Figure 3. Thermogram of fructan fractions of *Agave tequilana* cv. cenizo powder: LDP = Low degree of polymerization; IDP = Intermediate degree of polymerization; HDP = High degree of polymerization.

to the heterogeneity in the composition and distribution profile of fructans related to the study species, in addition to environmental conditions and extraction method (Aldrete-Herrera *et al.*, 2019).

It should be noted that the T_g shift in the ultrafiltered fractions, in addition to DP, appears to be related to the types of linkages present in the agave fructans. The β (2–6) bonds induce high flexibility in this type of biopolymer, as reported in the literature (Espinosa-Andrews and Urías-Silvas, 2012). Furthermore, the free bonds of the monosaccharides present (mostly fructose) interact with water molecules that induce a plasticizing effect, and consequently, reduce T_g (Goula and Adamopoulos, 2010). It is important to highlight that the thermograms of the HDP and IDP enriched fractions showed more than one inflection, which is related to the presence of fructans with different DP_a, this confirms what was previously observed in the dispersion profiles and with the DTP (Tables 1 and 2). From the above, the ability of ultrafiltration to selectively enrich DP from HDP, IDP and LDP fractions, based on the membrane molecular weight cutoff, can be confirmed. This enrichment provides differences in thermal stability and is directly related to the enriched DP_a, the higher the DP, the higher the thermal stability of the agave fructan fractions.

Rheological properties: Simple shear flow

Concentration effect

The rheological behavior of the fractions obtained in powder, and reconstituted in an aqueous medium (Figure 4A, B, C), show that the viscosity is mechanically stable to flow, *i.e.*, presented a Newtonian behavior in all the samples at the different concentrations studied. On the other hand, viscosity is concentration-dependent (Figure 4D), *i.e.*, an increase in concentration in the semi-dilute regime ($\geq 20\%$) due an interaction between the fructan chains, causing a cross-linking between them that restricts free movement, observing an increase in their viscosity at simple shear. In Figure 4C, it can be seen that the LDP fraction presents the lowest viscosity, followed by the IDP fraction (Figure 4B), and finally, the highest viscosity was observed in the

