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Thermal properties of agave fructans (*Agave tequilana* Weber var. Azul)

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ABSTRACT

Thermal properties of agave (*A. tequilana* Weber var. Azul) at different water contents were investigated. HP-TLC results showed a complex mixture of mono-, di-, oligo, and polysaccharides in agave fructans samples. The thermal decomposition temperatures were observed below to 200 °C. Modulated-differential scanning calorimetry studies showed a glass transition and a relaxation enthalpy processes in agave fructans. Samples with the highest moieties of monosaccharides showed the lower glass transition temperatures (*Tg*). The moisture sorption isotherm of agave fructans was determined at 20 °C and fitted to the GAB model. Gordon–Taylor equation was used to fit the *Tg* experimental data as a function of water content. Agave fructans was found to be an amorphous material. At low water activity (a_w) values (<0.4), agave fructans remained in a powdered amorphous state; and at intermediate a_w (0.4–0.75) collapsed and caked; and at high a_w (>0.75) changed in a highly viscous liquid-like solution.

1. Introduction

The use of fructans as a healthy food ingredient continues to grow in the food industry because of its benefits such as natural prebiotic, dietary fiber, and their technological functions (stabilizer, sweetener, moisturizer, gelling among others). Fructans are naturally present in many plants as storage carbohydrates and are claimed to enhance the cold and drought tolerance of plants (Ritsema & Smeekens, 2003). While up to 100,000 fructose moieties can be linked in a single molecule of bacterial fructans, in plant counterpart's fructosyl units range from 10 to 200. Among the plants that store fructans are many of significant economic importance, such as cereals (e.g. barley, wheat, and oat), vegetables (e.g. dahlia and tulip), forage grasses (e.g. *Lolium* and *Festuca*), and agave plants (Sánchez-Marroquín & Hope, 1953; Vijn & Smeekens, 1999). Because of the beta configuration of the bonds between fructose monomers, inulin-type fructans resist enzymatic hydrolysis by human salivary and small intestinal digestive enzymes, specific for alpha-glycosidic bonds. As a result, inulin-type fructans are indigestible and are fermented in the colon (Ritsema & Smeekens, 2003; Roberfroid, 2007). In Mexico, fructans can be obtained from the plants of *Agave* genus, particularly, there is great interest in *Agave tequilana* Weber var. Azul beside of other species due to Mexico is considered the origin center and biodiversity of this genus. Mancilla-Margalli and López (2006) reported that agave fructans consists of a complex mixture containing mainly graminans and branched neo-fructans. Urías-Silvas, Cani, Delmeé, López, and Delzenne (2008) evaluated the potential of agave vs. inulin-type fructans to modulate glucose and lipid metabolism and GLP-1 secretion in mice. They reported a positive influence of the agave fructans on body weight control, which might be of interest in the control of obesity and emphasized their potential of improving glucose and lipid homeostasis as well as the modulation of GLP-1 and proglucagon expression.

The physical, chemical and microbiological stability of food depends highly on the water content and its interaction with food ingredients (Sablani, Kasapis, & Rahman, 2007). The branched structure in agave fructans may confer different technological properties to the reported to the inulin-type fructans. Stickiness and caking are phenomena that may occur when amorphous food are heated or exposed to high humidity. Furthermore, stickiness and caking are frequently undesirable during manufacturing operations causing lower product yields and operational problems (Torres, Bastos, Goncalves, Teixeira, & Rodríguez, 2011). These changes in physical states are controlled by the glass transition temperature (*Tg*), which determines precisely the critical moisture content of the system where these changes begin to occur (Labuza & Altunakar, 2007). Below this region of water activity (a_w) or *Tg*, a material is glassy or bright/hard, while above this region, the amorphous materials show a texture from chewy to soft-sticky. Thus, the plasticizing effect of water is a major factor contributing to the characteristics and storage stability of fructans powder.

The applications of agave fructans in the food industry has a promising future, however, until today, there are no studies on their thermal properties and water sorption properties, which allow

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assessing the conditions of storage stability of the agave fructans as a function of water activity. For this reason, the aim of this study was to determine the thermal properties of agave (*A. tequilana* Weber var. Azul) at different water contents.

