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Assessment of agave fructans as lyoprotectants of bovine plasma proteins concentrated by ultrafiltration

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ABSTRACT

The effectiveness of fructans as protectant agent to prevent protein denaturation was evaluated during freeze-drying and storage. Native agave fructans and two fractions obtained by ultrafiltration were assessed as protective agents. Since the protein denaturation during freeze-drying can alter the functional properties of proteins, the evaluation of bovine plasma protein properties was performed with different concentration of fructans, pH and freezing temperatures. The results showed that the incorporation of fructans as lyoprotectants improved functional properties, due to the reduction of protein denaturation with maximum stabilization of plasma bovine protein at a fructan concentration between 5% and 10% (w/v) for native fructans and 10% (w/v) for the fractions of fructans at −40 °C. Moreover, when freezing at −4 °C, fructans fraction at 15% (w/v) prevented protein denaturation while native fructans exerted no acceptable protection ($P < 0.001$). This behavior could be explained by a microstructure study of fructans, where the higher heterogeneity in size of native fructans may be the reason for the lower protective effect. Furthermore, the higher heterogeneity of the samples affected the extent of non-enzymatic browning in the range of the temperatures assessed. The shelf life of freeze-dried proteins was improved from 1.7 months for the control sample to 7–11, depending on the saccharide considered. Taking into account the health benefits of fructans, since they are categorized as prebiotic, the protein–saccharide mixture may be valuable functional ingredients for food formulations.

1. Introduction

Fructans as non-digestible fermentable saccharide are among the most studied and well established prebiotics (Gibson, Probert, Van Loo, Rastall, & Robertfroid, 2004; Henelly, Dunne, O'Sullivan, & O'Riordan, 2006; Niness, 1999). A natural source rich in these compounds are the *Agave plants*, abundant in arid regions of Latin America, being Mexico considered as the center of origin and biodiversity of *Agave* species. The taxonomic diversity of that country has led to the development of industries for the production of alcoholic beverages, and recently, the production of syrup and agave fructans. The production of *agave* oligofructans and/or syrup is carried out by the hydrolysis of agave fructans (Ávila Fernández, Galicia-Lagunas, Rodríguez Alegría, Olvera, & López Munguía, 2011). *Agave tequilana* Weber var. blue is the most used for syrup and fructan production. This plant accumulates between 13% and 17% (w/w) fresh weight fructan in mature plants, being similar to the amount found in chicory (15.2–20.5% (w/w) fresh weight), the current source of inulin (Ávila Fernández et al., 2011; Mellado Mojica & López, 2012; Van Loo, Coussement, de Leenheer, Hoebregs, & Smits, 1995). While chicory inulin is a rather linear fructan in which fructose molecules are joined through β(2–1) linkages, fructans present in agave, particularly in *A. tequilana*, have a degree of polymerization (DP) ranging from 3 to 29 (López, Mancilla Margalli, & Mendoza Diaz, 2003; Mellado Mojica & López, 2012).

Fructans have many technological advances as food additive. Thus, higher DP fructans are more suitable as fat replacers (Verraest, Peters, Van Bekkum, & Van Rosmalen, 1996). Van den Ende et al. (2006) reported that different applications required fructans with a different DP and Gonzalez Tomas, Coll Marqués, and Costell (2008) reported how the different viscoelasticity of inulin-starch based dairy system was influenced by the inulin average chain length.

It is known that the presence of saccharides during the freeze-drying process preserves the native structure of proteins in the dried state. The mechanisms by which saccharides stabilize proteins during this process are due to their ability to form a glassy state that inhibits crystallization and influences the kinetics of deteriorative reactions upon storage (Buera, Schebor, and Elizalde (2005); Carpenter, Prestrelski, and Arakawa (1993); Minson, Fennema, & Amundson, 2006). The saccharides should have a high glass transition temperature (T_g) for a better protection (Rodriguez Furlán, Lecot, Pérez Padilla, Campderrós, & Zaritzky, 2011, 2012). They also stabilize proteins by direct interaction,

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through hydrogen bonding (Wolkers, van Kilsdonk, & Hoekstra, 1998). Previous studies demonstrated that oligosaccharides like fructans stabilize cellular membranes during dehydration (Hincha et al., 2007).

In many regions of the world, especially in countries such as Argentina and Mexico, the majority of livestock production corresponds to cattle, being the animal blood the main by-product. Taking into account that, on average, 17% (w/w) of blood are proteins, the need to recover and use this resource is understood (Cheftel, Cuq, & Lorient, 1989). Bovine plasma production requires the separation of components by centrifugation of two fractions: the plasma and red blood cells. The proper treatment of these proteins increases profitability in the meat industry. Bovine plasma proteins are valuable from the nutritional point of view due to the presence of amino acids that are essential in small amounts in other foods. Moreover, due to the good functional properties of the plasma fraction, it is used as ingredient in a variety of foodstuffs (Del Hoyo, Rendueles, & Díaz, 2008; Silva & Silvestre, 2003).

However, these proteins, as many others, are structurally unstable in solution, and are susceptible to conformational changes due to several stresses encountered during purification, processing and storage. Freeze-drying is commonly used in the manufacture of protein products, since the proteins in dried state are less prone to denaturation and precipitation during transportation and storage (Ohtake, Kita, & Arakawa, 2011).

As was previously demonstrated, inulin protects the plasma bovine protein structure during freeze-drying, preserving, thus, the functional protein properties (Rodriguez Furlán et al., 2012). Hence, the aim of this study was to assess the protective capacity of Agave native fructans (NF) and two different fractions: high performance fructans (HPF) and high degree of polymerization fructans (HDPF) during freeze-drying process. The process of freeze-drying and self-life of the matrix (or mixture) formed by the plasma protein and fructans of Agave, and the characterization of the mixture were studied in order to optimize and predict its behavior in potential food applications.

2. Materials and methods

2.1. Raw materials

Commercial products of *A. tequilana* native fructans (NF) in powder and 72°Brix was donated by the company Agaviótica SA, Monterrey, Mexico. Two fractions of fructans concentrated were obtained by tangential ultrafiltration process at the Instituto Tecnológico de Tepic, Nayarit, Mexico. To obtain the high performance fructans (HPF) and high degree of polymerization fructans (HDPF) a solution to 20°Bx was ultrafiltered in commercially membrane modules Pellicon-2 (Millipore, MA, USA), with a nominal molecular cut off (MWCO) of 1 and 10 kDa respectively (20 °C, 3 bar y 4 L.min^{-1}). After separation, the fructan fractions retained on each membrane was spray-dried in LPG5 Model, CIMA Industries Inc., Chinese (inlet/outlet temperature: 100/80 °C, atomizer speed: 30,000 rpm and feed flow: 17.5 mL/min), the characteristic of powders and NF are reported in Table 1 (Aldrete Herrera, 2013; Espinosa Castrejón, 2012).

Table 1
Characteristic of *Agave tequilana* fructans additives (g·100 g^{-1}).

Characteristic (g·100 g^{-1})	NF	HPF	HDPF
Humidity	4.62 ± 0.2	3.01 ± 0.03	2.52 ± 0.23
Ash	0.1 ± 0.02	0.15 ± 0.05	0.1 ± 0.05
Total carbohydrate	97.5 ± 1.7	98.2 ± 0.7	98.6 ± 0.5
Reducing sugars	13.6 ± 0.5	0.8 ± 0.06	0.4 ± 0.04
Fructans	83.9 ± 0.3	97.4 ± 0.5	98.2 ± 0.3
Aw	0.41 ± 0.02	0.33 ± 0.001	0.3 ± 0.003
Enriched DP	–	4–80	24–80

HPF, high performance fructans; HDPF, high degree of polymerization fructans and NF, fructans natives.

Spray dried bovine plasma (Yerubá S.A., Argentine) has been used. The molecular weights of proteins are in the range of 15,000 to 80,000 Da. The proximate composition provided by the manufacturer was: 76 ± 5% proteins, 0.1% fat, 10% ash, 4% water, 1% low molecular weight compounds.

2.2. Plasma protein concentration by membrane technology

The micro and ultrafiltration processes show many advantages over traditional methods for protein concentration and purification (Noordman, Ketelaar, Donkers, & Wesselingh, 2002; Torres, Marín, Ramos, & Soriano, 2002). Indeed in a previous paper Rodriguez Furlán, Pérez Padilla, and Campderrós (2010a) demonstrated that the UF-DD step allows concentrating protein, eliminating insoluble macroscopic components, and reducing the saline content. In this work, improvements were made to the equipment using two membrane cassettes (Pellicon modules, Millipore, Bedford, MA, USA), doubling the membrane area to 1 m^2 employing a new centrifugal pump (Fluid-o-Tech, Milano Italy, PA 1001, caudal of 1200 L/h) so the experience of bovine plasma concentration was faster without refrigeration needs. The feed solution of bovine plasma which was dissolved in deionized water to 3% w/v using a mixer at low speed to avoid the formation of vortex and to minimize the appearance of foam. The feed solution (5 L) was driven first through a porous support (Viledon F 02431 D, Germany) to remove macroscopic aggregates, and then through to a frontal flow filter of microfiltration (MF) of 8 μm (Hidroquil, Argentina). This pretreatment process of the solution reduces fouling in the subsequent step of ultrafiltration (UF). The filtered solution was pumped to the UF module, which contains modified polyethersulfone membranes with a molecular weight cut-off (MWCO) of 10 kDa. The protein concentration by UF was carried out by continuously removing the permeate stream until the desired concentration of 4.2% (w/v) (VCR = 1.4), was achieved. The operating conditions were the following: transmembrane pressure (ΔP) of 1.8 bar, flow rate of (0.71 ± 0.08) L/min. A discontinuous diafiltration (DD) process was applied to remove salts and other contaminant of low molecular weight. For this operation the feed solution was the UF concentrate diluted to the initial volume (5 L) with deionized water in a single state and ultrafiltrated to the desired concentration range. The cleaning of the fouled membrane was performed by applying a "Cleaning in Place" (CIP) procedure according to the manufacturer's instructions. Measurements of normalized water permeability were performed in order to verify recovery of flow through the membrane and the optimal performance during the separation process.