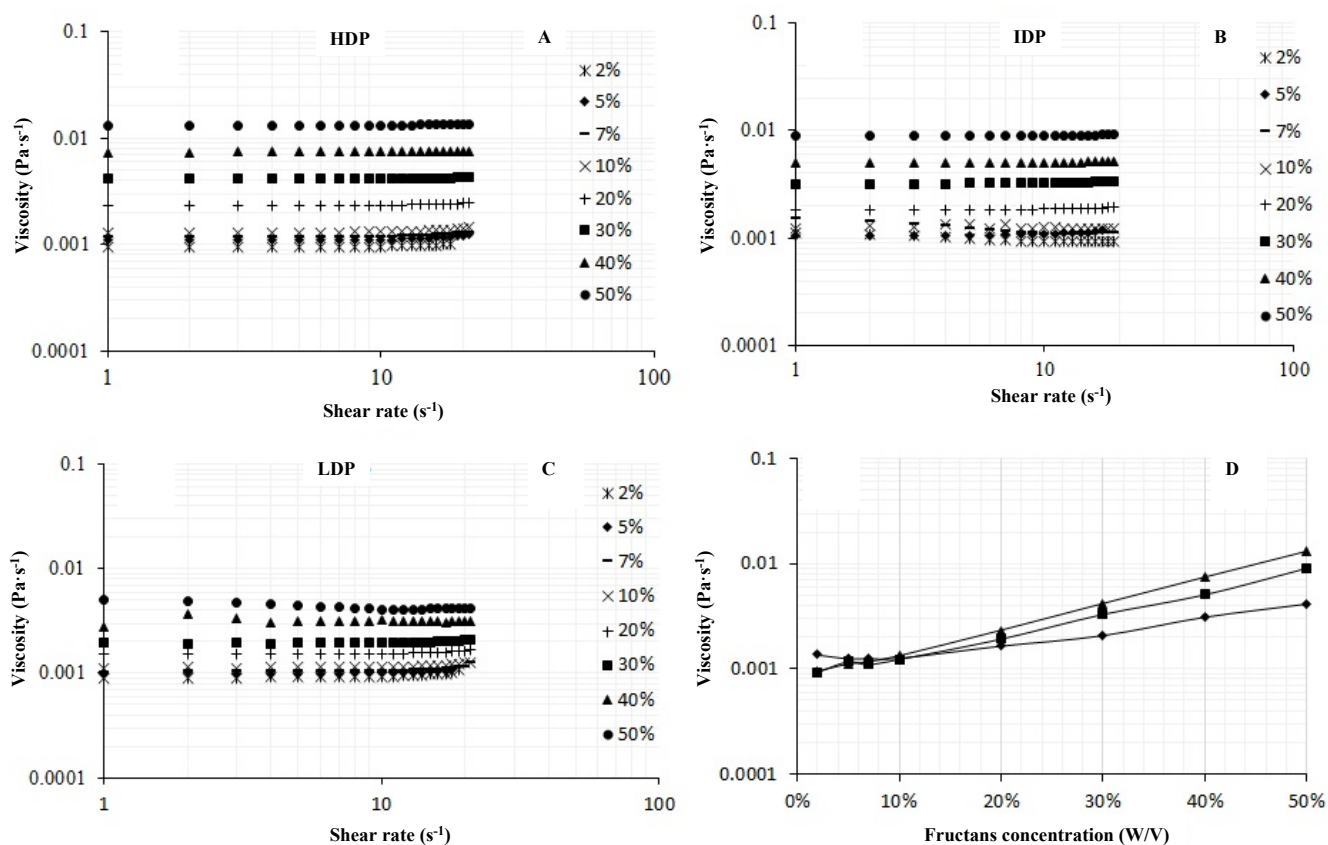


Figure 4. Effect of concentration of *Agave tequilana* cv. cenizo fructan fractions on viscosity (μ) over a single shear rate (γ) at 25 °C. (A) HDP = High degree of polymerization fraction; (B) IDP = Intermediate degree of polymerization fraction; (C) LDP = Low degree of polymerization fraction; (d) Comparison of viscosity of HDP (▲), IDP (■) and LDP (◆) fructans at different concentrations.

HDP fraction (Figure 4A). This is possibly due to the formation of a complex and free-flowing structure of the agave fructan chains with their corresponding DP in the medium.

This mechanical flow behavior of the three fructan fractions at concentrations of less than 20 % presupposes weak structural arrangements, and results in viscosity values very similar to water at a temperature of 25 °C.

Temperature effect

The thermal stability to flow of the fructan fractions is shown in Figure 5. The HDP and IDP fractions had similar behavior with increasing temperature, while the LDP fraction had a decrease in viscosity magnitude. This is mainly attributed to a higher concentration of mono- and disaccharides present in the LDP fraction. In all the samples of the agave fructan fractions a Newtonian behavior is exhibited, this behavior allows to assume that there are no significant structural changes with respect to the temperature imposed to the simple shear flow, which corresponds to what was observed by calorimetry.