2. Materials and methods

2.1. Materials

Agave fructans were obtained according to Mancilla-Margalli and López (2006) with some modifications. Briefly, agave fructans were extracted with hot water at 80 °C from *Agave* heads (*A. tequilana* Weber var. Azul) of 7 years old recollected from Jalisco (AtJ) and Nayarit (AtN), samples were filtered and spray dried at 150 °C. The fructose (F) and glucose (G) standards, and salts: LiCl, CH_3COOK, MgCl, K_2CO_3, $Mg(NO_3)_2$, NaBr, KI, NaCl, KCl and K_2SO_4 were purchased from Sigma Aldrich (Xalostoc, Estado de Mexico, Mexico). Pre-coated high performance-thin layer chromatography (HP-TLC) plates (Silica gel 60, 10 × 20 cm) and 1-kestose were obtained from Machenary-Nagel (Germany) and Megazyme (Ireland), respectively.

2.2. High performance-thin layer chromatography (HP-TLC)

High performance-thin layer chromatography was chosen as it is often used for the separation and identification of carbohydrates from plants. In fact, it is a simple, low cost analytical method, offering enormous flexibility and parallel separation of many samples with minimal time requirement. Three microliter of 2.0% fructans solutions were applied to pre-coated HP-TLC plates by means of a Camag (Switzerland) Linomat V sample applicator with a 100 μL Syringe (Hamilton, Bonaduz, Switzerland), and were developed and carbohydrates spot were visualized using the method according to Mancilla-Margalli and López (2006) and Reiffova and Nemcova (2006). After chromatography, densitometric scanning was performed with a TLC Scanner 3 (Camag) at 370 nm for all measurements. The scanner was operated by Wincats Software v1.4.4.6337. The source of radiation was a D2&W lamp and the scanning speed was 20 mm/s.

2.3. Thermogravimetric analysis (TGA)

The TGA of fructans samples were carried out under N_2 atmosphere with a heating rate of 10 °C/min in the temperature range from 30 to 300 °C, using a TGA 2950 TA-Instrument (New Castle, DE, USA). Weight loss over temperature was recorded, the derivative of weight loss as a function of temperature (dX/dT) was calculated using the Universal Analysis Software 4.7A (TA Instruments, New Castle, DE, USA).

2.4. Modulated differential scanning calorimetry (MDSC)

Anhydrous fructans transitions were estimated using a MDSC, Q2000 TA-Instruments (New Castle, DE, USA). Fifteen mg of agave fructans was dried in aluminum no-hermetic pans on the MDSC chamber. The heating cycle used was as follows: first, isothermal at 110 °C by 5 min and then equilibrated at 35 °C, a second modulated heating rate from 35 °C to 200 °C at 3 °C/min, period of 40 s and modulate ±1.5 °C. An empty aluminum no-hermetic pan was used as reference. The MDSC was calibrated for temperature using sapphire, indium, and distilled water standards. The instrument was purged with nitrogen at a flow rate of 50 mL/min. All measurements were made by triplicate. The data was analyzed using Universal Analysis 2000 software, version 4.7a (TA Instruments, New Castle, USA) and the *Tg*(I) calculated using the inflection point of the reversible heat flow signal.

2.5. Moisture sorption isotherms

The water sorption isotherms were calculated at 20 °C using an isopiestic method (Schaller-Povolny, Smith, & Labuza, 2000). Dried samples were placed in open weighing bottles and stored in air-sealed glass jars while maintaining equilibrium relative humidity with saturated salt solutions. Twice per week, the samples were removed and weighed until the mass gain reached 0.001 g for two successive weighing. Once equilibrium was reached, water activity of the sample was measured with water activity measurement aqualab (Model 3, Decagon, Device, Inc., Pullman, WA, USA) at 20 °C. Then, the equilibrium moisture content of the sample was measured gravimetrically by drying in an oven at 105 °C for at least 48 h to determine the solid mass in the sample. The results were adjusted to the GAB (Guggenheim–Anderson–De Boer) model:

$$X = \frac{a_w X_m C K}{1 - C a_w [1 + (K - 1) a_w]} \quad (1)$$

where X_m, C, and K represent the moisture content of the monolayer, a factor correcting the sorption properties of the first layer with respect to the bulk liquid, and a factor correcting the properties of the multilayer with respect to the bulk liquid, respectively. The GAB model has been widely used to describe the sorption behavior of foods, represent adequately the experimental data in the range of water activity of most practical interest in foods, i.e., 0.10–0.90 (Al-Muhtaseb, McMinn, & Magee, 2002; Labuza & Altunakar, 2007; Timmermann & Chirifeh, 1991).