2.3. Freeze-drying stage

The bovine plasma protein concentrate (concentration: 4.2% w/v), obtained by UF was fractioned into four fractions. A fraction as witness sample (control) was reserved and the fructans (NF = native fructans; HDPF = high degree of polymerization fructans; HPF = high performance fructans) used as protective agents were added to the rest, in concentrations of 5%, 10%, 15% and 20% (w/v). The protein concentrates were placed on stainless steel trays and frozen in a freezer at −4 °C, −20 °C and −40 °C and freeze-dried using a lyophilizer (Rificor S.A., Argentina) at 1 bar of pressure for 48 h. The samples temperature was controlled by a temperature sensor.

2.4. Determination of denatured protein content

The soluble protein content was determined after isoelectric precipitation of denatured/aggregated proteins (de Wit, 1981, 1990; Meza, Verdini, & Rubiolo, 2009; Rodriguez Furlán et al., 2010a; Verheul, Roefs, & de Kruif, 1998). Solutions of 1% (w/v) protein concentrate were adjusted to pH 4.8 using 0.1 N of NaOH and HCl. An aliquot of the solution was centrifuged (Rolco 2070 centrifuger, Argentina) at

3000 rpm for 30 min. Protein concentration in the supernatants was determined by measuring absorption at 280 nm after appropriate dilution in a dissociating buffer (EDTA 50 mM, urea 8 M) and reported as percentage of the total protein concentration (Giroux & Britten, 2004). Insoluble protein content of suspensions at pH 4.8 was defined as the difference between total protein (*TP*) and soluble protein (*SP*) contents and was used to estimate the extent of denaturation/aggregation of plasma protein (Anandharamakrishnan, Rielly, & Stapley, 2008). The percentage of denatured protein (*DP*) content was calculated with the following equation (Morr, 1990):

$$DP = \frac{(TP - SP)}{TP} 100 \quad (1)$$

The measures have been done in the protein concentrates with and without protectant agent (fructans: NF, HDPF, HPF) at different concentrations (5%, 10%, 15%, 20% w/v) and freezing temperatures (−40 °C, −20 °C, −4 °C).

2.5. Shelf life time study

The stability of the products defines the commercial value. For the study of the thermodegradation mechanism and kinetics, the influence of composition and/or process of the less stable component which determines the product quality was evaluated. In this sense, the kinetics of the native protein thermodegradation and the shelf life time prediction under storage was assessed.

The following procedure was used: 10 g freeze-dried samples were placed in flexible packaging material (Aluflex S.A., Argentina) consisting of different layers: PE, PET and Aluminum. The film protected the product from contact with oxygen, water vapor and light exposure. Samples were stored in ovens at constant temperatures of 28, 40 and 60 °C, for 33 days.

2.5.1. Kinetic degradation model

The order of a reaction in a kinetic model is a parameter that deals a mathematical description of time or concentration dependence. It is useful for predicting the behavior of a particular reaction in a food product, and therefore is suitable for modeling the shelf life of a product. The best known models referred to shelf life studies are zero-order reactions that represent a linear evolution (retention or degradation) of the parameter, and first-order reactions that represent an exponential evolution of the parameter (Bosch et al., 2013; Labuza and Riboh, 1982).

The kinetic degradation model employed to predict the loss of native protein in the concentrate has been provided by Labuza and Riboh (1982), by the following general equation:

$$-\frac{d[F]}{dt} = k[F]^n \quad (2)$$

where: $[F]$ = quality factor concentration; k = degradation velocity constant; n = reaction order; t = storage time.

Integrating Eq. (2), for a first order kinetics, n = 1:

$$-\ln\left(\frac{[F]_t}{[F]_0}\right) = -kt \quad (3)$$

where the subscripts *0* and *t* were at initial time and at time (t), after the degradation reaction, respectively.

The relationship between the reaction constant and the temperature is quantified by the Arrhenius equation (Labuza and Riboh, 1982; Robertson, 1993):

$$k = k_0 e^{\left(-\frac{Ea}{RT}\right)} \quad (4)$$

where: Ea = activity energy of the reaction (cal/mol); R = gases universal constant (1.987 cal/mol K); T = absolute temperature (K); k_0 = pre-exponential constant or frequency factor (1/min).

2.6. Nonenzymatic browning measurement

2.6.1. Free browning compounds

Triplicate samples were stored at 28 °C, 40 °C and 60 °C for 33 days and then were subsequently stored at 4 °C to avoid further browning before analysis. The extent of browning was determined spectrophotometrically (UV visible spectrophotometer, Double Beam Shimadzu Spectrophotometer, USA) using the technique described by Delgado Andrade, Rufián Henares, and Morales (2009): 0.5 g of sample were suspended in 5 mL of deionized water, the tube was shaken vigorously for 1 min and clarified with 0.25 mL each of Carrez I (potassium ferrocyanide, 15% w/v) and Carrez II (zinc acetate 30% w/v) solutions. The resulting mixture was centrifuged (25 min at 3000 rpm, Rolco 2070 centrifuge, USA), the supernatant was collected in a 10 mL volumetric flask, and two further extractions were performed using 2 mL of deionized water. The supernatants were mixed and the volume was made up to 10 mL with deionized water. Solutions were filtered, adequately diluted if necessary, and measured at 280 nm (wavelength to detect the products at an early stage of browning, and low molecular weight Maillard compounds) and at 420 nm (to detect the final and high molecular weight Maillard compounds) (Vercet, 2003). The rate constant of browning, k_b, was calculated from zero-order kinetics using a linear regression analysis (Miao & Roos, 2006).

2.6.2. Color determination

The color of the freeze-dried samples, based on the L^*, a^*, b^* color system, was determined with a digital spectrophotometer (MiniScan EZ) provided with the software. The Chroma meter was calibrated with a standard white tile ($L^* = 96.94$, $a^* = -0.09$, $b^* = +1.02$). The total color difference $\Delta E = [(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2]^{1/2}$ was calculated from values for L^* (lightness), a^* (redness), b^* (yellowness), (Aidoo, Sakyi Dawson, Tano Debrah, & Saalia, 2010).

2.7. Functional properties

The method of Lee, Morr, and Ha (1992) with several modifications was used to determine the protein solubility at room temperature (22 ± 1)°C. Solution of each protein–saccharide sample at 1% (w/v) (pH adjusted between 3 and 7.2 with either 1 N HCl or 1 N NaOH, respectively) was prepared and stirred for 5 min. Then, the samples were centrifuged at 3000 rpm for 60 min. The protein content of the supernatant was determined by absorption spectroscopy at 280 nm, bovine serum albumin (BSA, Sigma-Aldrich, USA) was used as standard. The protein solubility was calculated as the percentage of protein in supernatant divided by the percentage of total protein in the original solution (Ee, Rehman, Agboola, and Zhao (2009)).

The emulsifying capacity (EC) was performed as described by Yu, Ahmedna, and Goktepe (2007) with minor modifications. One gram of protein of each sample was mixed with 200 mL of deionized water for 2 min using a mixer at high speed before slowly addition of 500 mL of vegetal oil under continuous blending. The pH of solutions was adjusted to 3–7.2 with 1 N NaOH and HCl. Blending was stopped every 2 min to check for emulsion breakage. When a clear emulsion breakage was observed, the total volume of oil added was recorded and used to calculate EC as volume (mL) of oil emulsified per gram of protein concentrate.

The foaming capacity (FC) of the protein concentrates was investigated using a simple technique as described by several works (Chove, Grandison, and Lewis (2007); Makri, Papalamprou, and Doxastakis (2005)). Protein solutions 1% (w/w) were prepared in deionized water and the pH was adjusted between 3 and 7.2 with 1 N NaOH and HCl. Samples of the dispersions (V_I = 100 mL) were blended in a graduated measuring jug using a mixer or 30 s (1000 rpm). The final solution volume including the produced foam (V_F) was immediately reported. FC was calculated as the ratio of V_F to V_I. The samples were reserved at room temperature (22 ± 1)°C and the foam volumes read

at 1 h. The foaming stability (FS) was calculated as the percent ratio between at 1 h to that initial (Marco and Rosell (2008); Yusuf, Ayedun, and Sanni (2008)). The equipment for characterization assays was hermetically closed as to maintain a constant air volume in each run. The agitation time and volume used were standardized.

The experiences were carried out in triplicate.

2.8. Heat-induced gels preparation and rheological measurement

Gel strength was determined in three replicates according to the procedure described by Chakraborty (1986) and Yu et al. (2007). Protein suspensions containing 12.5% (w/v), 15% (w/v) and 17.5% (w/v) of protein were prepared, and the pH was adjusted between 3 and 7.2 with 1 N NaOH or HCl. Viscosities of these protein suspensions were measured by a programmable Brookfield DV-III + Viscometer (Brookfield, USA) at room temperature (22 ± 1)°C. Samples were heated at 90 °C in a shaking water bath and kept for 30 min, then cooled to room temperature without stirring. Gel viscosity was determined at different shear rates (0.1 s^1 to 40.0 s^{-1}) at room temperature. The rheological behavior of the fluid was characterized by means of Power-Law:

$$\eta = K\,\gamma^{n-1} \tag{5}$$

where η: apparent viscosity of the fluid (Pa s); γ: shear rate (s^{-1}); K: flow consistency index (Pa s^n); n: flow behavior index (<1 for pseudoplastics or shear-thinning fluids).