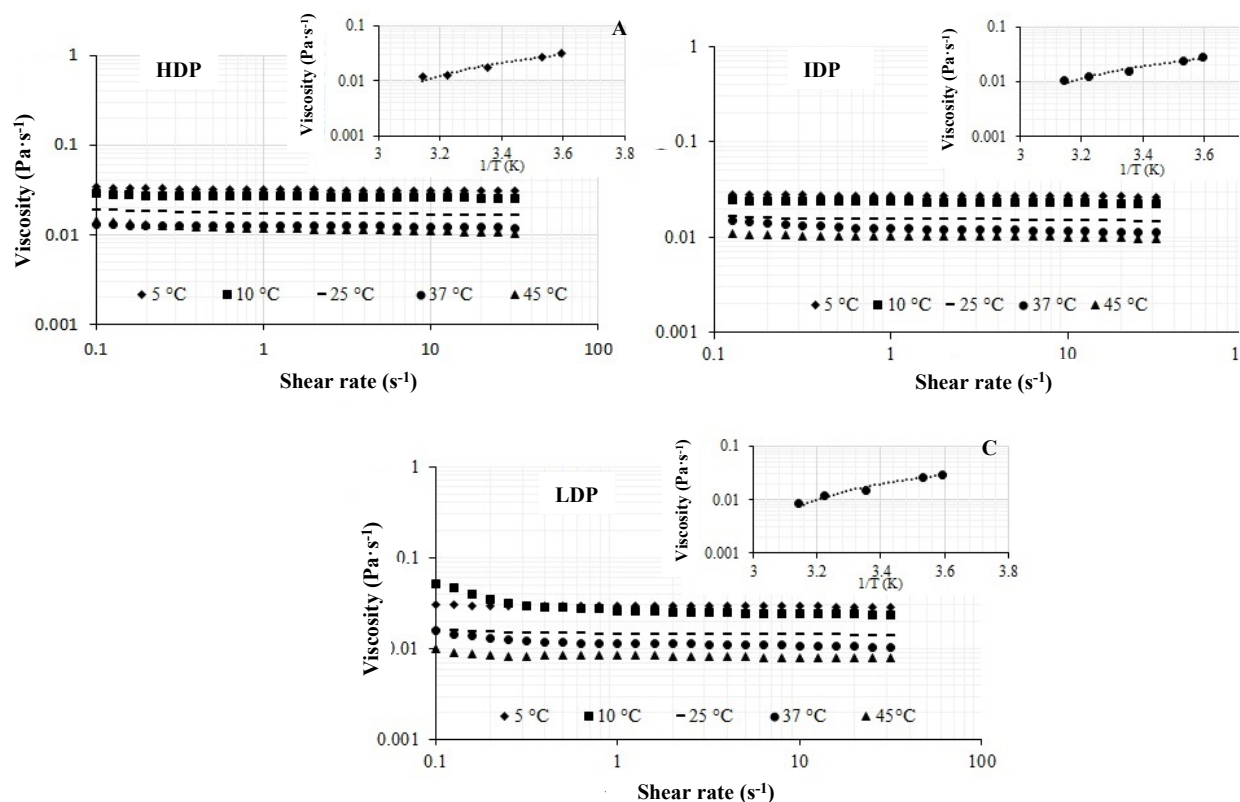


Figure 5. Effect of temperature of *Agave tequilana* cv. cenizo fructan fractions on viscosity (μ) over a single shear rate (g) at 30 % (w/v); 25 °C. (a) HDP = High degree of polymerization fraction; (b) IDP = Intermediate degree of polymerization fraction; (c) LDP = Low degree of polymerization fraction.

Finally, the activation energy (E_a) at flow of the three fractions had similar values, i.e., they are thermally stable at flow in the temperature range studied. That is, for example, at a concentration of 30 % (w/v), its relationship to DP is as follows: HDP = 0.089 Kcal·mol⁻¹, IDP = 0.074 Kcal·mol⁻¹, LDP = 0.094 Kcal·mol⁻¹. Where, a higher E_a translates as a faster change in viscosity with respect to temperature, so that, according to the results obtained, the fructan fractions show a thermo-mechanically stable behavior to the flow in the temperature range studied.

Effect of pH

The effect of pH on the fractions of the reconstituted powders was evaluated in aqueous medium between pH 2 to 9 (Figure 6D) at a fixed concentration and temperature of 30 % (w/v) and 25 °C. No significant dependence of the fractions on pH was observed in the acidic and alkaline regions; i.e., they do not exhibit an isoelectric point (Figure 6 A, B, C). This might suggest that the fructan chains have a net charge of zero. Ponce *et al.* (2008) concluded that the pH and conductivity behavior of agave fructans is