2.6. Glass transition at different moisture content by DSC

Agave fructans glass transitions at different moisture content were estimated using a DSC, Q2000 TA-Instruments (New Castle, DE, USA). In general, fifteen milligrams of agave fructans equilibrated at different a_w were heated in aluminum hermetic pans. Thermal program consisted in a heating ramp of −90 at 130 °C at 10 °C/min. An empty aluminum hermetic pan was used as a reference. The DSC was calibrated for temperature using indium, and distilled water standards. The instrument was purged with nitrogen at a flow rate of 50 mL/min. All measurements were made by triplicate. The data was analyzed using Universal Analysis 2000 software, version 4.7a (TA Instruments, New Castle, USA) and the *Tg*(I) calculated using the inflection point of the heat flow signal. The dependence of *Tg* as a function of moisture content was predicted using the Gordon–Taylor equation:

$$Tg = \frac{x_f Tg_f + k x_w Tg_w}{xf + k x_w} \quad (2)$$

where Tg, Tg_f, and Tg_w are glass transition temperatures of the sample, fructans and water, respectively, x_f and x_w the corresponding percent of solid and water contents and k an empirical parameter. Glass transition temperature of pure water was taken as $Tg_w = -135$ °C.

2.7. Statistical analysis

All the measurements were done by triplicate, and their means and standard deviations were reported. The parameters of nonlinear equations (1) and (2) were adjusted using the software Prism 5 for Windows v5.01 (GraphPad Software, San Diego, California, USA).

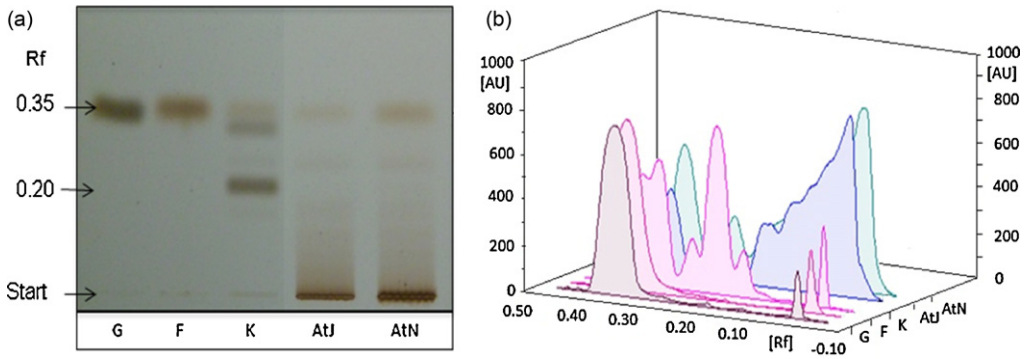

Fig. 1. (a) HP-TLC silica gel plates of carbohydrates samples, (b) densitogram obtained from carbohydrates standards and fructans samples. Glucose (G), fructose (F), 1-kestose (K), agave fructans from Jalisco (AtJ), and agave fructans from Nayarit (AtN).

3. Results and discussion

3.1. HP-TLC

Fig. 1 shows the results of HP-TLC of carbohydrates standards and fructans samples. The analysis of carbohydrates revealed the presence of mono-, di-, oligo, and polysaccharides in both agave fructans samples (Fig. 1a). Fructose and glucose could be identified easily with this technique by mean the color, due that is possible to distinguish between aldoses and kestoses. Generally, monosaccharides deliver the highest retention factor (R_f) values in this system (0.35). With increasing size of carbohydrates (di-, tri-, tetrasaccharide, etc.) reduced R_f values were obtained. The reason for this behavior lies in the nature of the separation system used, as higher carbohydrates show stronger interaction with the polar stationary phase. In addition, the existence of carbohydrate of higher degree of polymerization is also indicated. The resulting densitogram for a carbohydrates standards and fructans samples is presented in Fig. 1b.