2.9. Water sorption

The water sorption isotherms were calculated by using an isopiestic method (Espinosa Andrews & Urias Silvas, 2012). Freeze-dried samples were placed in open weighing glassware and stored in air-sealed containers while maintaining equilibrium relative humidity with saturated salt solutions. About 0.50 g of each sample was placed in glass flask and was stored in a vacuum desiccator over P_2O_5 for 8 days. After storage, the samples were considered anhydrous (Miao & Roos, 2006). Water sorption of dehydrated samples was studied for 169 h at room temperature (22 ± 1 °C) in desiccators over saturated solutions of LiCl, K_2CO_3, $NaNO_2$, and NaCl at a relative water vapor pressure (RVP) of 11.4%, 44.1%, 65.6% and 76.1%, respectively (Haque & Roos, 2006), until the sample weights leveled off, indicating steady-state water contents. The samples were weighed at 4, 6, 8, 15, 22, 25 h, and then at 24 h intervals for 11.4% and 44.1% RVP, at 1, 4, 5, 6, 8, 15, 22, 25 h, and then at 24 h intervals for 65.5% and 76.1% RVP. All samples were kept closed with caps after the vacuum was released in the desiccators prior to weighing. Water content of each sample was measured as a function of time and the mean weight of triplicate samples was calculated.

The GAB (Guggenheim–Anderson–De Boer) model has been widely used to describe the sorption behavior of foods, since it represents adequately the experimental data in the range of water activity of most practical interest in foods, i.e., 0.10–0.90 (Espinosa Andrews & Urias Silvas, 2012). The GAB isotherm model is given by the following equation:

$$\frac{m}{m_0} = \frac{CKa_w}{(1-Ka_w)(1-Ka_w+CKa_w)} \tag{6}$$

where m_0, C, and K represent the water 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. Second-order polynomial was obtained from Eq. (6):

$$\frac{a_w}{m} = \alpha a_w^2 + \beta a_w^2 + \gamma \tag{7}$$

2.10. Scanning electron microscopy

Surface structures of the amorphous freeze-dried fructans–protein samples and spray-dries agave fructans were observed by scanning electron microscopy (SEM) using an LEO1450VP equipment (Zeiss, Germany). Samples were mounted on specimen stubs using double-side adhesive tape and then gold-coated to obtain surface micrographs. The samples were systematically viewed at 50 to 400× magnifications (Haque & Roos, 2006). The images were analyzed by Image-Pro Plus 6.0 (Media Cybernetics Inc, Bethesda, USA) software.

2.11. Statistical analysis

The significance of differences among thermodynamic magnitudes was determined by using Tukey's procedure at $P < 0.05$ (SAS, 1989), using statistical GraphPad InStat software (1998).

3. Results and discussion

3.1. Protective effect of fructans during freeze-drying

Fig. 1 represents the amount of denatured protein of bovine plasma protein (BPP) freeze-dried concentrates with different fructans (NF, HDPF, HPF) at different concentrations and freezing temperatures. Denatured protein content of the sample without protective agent after the freeze-drying is of 62.00 ± 3.69%. In the freeze-dried samples at a freezing temperature of −40 °C (Fig. 1A), this percentage decreased ($P < 0.001$), and an optimum concentration at 10% (w/v) for BPP/HDPF (23.88 ± 3.22%) and BPP/HPF (26.85 ± 1.74%) blends, and between 5% and 10% (w/v) for BPP/NF (19.02 ± 4.0%) was observed. Similar results were obtained in a previous work for plasma bovine proteins using inulin as protective agent, in which a denatured protein content of 29.85 ± 0.65% was found (Rodriguez Furlán et al., 2010a).

A stabilization mechanism of proteins during the freeze-dried procedure is the water replacement hypothesis. This mechanism involves the formation of hydrogen bonds between a protein and a saccharide(s) to satisfy the hydrogen bonding requirement of polar groups on the protein surface (Allison, Chang, Randolph, and Carpenter (1999); Hinrichs, Prinsen, and Frijlink (2001); Hinrichs, Sanders, De Smedt, Demeester, & Frijlink, 2005; Hinrichs et al., 2006; Wang, 2000). The presence of these saccharides supplants water forming hydrogen bonds with the dry protein, preserving the native structures of proteins (Buera et al., 2005; Carpenter et al., 1993). In this regard, saccharides protect the proteins by direct interaction through the formation of hydrogen bonds. The maximum stabilization is obtained when the concentration of saccharide is sufficient to form a monomolecular layer on the protein surface (Allison et al., 1999; Wang, 2000). Another mechanism of protein stabilization by lyoprotectants is the formation of an amorphous glass during freeze-drying (Hinrichs et al., 2005; Rodriguez Furlán et al., 2012; Tattini, Parra, Polakiewicz, & Pitombo, 2005). However, the crystallization may occur in the presence of excess of saccharides (Rodriguez Furlán et al., 2012), which may be because the amorphous state favors hydrogen bonding between protein and saccharides. Thus, during freeze-dried crystallization inefficient hydrogen bonding would occur (Allison et al., 1999; Buera et al., 2005; Carpenter et al., 1993; Murray & Liang, 1999; Wang, 2000). Therefore, based on the results previously exposed, the stabilization of bovine plasma proteins was found to depend on the concentration of fructans. Maximum stabilization of plasma bovine protein was reached at a fructan concentration of 10% (w/v). The increased fructan concentration did not improve the protein stability, which can be attributed to the collapse of the formulation structure. This may be due to crystallization and loss of intimate interaction with the protein during the freeze-drying of the saccharides at high concentrations (Carpenter et al., 1993; Wang, 2000). The difference observed among saccharides in stabilization of

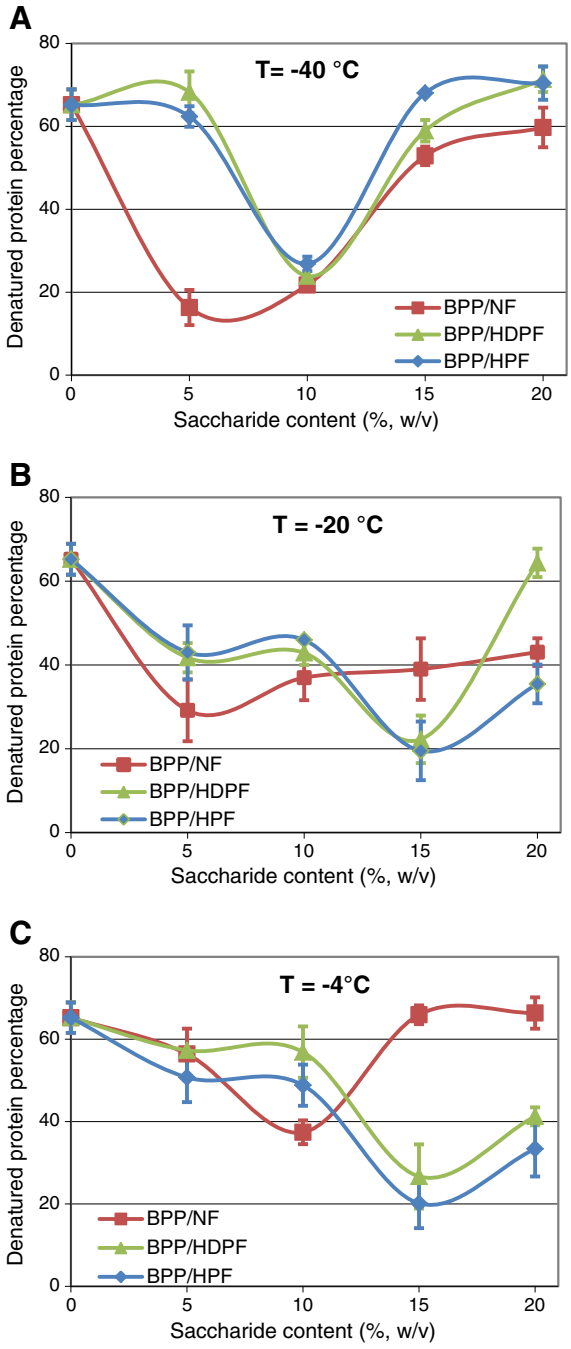

Fig. 1. Denatured protein content of bovine plasma proteins protected with NF, HDPF HDPF during freeze-drying at different concentrations and freezing temperatures: A = −40 °C, B = −20 °C, C = −4 °C.

proteins may be partially because of the difference in the extent and intimacy of hydrogen bond formation (Wang, 2000).

Freeze-drying is a relatively expensive process, since the high energy demands lead to high energy costs. For this reason, the effect of agave fructans as lyoprotective agents at higher freezing temperatures (−20 °C and −4 °C) was assayed. Fig. 1B and C shows that the freeze-dried samples at higher freezing temperatures present a shift in the optimal concentration towards higher concentrations: at −20 °C and −4 °C, BPP/HDPF and BPP/HPF showed a shift from 10% (w/v) to 15% (w/v), at −4 °C, the BPP/NF showed a shift in a range of 5 to 10%, with an optimum of 10% (w/v). This may be because, at higher concentrations of saccharides, T_g values are higher, giving greater stability to the product (Rodriguez Furlán et al., 2012). This behavior could be explained by a microstructure study of fructans.

Fig. 2 shows the SEM micrographs of the analyzed agave fructans (NF, HDPF and HPF), together with their respective particle size distribution (Fig. 2D), with a mean value of NF: 19.55 ± 2.74 μm, HDPF: 11.01 ± 0.73 μm and HPF: 10.00 ± 0.74 μm. No statistically significant difference between HDPF and HPF ($P > 0.05$) was found, but between them and NF, a significant difference was observed ($P < 0.001$). Fig. 2 shows that HDPF and HPF present a similar particle size distribution to NF (between 9 and 15 μm), with a higher concentration of diameters between 2 and 9 μm, and an absence of diameters greater than 40 μm, while native fructans (NF) exhibit diameters of 120 μm. Therefore, HDPF and HPF samples show less dispersion or higher homogeneity. Moreover, comparing the molecular weight distribution of HPF and HDPF, the latter had a lower dispersion with a lower concentration of particles of smaller diameter.