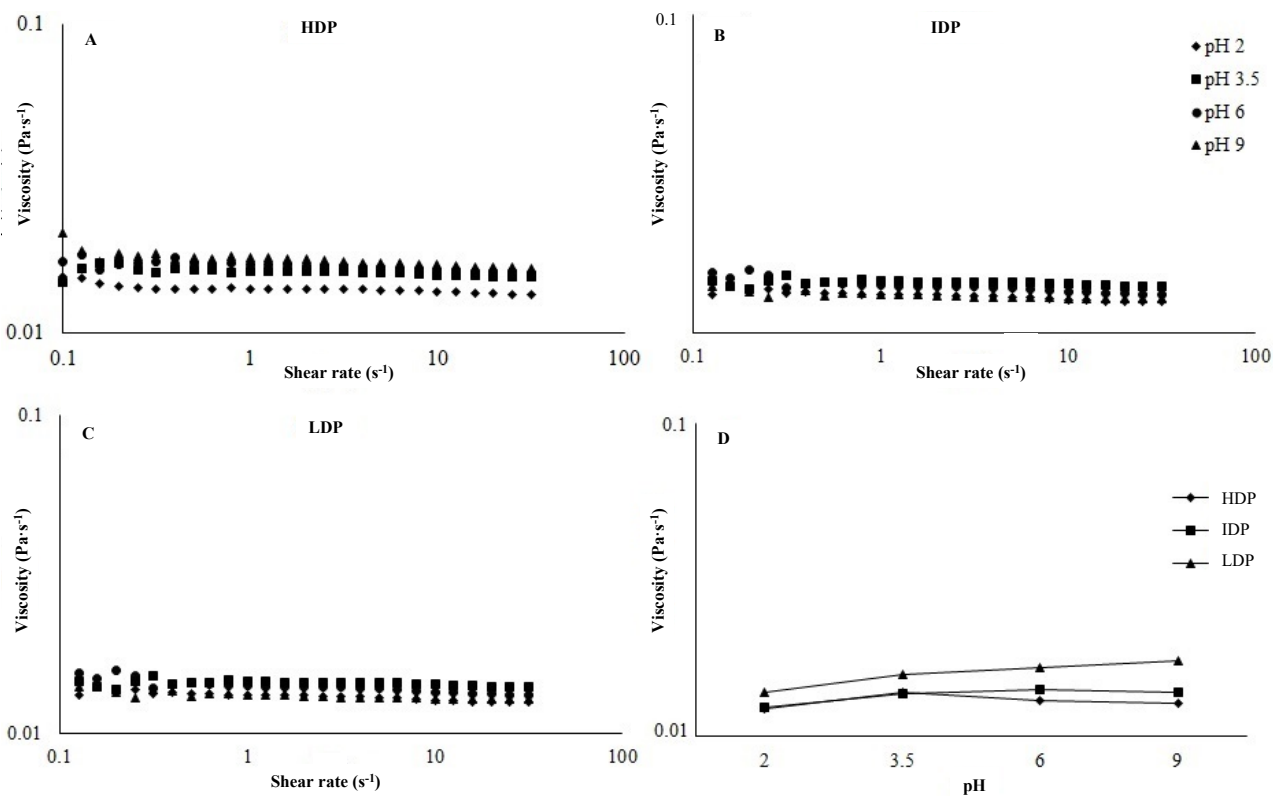


Figure 6. Effect of pH of *Agave tequilana* cv. cenizo fructan fractions on viscosity (μ) over a single shear rate (g) at 30 % (w/v); 25 °C. (a) HDP = High degree of polymerization fraction; (b) IDP = Intermediate degree of polymerization fraction; (c) LDP Low degree of polymerization fraction; (d) Comparison of viscosity of HDP, IDP and LDP fructans at different pH.

concentration-dependent only, and limits free water in solution, favoring molecule-molecule interactions, and decreasing the magnitude of pH, as well as the amount of impurities and other saccharides during separation or extraction, making this property of interest during the formulation of food matrices with weak hydrophobic interactions.

Finally, the physico-mechanical characterization of the ultradiafiltered agave fructan fractions allows them to be considered in the food and pharmaceutical industry, due to the properties that each of the agave fractions impart to the conditions studied, as a good stabilizing agent, cross-linker, wall material and prebiotic fiber.