3.2. TGA

Fig. 2 corresponds to TGA thermograms of fructans samples. At temperatures below 130 °C, both samples showed a first mass loss that was related to the evaporation of moisture. The moisture content was of 4.35 and 4.65 g of H_2O/g for AtJ and AtN, respectively. At temperatures around 200 °C, agave fructans showed a second mass loss associated with the thermal decomposition. The initial temperatures of thermal decomposition and the peak maximum temperature were calculated from the first derivative of weight loss (Fig. 2): AtJ (202.7 ± 1.0 and 220.1 ± 0.4 °C) and AtN (200.2 ± 1.5 and 222.7 ± 0.1 °C). Particularly, the agave fructans showed a decrease in the thermal decomposition rate around 230 °C; that could be associated with the decomposition of branched chains of agave fructans. Chui, Hsu, and Lin (2002) reported that the decomposition rates decreased for cross-linked inulin in comparison with native inulin.

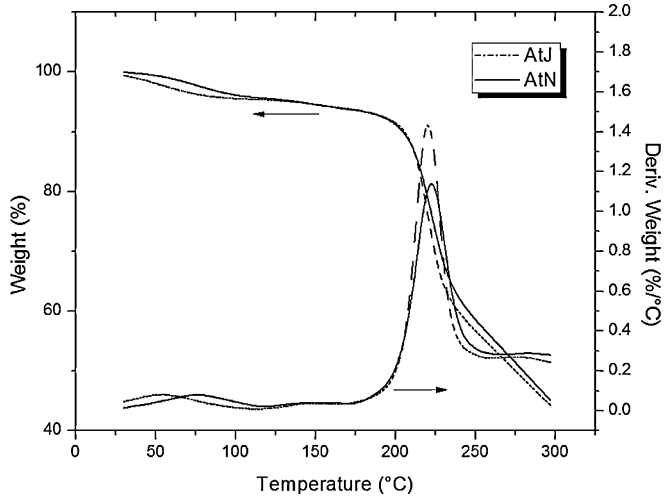

Fig. 2. Thermogravimetric analysis from fructans samples: (a) agave fructans from Jalisco (AtJ) and (b) agave fructans from Nayarit (AtN).

3.3. Glass transition temperature of anhydrous fructans samples

Fig. 3 shows the thermogram obtained from MDSC of anhydrous fructans samples. The reversible heat flow signal shows a glass transition event between 101.8 and 118.1 °C for AtN, and 119.8 and 131.3 °C for AtJ. The *Tg*(I) values were 109.6 and 125.7 °C for AtN and AtJ anhydrous samples, respectively. The effect of branching structure as a *Tg* depressor is known and is often described in terms of internal plasticization (Bizot et al., 1997). The presence of β(2-6) linkages in agave fructans induces a higher flexibility of biopolymer chains decreasing the *Tg* value. However, it must be remembered that agave fructans are a complex mixture with have different degree of polymerization and chemical structures (Mancilla-Margalli & López, 2006). Furthermore, the presence of monosaccharides (mainly fructose) has a negative impact on the *Tg*. Particularly, AtN fructans showed more intensity spot in the region of glucose and fructose compared to AtJ, which was corroborated by the relative area values obtained with the densitometric analysis (Fig. 1b). This could explain the fact that the glass transition temperature of the AtN fructans was lower than that observed in AtJ fructans.

When a glass material is stored below its glass transition temperature, it will spontaneously approach the more stable state, due to its nonequilibrium nature, this kind of change is called enthalpy relaxation or structural relaxation or physical aging (Liu, Bhandari, & Zhou, 2006). Some macroscopic properties, such as density, mechanical strength, and vapor permeability can be affected by enthalpy relaxation in amorphous glassy materials (Haque, Kawai, & Suzuki, 2006). The non-reversing heat flow showed the enthalpy relaxation process in agave fructans with an on-set temperature of 96.9 and 113.7 °C and relaxation enthalpy of 1.52 and 1.65 J/g for AtN and AtJ, respectively. These property changes may also affect the performance and utilization of the aged polymer (Le Meste, Champion, Roudaut, Blond, & Simatos, 2002).