Fig. 1 shows that, at the higher temperatures of freezing (−20 and −4 °C), the samples with a smaller particle size dispersion (HDPF and HPF) have a lower denatured protein content compared to the sample with a higher heterogeneity (NF), (Fig. 2). In addition, samples of BPP/HDPF and BPP/HPF at their optimal concentrations at −20 and −4 °C do not present statistically significant differences with their respective optimal values of denatured protein content at −40 °C ($P > 0.05$). This may be due to the greater homogeneity of the sample that leads to a better integration of bovine plasma proteins into the amorphous matrix, resulting in a lower denatured protein content (Fig. 1). Therefore, a product of similar quality was obtained for the samples with HDPF and HPF at 15% (w/v) (analogous denatured protein percentage, $P > 0.05$) with a significant decrease of energy consumption, since it could be frozen at a higher temperature, −20 °C and −4 °C.

The following studies were conducted at a freezing temperature of −40 °C and at the optimal concentration of 10% (w/v).

3.2. Shelf life time of freeze-dried samples

The native protein content as a function of time and temperature was analyzed to find the kinetics of the reaction and predict the shelf life of the freeze-dried product. The experimental results are presented in Table 2. Experimental data were analyzed with rate equations of different orders (Eq. (2)), and a first-order equation was found to provide the best fit to describe the loss of native protein under different storage temperatures. This fact was in agreement with other authors who indicated that protein degradation reaction in dehydrated foods follows first-order kinetics (Kaanane & Labuza, 1985; Labuza & Schmidl, 1985; Robertson, 1993). Then, linear regressions were calculated from experimental data of native protein percentage of the samples at 28, 40 and 60 °C (Fig. 3), and the values of k for each temperature were obtained. They are reported in Table 2. The model fit was assessed by the magnitude of the coefficients: R^2.

From the representation of ln k versus the inverse of temperature, a linear plot with a slope representing $-Ea/R$ was obtained. The values of Ea (Eq. (4)) were 3.27 kcal mol^{-1}, 3.61 kcal mol^{-1}, 3.55 kcal mol^{-1} and 3.50 kcal mol^{-1} for the control, BPP/NF, BPP/HDPF and BPP/HPF, respectively. These results confirmed the temperature-dependent degradation of proteins, and the capacity of fructans as protein lyoprotectors.

The shelf life ($t_{1/2}$) was defined as the storage time until 30% of undernaturated protein remained. The shelf life of the products at different temperatures was calculated by Eqs. (2) and (3), considering 30% (w/v) as final quality factor, and using the calculated Ea value. The results are shown in Table 2. Thus, by studying a reaction and measuring k at three temperatures, the degradation rate may be predicted at any desired temperature. And so, the product shelf life may be estimated. Therefore, at stored temperature of 2 °C, a protein concentrate protected with NF, HDPF and HPF had a shelf life of approximately 11 months, 8.2 months and 7.1 months respectively, resulting in higher than that of the plasma without fructans (1.7 months). This longer shelf

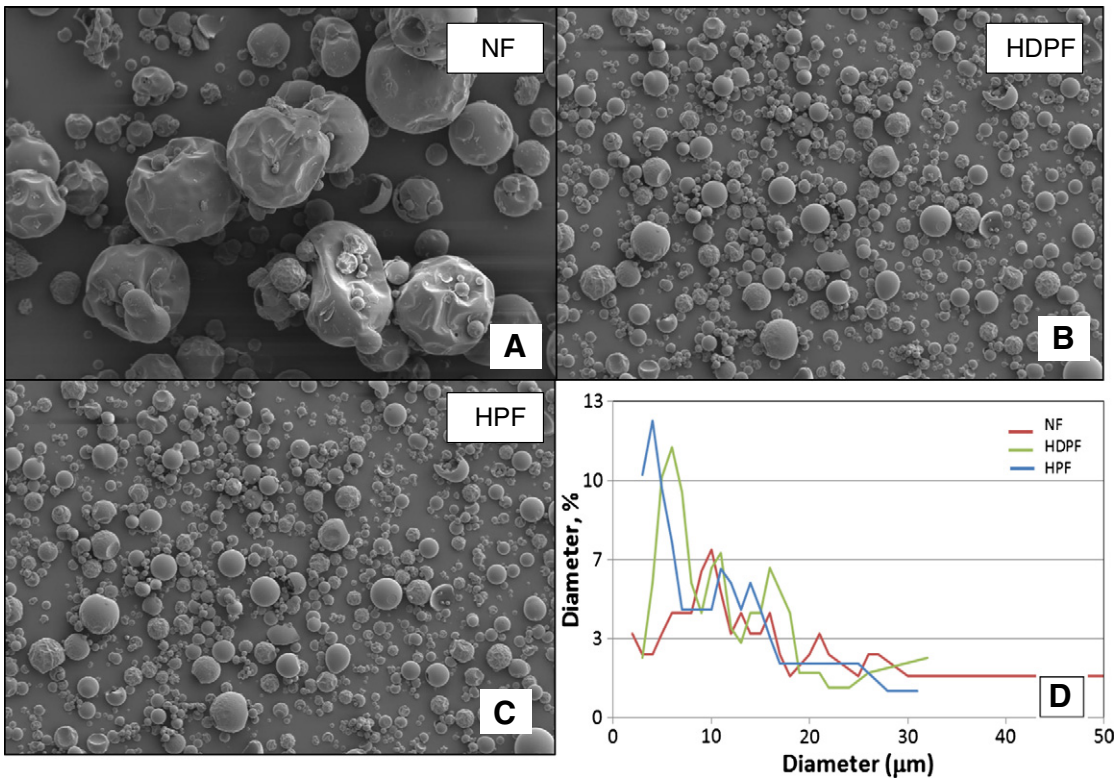

Fig. 2. A–C: Scanning electron micrographs of powdered agave fructans (magnification: 200×). D = Particle size distribution.

life period would be due to the fact that oligosaccharides form glassy structures with high T_g values, and low crystallization tendency, increasing the product stability during storage (Hinrichs et al., 2005). Similar results were found by Rodriguez Furlán et al. (2010a) when studying the stabilizing effect of the oligosaccharide inulin on bovine plasma proteins, and obtaining a shelf-life period of 14.5 months.

3.3. Nonenzymatic browning

Nonenzymatic browning (Maillard's reaction) occurring in the freeze-dried bovine plasma protein and fructose from fructans was evaluated under the conditions studied (28–60 °C). Measurements at 280 nm and 420 nm against storage time are shown in Table 3. An apparent kinetic reaction order of zero-order during storage was assumed, taking into account other studies in saccharide–protein systems similar to ours (Labuza & Baisier, 1992; Lievonen, Laaksonen, & Roos, 1998; Miao & Roos, 2006).

Table 3 shows that the browning rates of BPP/NF, BPP/HDPF and BPP/HPF increased with increasing temperature. Furthermore, the results indicated that the control sample (protein concentrate lyophilized without protective agent) did no present appreciable browning over time at different temperatures tested. This behavior could be explained by the fact that the sample had not saccharides that, together with proteins, could produce these reactions. Moreover, while the freeze-dried material is below its T_g (glass transition temperatures) value; that is, in an amorphous state with restricted mobility, it does not cause a nonenzymatic browning. However, because of heterogeneities of the materials as well as defects and porosity of the matrix structure, nonenzymatic browning could occur below T_g (Miao & Roos, 2006). In this respect, the values obtained at 280 and 420 nn were higher for the samples with fructans having a higher heterogeneity (Fig. 2). Nevertheless, the browning rates were very low or null at low temperatures (Table 2, T = 28 °C and 40 °C), indicating that most of the systems were in an amorphous or glassy state with extremely low mobility and diffusion. However, at higher temperature, the browning

Table 2
Native protein percentage (with 95% confidence limits) at different storage temperatures of bovine plasma protein (BPP) with and without agave fructans.

	Control			BPP/NF			BPP/HDPF			BPP/HPF		
	Temperature (°C)											
	28	40	60	28	40	60	28	40	60	28	40	60
Time (days)	Native protein percentage											
0	50.0 ± 3.7^{a}	50.0 ± 3.7^{a}	50.0 ± 3.7^{a}	78.3 ± 0.8^{a}	78.3 ± 0.8^{a}	78.3 ± 0.8^{a}	72.9 ± 3.2^{a}	72.9 ± 3.2^{a}	72.9 ± 3.2^{a}	73.1 ± 1.7^{a}	73.1 ± 1.7^{a}	73.1 ± 1.7^{a}
15	44.5 ± 1.6^{a}	34.9 ± 5.2^{b}	6.2 ± 0.3^{b}	66.6 ± 3.7^{b}	72.3 ± 3.3^{a}	53.3 ± 5.2^{b}	56.0 ± 2.9^{b}	51.2 ± 5.1^{b}	53.7 ± 2.1^{b}	59.0 ± 2.7^{b}	52.8 ± 2.8^{b}	46.0 ± 2.1^{b}
23	36.1 ± 3.3^{b}	26.8 ± 1.4^{c}	2.0 ± 0.8^{c}	51.0 ± 1.3^{c}	60.0 ± 2.8^{b}	46.0 ± 4.7^{b,c}	53.3 ± 1.2^{b}	47.5 ± 3.8^{b}	43.0 ± 3.4^{c}	52.1 ± 0.6^{c}	44.4 ± 5.1^{b,c}	41.0 ± 4.2^{b}
33	26.5 ± 2.3^{c}	16.1 ± 0.1^{d}	0.0^{d}	50.7 ± 1.8^{c}	55.2 ± 2.6^{b}	40.0 ± 0.3^{c}	50.8 ± 1.8^{b}	44.7 ± 0.2^{b}	30.0 ± 0.6^{d}	49.2 ± 2.1^{d}	39.7 ± 0.9^{c}	27.0 ± 1.4^{c}
R^2	0.91	0.96	0.94	0.95	0.91	0.98	0.90	0.90	0.98	0.97	0.98	0.98
k (*days*)	0.52	0.99	5.18	0.32	0.43	0.69	0.40	0.52	0.82	0.41	0.65	0.95
$t_{1/2}$ (months)	0.57	0.40	0.22	3.55	2.17	1.12	2.70	1.72	0.87	2.36	1.51	0.77