CONCLUSIONS

The present rheological and physicochemical characterization of fructans from *Agave tequilana* cv. cenizo revealed that the fractions of high and intermediate degree of polymerization presented a modal particle size distribution, which improved the flow properties and mechanical stability to single shear flow with respect to the native fructans.

Differential scanning calorimetry studies revealed that the thermal stability of the fractions is related to their degree of polymerization. While its activation energy revealed a stable thermo-mechanical behavior to the flow.

The fractions reconstituted in aqueous medium showed Newtonian behavior at different concentration, temperature and pH conditions, i.e., they maintain a constant viscosity in the single shear flow range, with a magnitude and dependence on the degree of polymerization.

The stability and technofunctional properties of the enriched fractions improve those of native agave fructans, which is of potential interest in their use the food and nutraceutical industry, as thickening agents, stabilizers, crosslinkers, wall material and soluble prebiotic fiber, among others.

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REFERENCES

- Aldrete-Herrera PI, López MG, Medina-Torres L, Ragazzo-Sánchez JA, Calderón-Santoyo M, González-Ávila M, Ortiz-Basurto RI. 2019. Physicochemical composition and apparent degree of polymerization of fructans in five wild agave varieties: Potential industrial use. *Foods* 8 (9): 404–415. <https://doi.org/10.3390/foods8090404>
- Anandharamakrishnan C, Ishwarya SP. 2015. Spray drying for encapsulation. In Anandharamakrishnan C, Ishwarya SP (eds.) *Spray Drying Techniques for Food Ingredient Encapsulation*. John Wiley & Sons: Hoboken, NJ, USA, pp. 65–76. <https://doi.org/10.1002/9781118863985.ch3>
- Ceja-Medina LI, Ortiz-Basurto RI, Medina-Torres L, Calderas F, Bernad-Bernad MJ, González-Laredo RF, Ragazzo-Sánchez JA, Calderón-Santoyo M, González-Ávila M, Andrade-