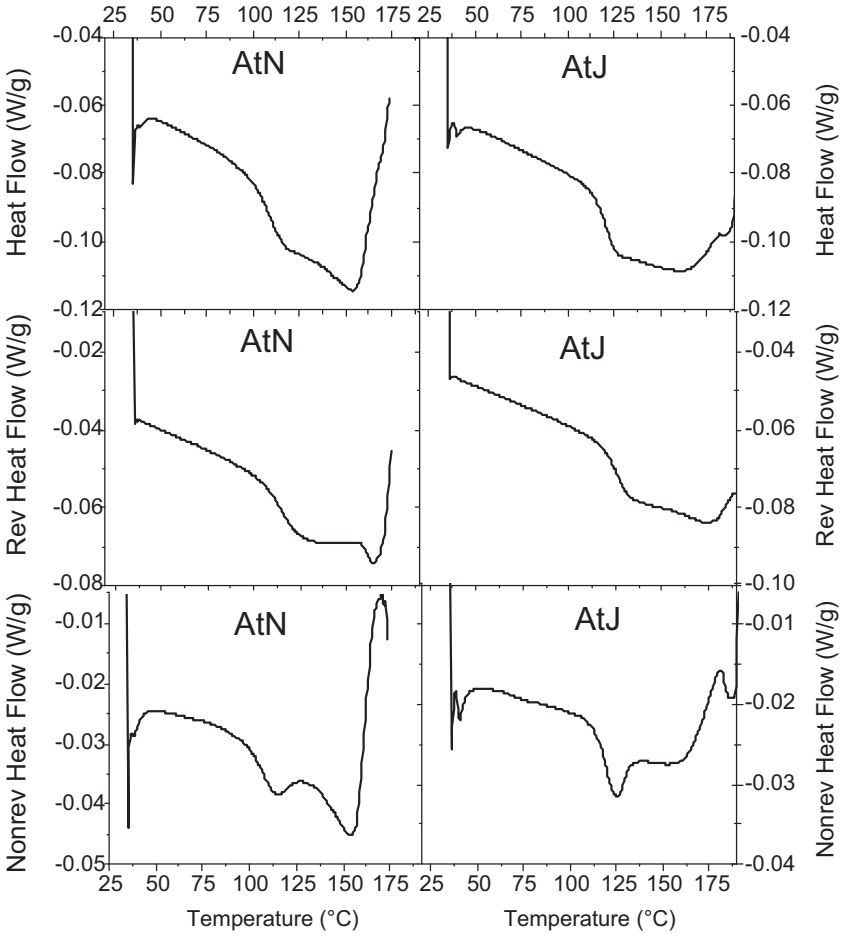

Fig. 3. Modulated differential scanning calorimetry from anhydride fructans samples: (a) agave fructans from Jalisco (AtJ) and (b) agave fructans from Nayarit (AtN).

3.4. Moisture sorption properties of fructans

The agave fructans showed higher water absorption capacity, compared with data reported for chicory fructans (lineal structure) (Kawai, Fukami, Thanatuksorn, Viriyarattanasak, & Kajiwara, 2011; Schaller-Povolny, Smith, & Labuza, 2000; Zimeri & Kokini, 2002). The results showed evidence that X_m depended of the structure and composition of fructans samples. The branched chains and the presence of moieties of free fructose contains a higher amount of hydroxyl groups available to bind water, this could explain the higher values of X_m for agave samples. Caking is as a deleterious phenomenon by which a low-moisture, free flowing powder is first transformed into lumps, then into an agglomerated solid and ultimately into a sticky material, resulting in loss of functionality and lowered quality (Aguilera, del Valle, & Karel, 1995). It is well known that the factors controlling structure collapse, stickiness, and caking are temperature and water content and that these processes are time dependent (Le Meste et al., 2002). Fig. 4 shows the photographs of fructans powders equilibrated at different relative humidity. At a_w below 0.4, the water plasticizing effect was small and the mobility of the amorphous regions was restricted, both fructans samples were still in a powder form. At a_w around 0.44, both fructans showed the agglomeration of particles (first stages of caking). In this state, small interparticle bridges may be disintegrated under mild pressure. At a_w around 0.6 agave fructans caked and change into a sticky solid. At a_w of above 0.8, agave samples absorbed enough water to forms a high viscous liquid-like solution. The branched conformation of agave fructans allows the formation of a solution, unlike chicory fructans, which crystallize forming a gel at water activities up to 0.5 (Ronkart, Paquot, Fougnies, Deroanne, & Blecker, 2009; Zimeri & Kokini, 2002).