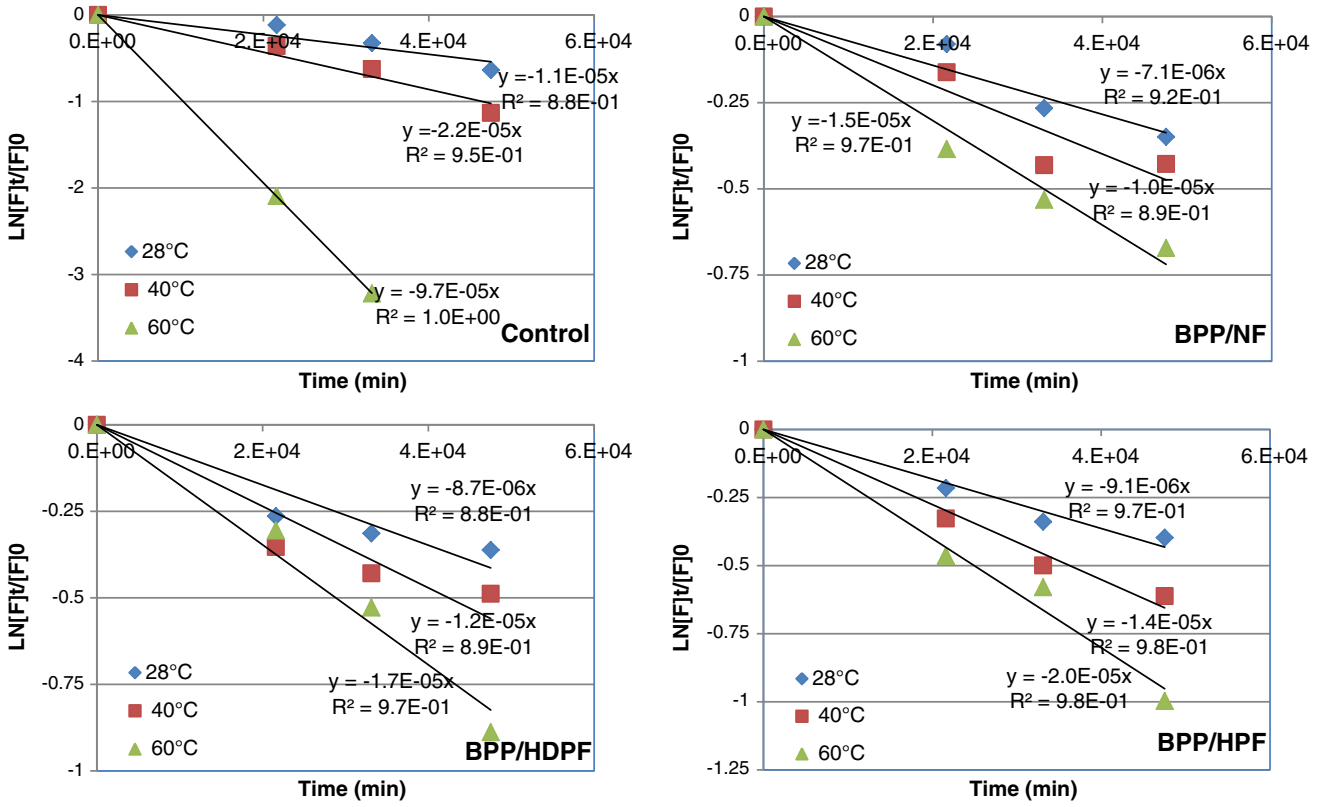

Fig. 3. Determination of the k value considering an order kinetics of $n = 1$ for the bovine plasma protein with fructans at 10% (w/v).

reaction accelerated ($P < 0.05$). This behavior could occur because, as 60 °C is probably higher than the T_g value of the system, the saccharide crystallization could cause the release of the absorbed water which contributes to the increase of the product browning (Miao & Roos, 2006).

Table 4 lists color parameters of bovine plasma protein/fructan systems at different temperatures (28 °C, 40 °C and 60 °C) for 33 days. Color parameters showed effectively that an increase in temperature led to an increase in brown color and a darkening of all samples, since a^* and b^* increased with the increase of temperature, while L^* decreased for BPP/NF, remaining constant for BPP/HDPF and BPP/HPF ($P > 0.05$). This difference in color might be attributed to nonenzymatic browning reactions between the reducing sugars and amino acids within the samples. Significant differences ($P < 0.001$) in all color parameters between the BPP/NF samples at 60 °C and 28 °C were found. However, for sample BPP/HPF an increase in the parameter b^* ($P < 0.05$), and for BPP/HDPF, both a^* and b^* increased ($P < 0.05$). These results suggest the formation of compounds of non-enzymatic browning. Table 3 also shows the color variation (ΔE) from the white tile. There was a significantly color variation with the increase of the temperature for samples with NF ($P < 0.001$). These parameters can be related to the absorbance values reported at 280 nm and 420 nm, and to the fact that the BPP/NF matrix had the major formation of browning compounds.

Table 3
Rate constants of nonenzymatic browning for freeze-dried BPP with and without agave fructans determined by the linear regression of the absorbance at 280 nm and 420 nm.

Temperature (°C)	BPP		BPP/NF		BPP/HDPF		BPP/HPF	
	Absorbance per gram of protein at 280 nm							
	0 days	33 days	0 days	33 days	0 days	33 days	0 days	33 days
28	0.023 ± 0.001 $k \approx 0$	0.024 ± 0.002	0.036 ± 0.003 $k = 0.01$	0.046 ± 0.002	0.021 ± 0.004 $k \approx 0$	0.023 ± 0.002	0.032 ± 0.014 $k = 0.01$	0.046 ± 0.005
40	0.023 ± 0.001 $k \approx 0$	0.023 ± 0.002	0.036 ± 0.003 $k = 0.01$	0.050 ± 0.004	0.021 ± 0.004 $k \approx 0$	0.020 ± 0.005	0.032 ± 0.014 $k = 0.01$	0.047 ± 0.006
60	0.023 ± 0.001 $k \approx 0$	0.020 ± 0.001	0.036 ± 0.003 $k = 0.11$	0.165 ± 0.008	0.021 ± 0.004 $k = 0.04$	0.073 ± 0.009	0.032 ± 0.014 $k = 0.07$	0.115 ± 0.016
	Absorbance per gram of protein at 420 nm							
	0 days	33 days	0 days	33 days	0 days	33 days	0 days	33 days
28	0.0045 ± 0.0003 $k \approx 0$	0.0043 ± 0.0002	0.0065 ± 0.0004 $k = 0.001$	0.0082 ± 0.0006	0.0043 ± 0.0004 $k \approx 0$	0.0045 ± 0.0008	0.0052 ± 0.0009 $k \approx 0$	0.0053 ± 0.0003
40	0.0045 ± 0.0003 $k \approx 0$	0.0044 ± 0.0001	0.0065 ± 0.0006 $k = 0.002$	0.0091 ± 0.0005	0.0043 ± 0.0004 $k = 0.001$	0.0055 ± 0.0004	0.0052 ± 0.0009 $k = 0.003$	0.0082 ± 0.0006
60	0.0045 ± 0.0003 $k \approx 0$	0.0044 ± 0.0002	0.0065 ± 0.0006 $k = 0.007$	0.0146 ± 0.0010	0.0043 ± 0.0004 $k = 0.005$	0.0102 ± 0.0011	0.0052 ± 0.0009 $k = 0.008$	0.0145 ± 0.0009

Table 4
Color parameters in BPP/fructans systems at different temperatures after 33 days.

Sample	Temperature	L^*	a^*	b^*	ΔE
BPP/NF	28 °C	76.73 ± 0.13^{a}	0.86 ± 0.12^{a}	14.69 ± 0.40^{a}	24.42 ± 0.14^{a}
	40 °C	75.76 ± 0.64^{a}	1.09 ± 0.13^{a,b}	17.30 ± 0.40^{b,e}	26.74 ± 0.27^{b}
	60 °C	62.89 ± 0.79^{b}	11.08 ± 0.05^{b}	31.57 ± 0.16^{c}	47.09 ± 0.50^{c}
BPP/HDPF	28 °C	77.00 ± 1.03^{a}	0.95 ± 0.31^{a}	14.88 ± 0.79^{a}	24.37 ± 0.51^{a,e}
	40 °C	74.08 ± 0.37^{a}	0.99 ± 0.10^{a}	14.86 ± 0.15^{a}	26.73 ± 0.32^{b}
	60 °C	74.50 ± 0.16^{a}	2.43 ± 0.13^{c}	21.95 ± 0.24^{d}	30.79 ± 0.07^{d}
BPP/HPF	28 °C	76.65 ± 0.20^{a}	0.99 ± 0.07^{a}	14.83 ± 0.32^{a}	25.40 ± 0.20^{e}
	40 °C	76.56 ± 0.17^{a}	1.13 ± 0.03^{a,d}	15.95 ± 0.17^{b}	25.50 ± 0.12^{e}
	60 °C	75.91 ± 0.21^{a}	1.25 ± 0.06^{d}	18.56 ± 0.32^{e}	27.42 ± 0.05^{b}

3.4. Functional properties

The solubility values of control (BPP) and BPP/fructan blends are shown at different pH in Table 5. As can be seen, the solubility was affected by pH, which was also reported by Álvarez, García, Rendueles, and Díaz (2012) and Silva and Silvestre (2003) with the minimum value at pH 5. This result is in agreement with the protein isoelectric point (pH 4.6–5.2), (Rodriguez Furlán et al., 2010a).