- González I, Manero O. 2020. Microencapsulation of *Lactobacillus plantarum* by spray drying with mixtures of *Aloe vera* mucilage and agave fructans as wall materials. *Journal of Food Process Engineering* 43 (8): e13436. <https://doi.org/10.1111/jfpe.13436>
- Cervantes-Martínez CV, Medina-Torres L, González-Laredo RF, Calderas F., Sánchez-Olivares G, Herrera-Valencia EE, Gallegos-Infante JA, Rocha-Guzmán NE, Rodríguez-Ramírez J. 2014. Study of spray drying of the *Aloe vera* mucilage (*Aloe vera* barbadensis Miller) as a function of its rheological properties. *LWT Food Science and Technology* 55 (2): 426–435. <https://doi.org/10.1016/j.lwt.2013.09.026>
- Cruz-Salas CN, Prieto C, Calderón-Santoyo M, Lagarón JM, Ragazzo-Sánchez JA. 2019. Micro- and nanostructures of agave fructans to stabilize compounds of high biological value via electrohydrodynamic processing. *Nanomaterials* 9 (12): 1659. <https://doi.org/10.3390/nano9121659>
- Espinosa-Andrews H, Urias-Silva JE. 2012. Thermal properties of agave fructans (*Agave tequilana* Weber var. Azul). *Carbohydrate Polymers* 87 (4): 2671–2676. <https://doi.org/10.1016/j.carbpol.2011.11.053>
- García-Gamboa R, Gradilla-Hernández MS, Ortiz-Basurto RI, García-Reyes RA, González-Avila M. 2020. Assessment of intermediate-and long-chains agave fructan fermentation on the growth of intestinal bacteria cultured in a gastrointestinal tract. *Revista Mexicana de Ingeniería Química* 19 (2): 827–838. <https://doi.org/10.24275/rmiq/Bio842>
- Goula AM, Adamopoulos KG. 2005. Spray drying of tomato pulp in dehumidified air: II. The effect on powder properties. *Journal of Food Engineering* 66 (1): 35–42. <https://doi.org/10.1016/j.jfoodeng.2004.02.031>
- Goula AM, Adamopoulos KG. 2010. Kinetic models of β -carotene degradation during air drying of carrots. *Drying Technology* 28 (6): 752–761. <https://doi.org/10.1080/07373937.2010.482690>
- Hernández-Granados MJ, Ortiz-Basurto RI, Jiménez-Fernández M, García-Munguía CA, Franco-Robles E. 2022. Dietary encapsulated *Bifidobacterium animalis* and agave fructans improve growth performance, health parameters, and immune response in broiler chickens. *Animal Bioscience* 35 (4): 587–595. <https://doi.org/10.5713/ab.21.0213>
- Ignot-Gutiérrez A, Ortiz-Basurto RI, García-Barradas O, Díaz-Ramos DI, Jiménez-Fernández M. 2020. Physicochemical and functional properties of native and modified agave fructans by acylation. *Carbohydrate Polymers* 245: 116529. <https://doi.org/10.1016/j.carbpol.2020.116529>
- Juárez-Trujillo N, Jiménez-Fernández M, Franco-Robles E, Beristáin-Guevara CI, Chacón-López MA, Ortiz-Basurto RI. 2021. Effect of three-stage encapsulation on survival of emulsified *Bifidobacterium animalis* subsp. *Lactis* during processing, storage and simulated gastrointestinal tests. *LWT-Food Science and Technology* 137: 110468. <https://doi.org/10.1016/j.lwt.2020.110468>
- León-Martínez FM, Rodríguez-Ramírez J, Medina-Torres L, Méndez-Lagunas L, Bernad-Bernad MJ. 2011. Effects of drying conditions on the rheological properties of reconstituted mucilage solutions (*Opuntia ficus-indica*). *Carbohydrate Polymers* 84 (1): 439–445. <https://doi.org/10.1016/j.carbpol.2010.12.004>
- Macías-Cortés E, Gallegos-Infante JA, Rocha-Guzmán NE, Moreno-Jiménez MR, Medina-Torres L, González-Laredo RF. 2020. Microencapsulation of phenolic compounds: technologies and novel polymers. *Revista Mexicana de Ingeniería Química* 19 (2): 491–521. <https://doi.org/10.24275/rmiq/Alim642>
- Mancilla-Margalli NA, López MG. 2006. Water-soluble carbohydrates and fructan structure patterns from *Agave* and *Dasyllirion* species. *Journal of Agricultural and Food Chemistry* 54 (20): 7832–7839. <https://doi.org/10.1021/jf060354v>
- Martínez-Gamiño D, García-Soto MJ, González-Acevedo O, Godínez-Hernández C, Juárez-Flores B, Ortiz-Basurto RI, Rodríguez-Aguilar M, Flores-Ramírez R, Martínez-Martínez M, Ratering S, Schnell S, Bach H, Martínez-Gutiérrez F. 2022. Prebiotic effect of fructans of *Agave salmiana* on probiotic lactic acid bacteria in children as a supplement for malnutrition. *Food and Function* 13: 4184–4193. <https://doi.org/10.1039/D1FO03852D>
- Martínez-Martínez MU, Vázquez-Maldonado D, Ratering S, Godínez-Hernández C, Ortiz-Basurto RI, Soria-Guerra RE, Schneider B, Juárez-Flores BI, Portales-Pérez DP, Schnell S,