Fig. 5 shows the moisture sorption isotherm for agave fructans at 20 °C. The results show the ability of fructans samples to binding water. Moisture sorption isotherms of most foods are nonlinear, generally sigmoidal in shape, and have been classified as Type II isotherms (Al-Muhtaseb et al., 2002; Labuza & Altunakar, 2007), and agave fructans are not the exception. Table 1 shows the results of nonlinear fitting of GAB model (Eq. (1)). The X_m value is recognized as the moisture content affording the longest time period with minimum quality loss at a given temperature. Above it, foods show a variety of undesirable consequences, such as caking of particles, collapse of dehydrated structures, loss of crisp textures, crystallization, etc. According to the GAB model, these values were 7.3 and 9.8 g H_2O/100 g for AtJ and AtN, respectively. Again, the presence of a higher concentration of monosaccharides increases the values of X_m in AtN compared with AtJ.

Table 1
Results of the non-linear regression parameters of the GAB and Gordon–Taylor models.

	GAB				Gordon–Taylor		
	X_m	C	K	R^2	Tg	k	R^2
AtN	0.098	7.3	0.86	0.990	109.6	4.3	0.980
AtJ	0.073	5.9	0.91	0.997	125.7	5.3	0.992

Table 2
Glass transition temperature of agave fructans as a function of water activity.

a_w	Anhydrous	LiCl	CH_3COOK	$MgCl_2$	K_2CO_3	$Mg(NO_3)_2$	KI	NaCl	KCl
AtN	109.6	62.1	46.1	35.4	16.7	5.0	−12.8	−23.2	−47.8
AtJ	125.7	63.9	50.0	44.4	34.0	17.2	−11.1	−23.3	−45.8

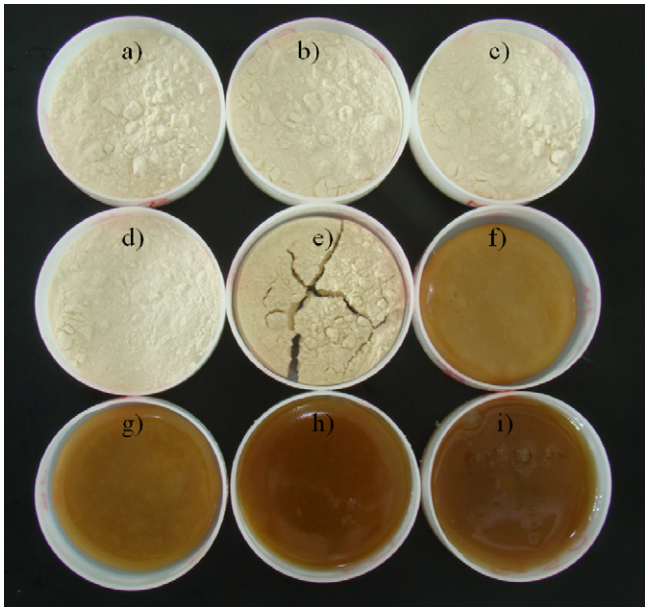

Fig. 4. Photographs of fructans powders equilibrated at different relative humidity: (a) LiCl, (b) CH_3COOH, (c) $MgCl_2$, (d) K_2CO_3, (e) $Mg(NO_3)_2$, (f) NaBr, (g) KI, (h) NaCl, (i) KCl, and (j) K_2SO_4.