With respect to the emulsion capacity (EC), all BPP samples were also affected by pH with the minimum at pH 7.2. Similar results were reported by Jeng Huh and Chin Wen (1998) with respect to the decolorization of porcine blood globin. As shown in Table 5, the protein concentrate with BPP/fructan mixtures presents a higher emulsion capacity than BPP, this could be due to the presence of the polysaccharide. Since the protein is an amphipathic molecule, the linking to fructans (which is strongly hydrophilic) suggests an increase of the emulsifying capacity. In effect, the protein hydrophobic groups are able to adsorb the lipid phase, and the fructans can easily solvate the water phase, while the EC depends on the protein–polysaccharide interaction strength (Álvarez et al., 2012; Leal Calderon, Thivilliers, & Schmitt, 2007).

With respect to foam capacity, only the sample with NF showed a significant difference ($P < 0.05$), suggesting that the presence of natural fructans with higher molecular weight that could retain more air facilitates the foam formation.

Fig. 4 shows the foam stability results. It is observed that, for all the studied pH, the highest stability was presented by the sample without addition of fructans. Furthermore, when analyzing the results between systems BPP/fructans, they have greater stability as well as control, at pH 5 (pI of proteins), presenting the following order of stability: BPP/HDPF > BPP/HPF > BPP/NF. At a pH above and below the pI, the sample BPP/NF exhibited a foaming stability equal or superior to the samples BPP/HDPF and BPP/HPF. The lower stability of the samples with fructans was at pH = 3; that is, at which more air was absorbed (> foaming). This behavior can be explained considering the higher gas volume fraction, which tends to destabilize faster the system and the variation of the bulk viscosity, as a function of the pH and fructan composition, influencing foam draining and destabilization (Rouimi, Schorsch, Valentini, & Vaslin, 2005). These results are in agreement with those reported previously by Rodriguez Furlán et al. (2010a), who described that the addition of inulin to bovine plasma proteins increased FC but decreased FS.

3.5. Rheological properties of PBP and PBP/fructan mixtures

Viscosity is an important property of foods that affects taste, flavor and texture. Gel formation occurs in two cases: (i) when the protein is at high concentrations, and (ii) as a consequence of a sol–gel transformation, due to protein denaturation, usually caused by a thermal treatment. Therefore, gel formation takes place because of a controlled aggregation of the protein molecules after cooling, forming a tridimensional matrix that confines the liquid (de Man, 1999; Jeng Huh & Chin Wen, 1998; Rodriguez Furlán, Pérez Padilla, & Campderrós, 2010b; Yu et al., 2007).

Figs. 5 and 6 show the viscosity of the different samples (control and PBP/fructan blends) at different pH and concentrations after heating. Upon heating to 90 °C and cooling to room temperature, in the suspension containing 12.5% (w/v) and 15% (w/v) of protein, a gelation producing a soft gel after heating was observed; while 17.5% (w/v) suspension turned into a firm gel. Fig. 5 shows that the samples PBP NF had higher viscosity values than other samples, especially at lower concentrations. This may be due to the presence of higher molecular weight polysaccharides that produced an increase in the gel strength (Lazaridou, Biliaderis, & Izydorczyk, 2003).

The viscosities of PBP/fructan and control solutions (Fig. 6) were sensitive to pH, presenting the highest viscosity values at pH 7.2, except for BPP/HPF which showed a maximum at pH of plasma protein isoelectric point (pI). At pH 3 for BPP/NF and BPP/HPF, gelification practically did not take place. This behavior may be due to the little or no aggregation of protein molecules in the state of pre-gel (Rodriguez Furlán et al., 2010b). However, the sample of BPP/HDPF showed gelification properties similar to the control, forming a gel in all the pH range studied. This

Table 5
Effects of pH on functional properties of freeze-dried BPP with and without fructans.

Functional property	Sample	pH		
		3	5	7.2
Solubility (%)	BPP	83.32 ± 3.37^{a,b}	64.36 ± 2.51^{a}	84.92 ± 1.63^{a}
	BPP/NF	77.11 ± 3.75^{a}	69.48 ± 3.47^{a}	76.26 ± 3.83^{a,b}
	BPP/HDPF	81.60 ± 3.89^{a,b}	51.71 ± 2.46^{b}	73.55 ± 3.71^{b}
	BPP/HPF	92.47 ± 4.32^{b}	70.78 ± 3.45^{a}	84.06 ± 4.10^{a,b}
EC (mL of oil g^{-1} of protein)	BPP	205 ± 11^{a}	308 ± 15^{a}	125 ± 6^{a}
	BPP/NF	657 ± 33^{b}	1428 ± 76^{b}	470 ± 22^{b}
	BPP/HDPF	1133 ± 54^{c}	1333 ± 69^{b,c}	609 ± 31^{c}
	BPP/HPF	480 ± 25^{d}	1142 ± 57^{c}	476 ± 28^{b}
FC (mL mL^{-1})	BPP	1.14 ± 0.06^{a}	1.12 ± 0.07^{a}	1.10 ± 0.06^{a}
	BPP/NF	1.44 ± 0.08^{b}	1.32 ± 0.07^{a}	1.16 ± 0.06^{a}
	BPP/HDPF	1.26 ± 0.04^{a,b}	1.14 ± 0.06^{a}	1.08 ± 0.05^{a}
	BPP/HPF	1.15 ± 0.05^{a}	1.12 ± 0.04^{a}	1.10 ± 0.06^{a}

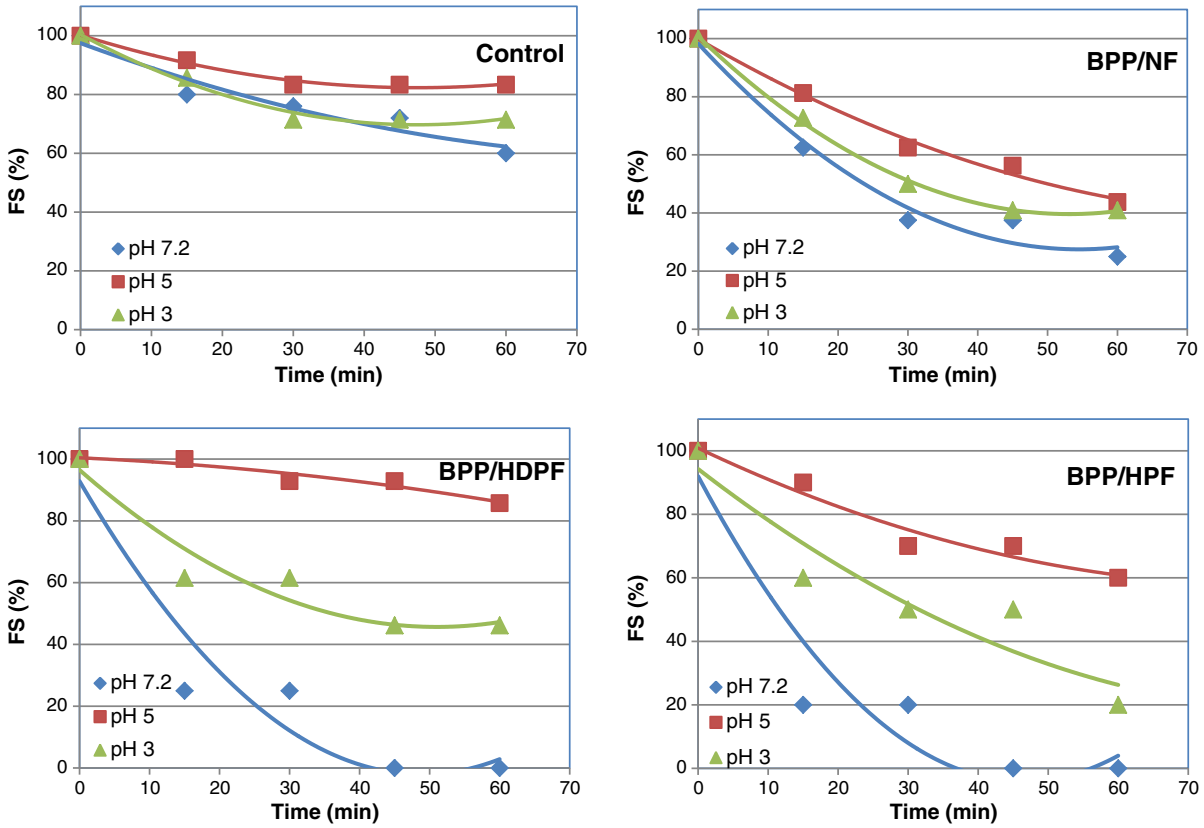

Fig. 4. Foam stability of bovine plasma protein with agave fructans at a protein concentration of 1% (w/v) at different pH values.

could be due to a higher size homogeneity of the fructan (Fig. 2), resulting in a more integrated gel matrix.