- Martinez-Gutierrez F. 2022. Fructans from agave enhance probiotic yoghurt by modulating gut microbiota on children with overweight or obesity. *Food Bioscience* (46): 101506. <https://doi.org/10.1016/j.fbio.2021.101516>
- Medina-Torres L, Calderas F, Minjares R, Femenia A, Sánchez-Olivares G, González-Laredo FR, Santiago-Adame R, Ramírez-Nuñez DM, Rodríguez-Ramírez J, Manero O. 2016. Structure preservation of *Aloe vera* (*barbadensis* Miller) mucilage in a spray drying process. *LWT - Food Science and Technology* 66: 93–100. <https://doi.org/10.1016/j.lwt.2015.10.023>
- Medina-Torres L, Núñez-Ramírez DM, Calderas F, González-Laredo RF, Minjares-Fuentes R, Valadez-García MA, Bernad-Bernad MJ, Manero O. 2019. Microencapsulation of gallic acid by spray drying with *Aloe vera* mucilage (*Aloe barbadensis* Miller) as wall material. *Industrial Crops and Products* 138 (5): 111461. <https://doi.org/10.1016/j.indcrop.2019.06.024>
- Mellado-Mojica E, González-De la Vara LE, López MG. 2017. Fructan active enzymes (FAZY) activities and biosynthesis of fructooligosaccharides in the vacuoles of *Agave tequilana* Weber Blue variety plants of different age. *Planta* 245 (2): 265–281. <https://doi.org/10.1007/s00425-016-2602-7>
- Nieto-Mazzocco E, Saldaña-Robles A, Franco-Robles E, Mireles-Arriaga AI, Mares-Mares E, Ozuna C. 2022. Optimization of gluten-free muffin formulation with agavin-type fructans as fat and sucrose replacer using response surface methodology. *Future Foods* 5: 100112. <https://doi.org/10.1016/j.fufo.2021.100112>
- Ortiz-Basurto RI, Rubio-Ibarra ME, Ragazzo-Sánchez JA, Beristain CI, Jiménez-Fernández M. 2017. Microencapsulation of *Eugenia uniflora* L. juice by spray drying using fructans with different degrees of polymerisation. *Carbohydrate Polymers* 175: 603–609. <https://doi.org/10.1016/j.carbpol.2017.08.030>
- Papadakis SE, Gardeli C, Tzia C. 2006. Spray drying of raisin juice concentrate. *Drying Technology* 24 (2): 173–180. <https://doi.org/10.1080/07373930600559019>
- Pereira-Bicudo MO, Jó J, Alves-Oliveira G, Chaimsohn PF, Sierakowski MR, Alves-Freitas R, Hoffman-Ribani R. 2015. Microencapsulation of juçara (*Euterpe edulis* M.) pulp by spray drying using different carriers and drying temperatures. *Drying Technology* 33 (2): 153–161. <https://doi.org/10.1080/07373937.2014.937872>
- Ponce JA, Macías ER, Soltero JA, Fernández VV, Zúñiga V, Escalona HB. 2008. Physical-chemical and non-linear rheological properties of aqueous solutions of agave fructans. *e-Gnosis* 6: 8. <https://www.redalyc.org/pdf/730/7301197008.pdf>
- Ramos-Hernández JA, Ragazzo-Sánchez JA, Calderón-Santoyo M, Ortiz-Basurto RI, Prieto C, Lagarón JM. 2018. Use of electrosprayed agave fructans as nanoencapsulating hydrocolloids for bioactives. *Nanomaterials* 8 (11): 868–879. <https://doi.org/10.3390/nano8110868>
- Rodríguez AJR, L. Pérez RL, Lafourcade PA, Escalona AJC, Irazoz CA. 2014. Formulation of hard capsules from *Tamarindus indica* L. *Revista Cubana de Farmacia* 48 (1): 118–128. <https://www.medigraphic.com/pdfs/revcubfar/rcf-2014/rcf141m.pdf>
- Sáyago-Ayerdi SG, Zamora-Gasga VM, Venema K. 2020. Changes in gut microbiota in predigested *Hibiscus sabdariffa* L calyces and agave (*Agave tequilana* Weber) fructans assessed in a dynamic *in vitro* model (TIM-2) of the human colon. *Food Research International* 132: 109036. <https://doi.org/10.1016/j.foodres.2020.109036>
- Shi Q, Fang Z, Bhandari B. 2013. Effect of addition of whey protein isolate on spray-drying behavior of honey with maltodextrin as a carrier material. *Drying Technology* 31 (13–14): 1681–1692. <https://doi.org/10.1080/07373937.2013.783593>
- Walton DE. 2000. The morphology of spray-dried particles a qualitative view. *Drying Technology* 18 (9): 1943–1986. <https://doi.org/10.1080/07373930008917822>