3.5. a_w vs Tg

Water plasticization is an important factor contributing to the characteristics and storage stability of the fructans powders. The *Tg* value of a given hydrophilic substance is decreased with an increase in water content, following a nonlinear function as described by the Gordon–Taylor equation (Fig. 6 and Table 1) (Liu et al., 2006). In general, agave fructans showed a greater heat capacity change (glass transition) than that reported for chicory fructans (Ronkart et al., 2009; Zimeri & Kokini, 2002). The *Tg* as a function of water activity of fructans is shown in Table 2. In the particular case of samples equilibrated at higher relative humidity ($a_w > 0.9$), an exothermic peak was observed during the cooling, then in the heating stage an endothermic peak related with the fusion of solution was observed,

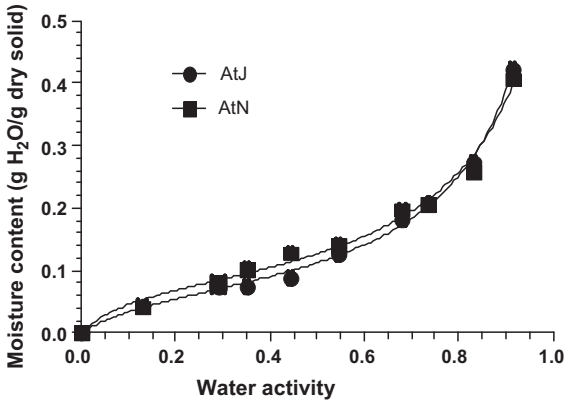

Fig. 5. Absorption curves from fructans samples adjusted to GAB model.

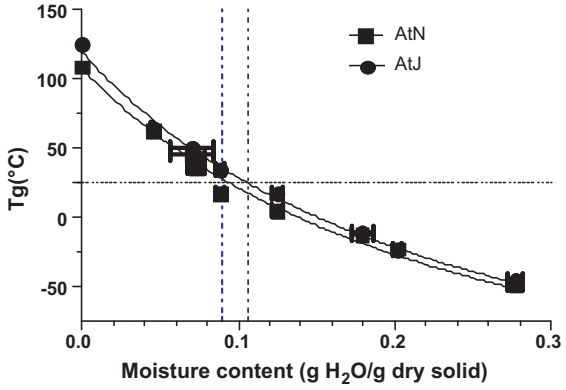

Fig. 6. Agave fructans glass transition temperature as a function of moisture content, adjustment of the Gordon–Taylor equation.

non glass transition was observed, even in MDSC mode scan (data not shown). One of the characteristics of glassy systems is that they have a collapse at a temperature above the *Tg*, showing a macroscopically visible changes in physical properties, which results in a dramatic change in the stability of matrices such as stiffness, structural collapse, caking, texture, and viscosity (Liu et al., 2006; Le Meste et al., 2002). The results show that the branched structure of agave fructans allowed a greater water absorption capacity, resulting in greater internal plasticization of fructans, leading to lower values of *Tg* at constant a_w. Kilburn et al. (2004) concluded that the sorption of water in amorphous carbohydrates and its ensuing plasticization proceeds via a complex mechanism involving both hydrogen bond formation and disruption, and changes in the matrix free volume.

4. Conclusions

Thermal properties and the effects of water uptake during storage of agave fructans properties have been investigated. HP-TLC results showed the presence of mono-, di-, oligo, and polysaccharides in the agave fructans samples. In general, agave fructans showed the behavior of an amorphous solid with thermal decomposition temperature below to 200 °C. At critical moistures contents agave fructans showed the agglomeration of the amorphous solid particles, while at high moisture content showed a highly viscous liquid-like behavior. Anhydrous agave fructans showed a glass transition and endothermic process, which was attributed to the relaxation enthalpy energy of the amorphous polymers chains, as confirmed by modulated differential scanning calorimetry. The carbohydrate profile allowed a high water uptake, decreased the glass transition temperature. These results may help us understand the interrelationship between the capacity of water absorption, glass transition temperature and physical changes of agave fructans. The results together allow us to identify the right conditions for storage, manipulation and storage of agave fructans.

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