Consistency and flow indexes are of practical value for identifying flow, and for engineering design. Both indexes can be used in

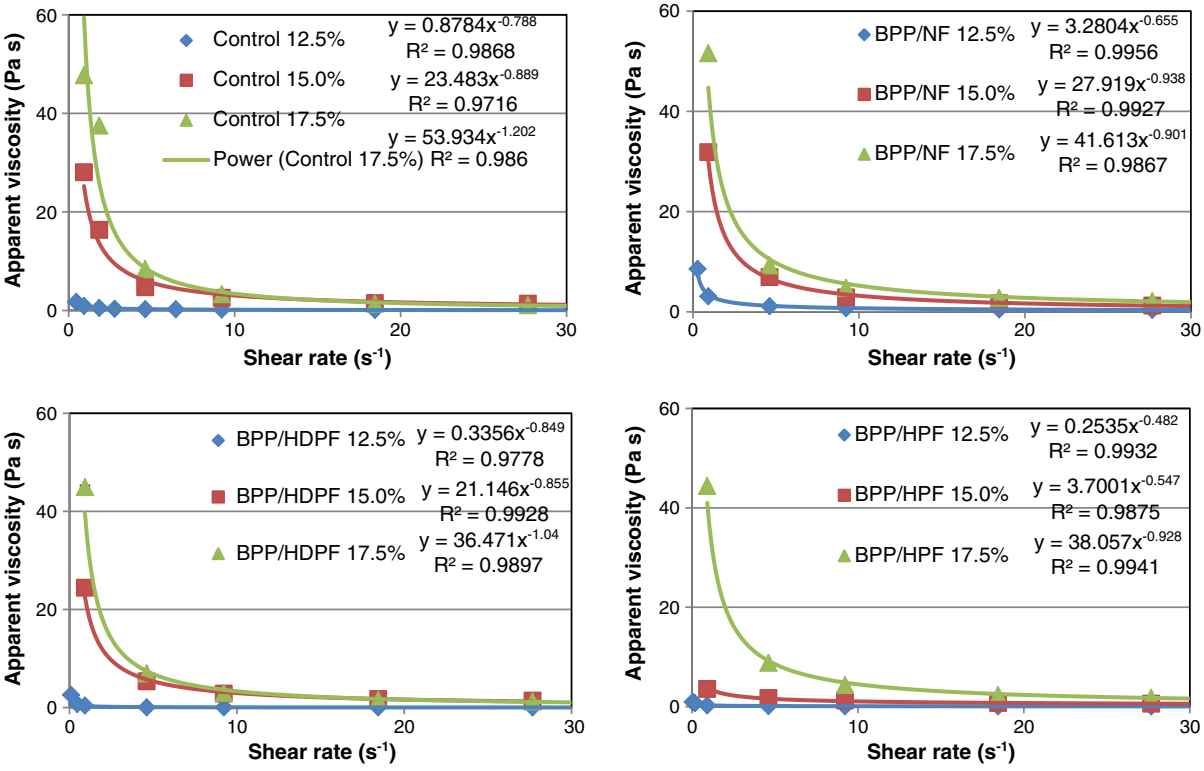

Fig. 5. Viscosity comparison between suspensions of bovine plasma protein (BPP) and BPP with fructans after heating at different concentrations.

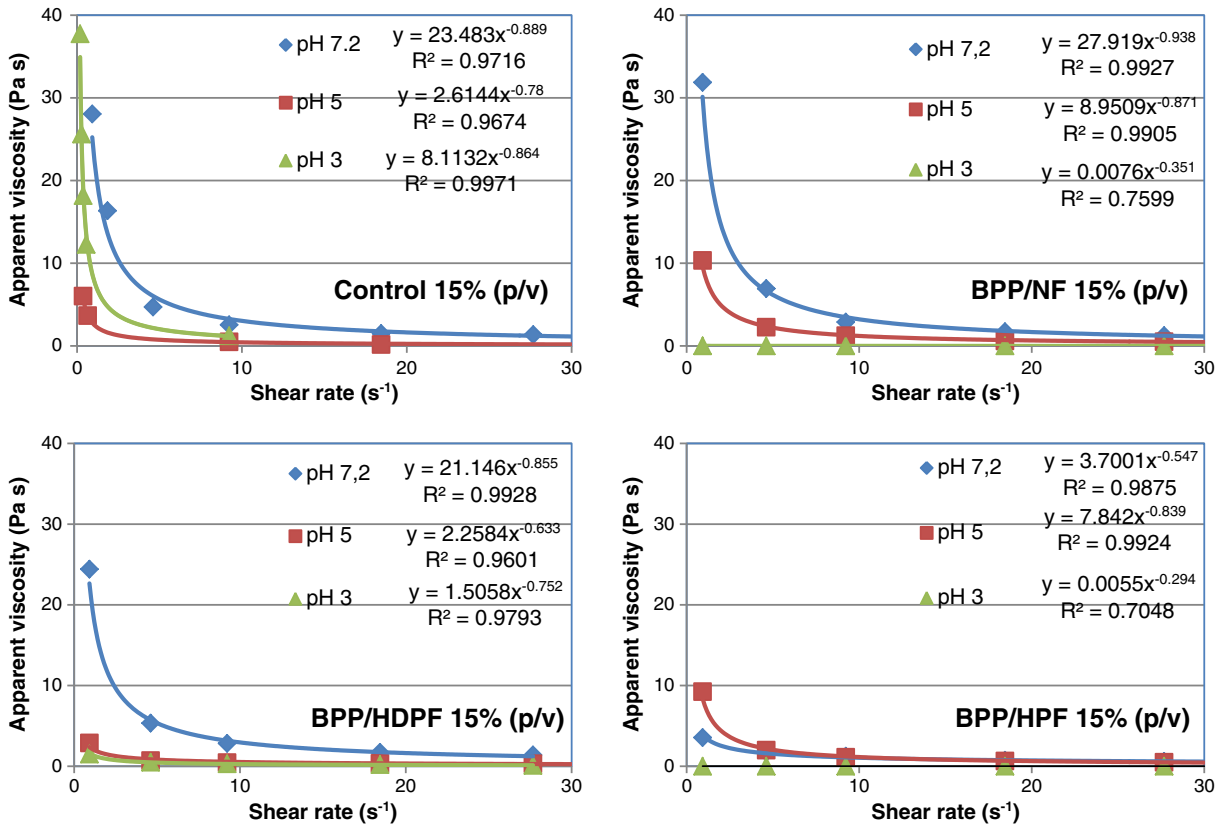

Fig. 6. Viscosity comparison between suspensions of BPP and BPP with fructans after heating at a protein concentration of 15% (w/v) and different pH.

determining power requirements (Wei, Wang, & Wu, 2001). The Power Law model (Eq. (5)) was applied to describe flow behavior of PBP and PBP/fructan mixtures, determining the flow behavior index, n, and the consistency index, K, the model fit was assessed by the magnitude of the coefficient R^2. The n values (<1) correspond to a pseudoplastic behavior. The samples presented a $n < 1$ corresponding to a pseudoplastic behavior. However, the samples BPP/NF and BPP/HPF at 15% (w/v) and pH = 3 did not form a gel (there were no statistic differences between values before and after heating).

In all samples, an increase in the consistency index along with the viscosity was identified as the concentration of solution increases. This result can be due to the fact that the bonds among the protein molecules rise the viscosity or the consistency index of the solutions as a function of the pH and concentration (Gauchea, Vieiraa, Ogliaria, & Bordignon Luiz, 2008).

3.6. Water sorption properties of bovine plasma protein/fructan blends

The crystallization of freeze-dried amorphous material is controlled by water content causing rapid product deterioration. Crystallization occurs depending on time when water content exceeds a critical value during storage (Haque & Roos, 2006).

Fig. 7 shows the water content of freeze-dried BPP/fructan mixtures as a function of storage time under RVP of 65.6% and 76.1%. The bovine plasma protein/fructan blends exhibited smaller water absorption capacity, compared with data reported by Espinosa Andrews and Urias Silvas (2012) for agave fructans. Samples containing native fructans (NF) presented the highest values for both RVP: 65.5% and 76.1%. The results indicated that the water absorption depended on the structure and the composition of fructans, and probably on the interaction with the plasma bovine proteins in the mixture. Several factors control the structure collapse or crystallization of freeze-dried material, such as the temperature ($>T_g$) and water content (plastic effect of water), and these processes are time dependent (Espinosa Andrews & Urias Silvas, 2012). The crystallization of the samples occurred at RVP ≥ 44.1% (data don't show) at room temperature. Freeze-dried BPP/HPF mixture at 65.6% and 76.1% RVP showed (Fig. 7) higher water content than that of BPP/HDPF and BPP/NF, before the loss of sobbed water. In those mixtures, maximum water sorption occurred between 8 and 22 h. Loss of sobbed water in these samples was observed after the same period of time (<12 h) at RVP ≥ 65.6%.

The results of nonlinear fitting of GAB model (Eq. (6)) for m_0 were 1.5, 1.66, and 2.10 g/100 g dry solid for the samples BPP/NF, BPP/HDPF and BPP/HPF, respectively. The smaller value of m_0, corresponding to a smaller moisture content on monolayer, provides the longest shelf life time with minimum quality loss. In this regard, both the life time as a function of temperature results, and m_0 measurements showed the same stability behavior during storage: BPP/NF > BPP/HDPF > BPP/HPF.

3.7. Morphology of the freeze-dried BPP/fructan blends

Fig. 8A and B represent the control and BPP/NF samples, respectively. They show structures mostly irregular, broken glass-like surfaces. Similar structures were reported by Miao and Roos (2006) who studied the morphology of freeze-dried systems of lactose, trehalose and lactose/trehalose containing L-lysine and D-xylose.

Porous structures of the freeze-dried systems are shown from Fig. 8C to F for the blends protein/fructans. The SEM micrographs revealed the interconnected network structure of solids and embedded pores formed by ice crystals during the pre-freezing step and vacated as consequence of ice sublimation. The wall membranes depended on the unfrozen solute phase formed during pre-freezing. Moreover, the formation of dendritic ice crystals in the direction of pore channels was observed. The incorporation of saccharides might lead to a product with more uniform, smaller and homogeneous pore structures, and with a thinner

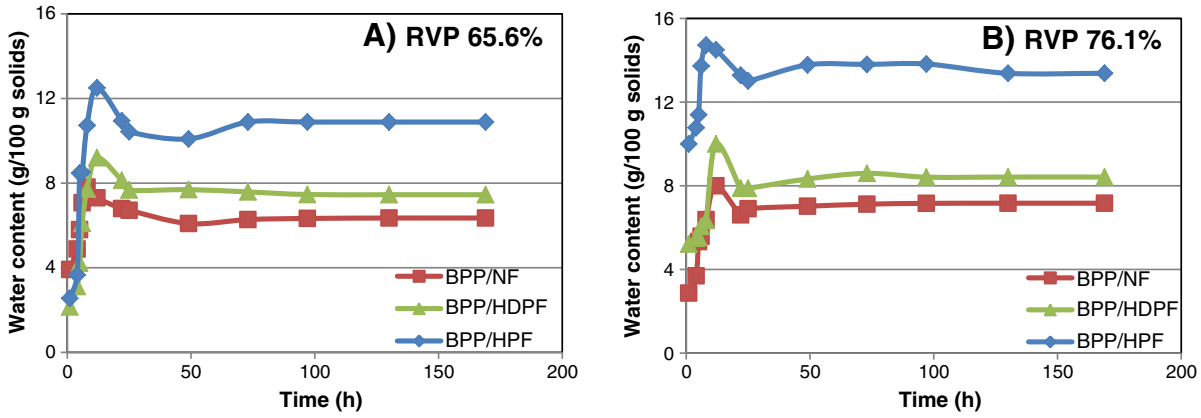

Fig. 7. Water content of freeze-dried bovine plasma protein with fructans as a function of storage time under RVP of 65.6% (A) and 76.1% (B).

wall membrane than that of the control sample. Similar structures were found by Harnkarnsujarit, Charoenrein, and Roos (2012) working with freeze-dried agar gels of maltodextrin (control sample) and maltodextrin-agar with fructose (control sample plus fructose).

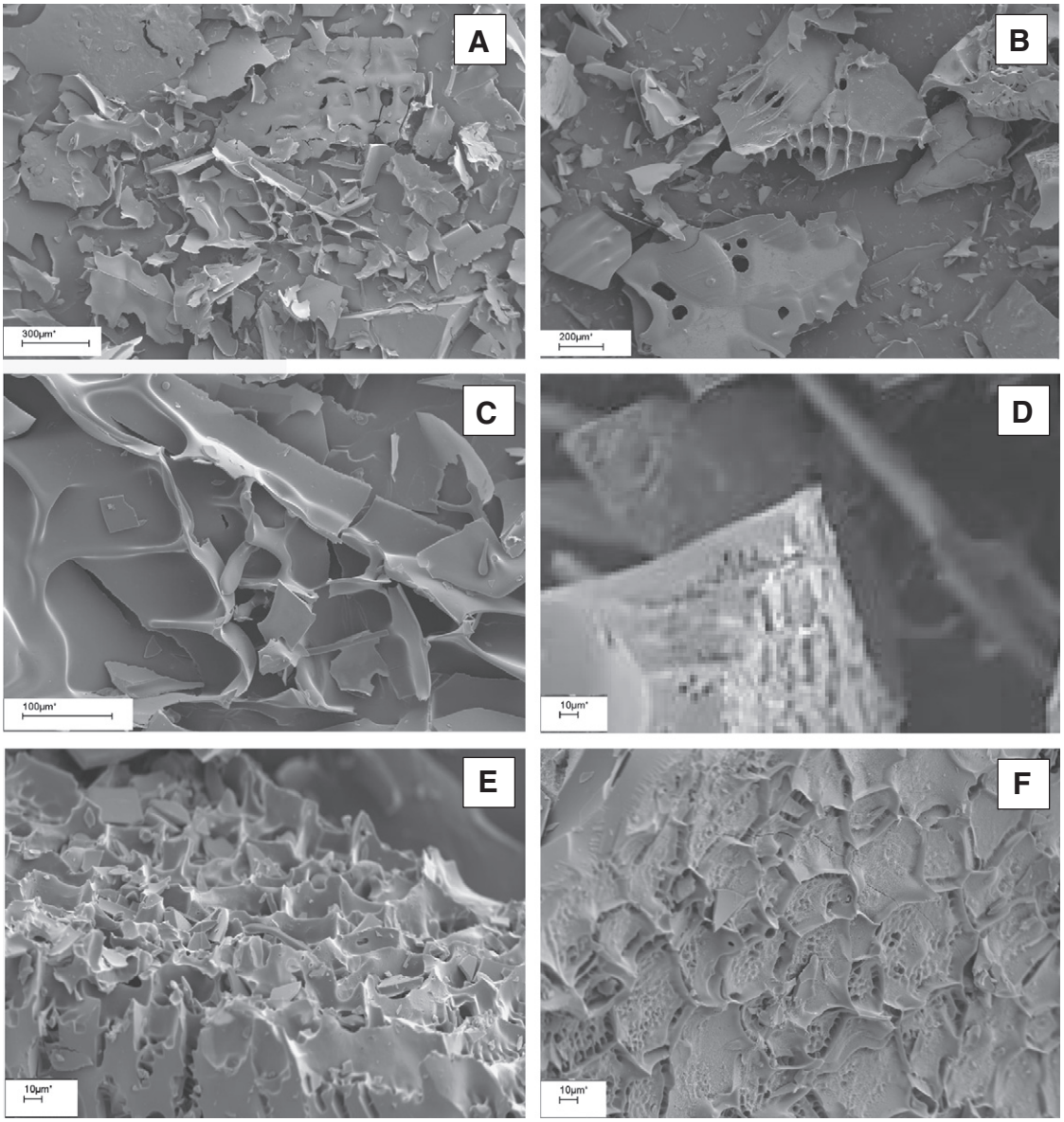

Fig. 8. SEM micrographs of freeze-dried BPP (A) solids with (B) FN frozen at −40 °C prior to freeze-dried (50× magnification). Micrographs of freeze-dried BPP (C) (200× magnification) and with fructans (D–F) (400× magnification).

Accordingly, the composition variations influenced the number of pores and wall thicknesses of the protein–saccharide matrix (Fig. 8). In their studies, Harnkarnsujarit et al. (2012) found that the entrapment of dispersed components could become better protected in thicker walls in a high molecular weight carbohydrate system. This may be because the molecular size affects the amount of unfrozen water during freezing, and the system viscosities could also differ affecting the T_g value, which, in turn, affects the product stability.

Our results show that the pore size is the same for BPP/fructan systems (≈38 μm), but the membrane thicknesses differ according to molecular weight of the saccharide (Fig. 2): NF (4.65 ± 0.08 μm) > HDPF (1.51 ± 0.10 μm) > HPF (0.67 ± 0.09 μm). These results are consistent with those obtained for stability as a function of temperature (kinetic study) and moisture (GAB model).

4. Conclusions

From the obtained results, it was found that tested agave fructans perform a protective effect on bovine plasma proteins during the freeze-dried process, reducing by approximately 65% the denatured protein content to the optimum concentrations (5–10% (w/v) for NF and 10% for HDPF and HPF at a freeze temperature of −40 °C), compared to the control (without the protective agent). Moreover, at higher freeze temperatures (−4 °C), have no statistically significant difference with the optimal values obtained at lower freeze-dried temperature (−40 °C), with HDPF and HPF at 15% (w/v), while with NF a higher content of denatured protein was obtained ($P < 0.005$). This could be due to the higher homogeneity of BPP/HDPF and BPP/HPF. Thus, a freeze-dried product could reach a similar quality to that obtained at −40 °C with a considerable reduction in energy consumption.

The kinetic study of thermal degradation found that bovine plasma proteins protected with agave fructans had a longer lifetime than the control, being the evaluated efficacy: BPP/NF > BPP/HDPF > BPP/HPF.

It was observed that the higher heterogeneity of the samples affected the extent of non-enzymatic browning in the range of the temperatures assessed, resulting in the following order: BPP/NF > BPP/HPF > BPP/HDPF. However, the rates of non-enzymatic browning were fairly slow, suggesting that the samples were forming amorphous matrices with high viscosity. The sample of BPP/NF presented the most color variation (ΔE).

The different functional properties of the plasma proteins with agave fructans remained constant or were improved with respect to the control sample (plasma protein without the protective agent). Thus, a similar solubility to the control was obtained, while emulsifying and foaming capacities were improved in the samples with fructans.

Gelling property of the samples BPP/NF and BPP/HDPF was similar to the control at the concentrations tested. Meanwhile, over the pH range studied, only the BPP/HDPF sample presented a similar behavior to the control, possibly due to a its greater homogeneity.

The m_0 values (moisture content in the monolayer) related to the sample stability, obtained from a nonlinear fitting of GAB model, showed a similar trend to the thermodegradation kinetic study: BPP/NF > BPP/HDPF > BPP/HPF.

The morphology study of the samples revealed the presence of an interconnected network structure of solids and embedded pores. The addition of fructans produced a decrease in the pore size and in the membrane thickness when compared with the control sample. The membrane thickness is a parameter proportional to the system stability, and the results showed the following order of membrane thickness: BPP/NF > BPP/HDPF > BPP/HPF, in accordance with the order of the results previously obtained in the stability studies.

The finding about the agave fructans cryoprotectant role of food proteins is relevant considering that they are considered as soluble fiber from natural and abundant source, categorized as prebiotics. Thus, they become a valuable alternative as a functional ingredient for food formulation.

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