Thermodynamic sorption analysis and glass transition temperature of faba bean (*Vicia faba* L.) protein

E. Alpizar-Reyes¹ · J. Castaño² · H. Carrillo-Navas³ · J. Alvarez-Ramírez³ ·
R. Gallardo-Rivera⁴ · C. Pérez-Alonso¹ · A. Y. Guadarrama-Lezama¹

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Abstract Freeze-dried faba bean (*Vicia faba* L.) protein adsorption isotherms were determined at 25, 35 and 40 °C and fitted with the Guggenheim–Anderson–de Boer model. The pore radius of protein was in the range of 0.87–6.44 nm, so that they were considered as micropores and mesopores. The minimum integral entropy ranged between 4.33 and 4.44 kg H₂O/100 kg d.s., was regarded as the point of maximum of stability. The glass transition temperature of the protein equilibrated at the different conditions of storage was determined, showing that the protein remained in glassy state for all cases. The protein showed compact and rigid structures, evidenced by microscopy analysis.

Keywords Faba bean protein · Pore radius · GAB · Minimum integral entropy · Glassy state

Introduction

Legumes are good resources of proteins, carbohydrates, fiber, vitamins and minerals with functional and health-promoting activities contributing to human nutrition. Faba bean is composed of proteins (~ 30%), carbohydrates (60%) and fats (10%). Among the identified proteins in faba bean, the main are globulins (79%), albumins (7%) and glutelins (6%). The study of faba bean protein is of special interest because of its nutritional value, availability, and health benefits (Vioque et al. 2012). The protein extracted from faba bean can be used within a powder product, so it is important to dry the protein in order to decrease its moisture content until the required level, delay its deterioration and consequently have a hydrocolloid in powder of easy use and application such as emulsifier or stabilizer in different food products (Karaca et al. 2011). Among the different drying processes to this purpose, the freeze drying is often used to dehydrate heat-sensitive foods because of the absence of heating in this process preserving nutrients and their sensory characteristics. Hence, freeze drying is a good alternative to remove free water from proteins (Ghribi et al. 2015).

The preservation and stability of faba bean protein (FBP) involve the knowledge of the appropriate conditions to store it in order to predict deteriorative reactions, and enhance its stability in function of moisture content, water activity and temperature. This task is achieved by relating the above three parameters in the sorption isotherms. Water sorption isotherms provide information about the stability of food powder, and is also helpful to understand the structural features, such as sorption properties and thermodynamic functions of vapor water sorption (Silva et al. 2014).

✉ A. Y. Guadarrama-Lezama
ayguadarramal@uaemex.mx

¹ Facultad de Química, Universidad Autónoma del Estado de México, Paseo Colón esq. Paseo Toluca s/n, Col. Residencial Colón, C.P. 50120 Toluca, Estado de México, Mexico
² Unidad de Desarrollo Tecnológico, Universidad de Concepción, Avenida. Cordillera, C.P 2634 Coronel, Republic of Chile
³ Departamento de Ingeniería de Procesos e Hidráulica, Universidad Autónoma Metropolitana-Iztapalapa, San Rafael Atlixco No. 186, Col. Vicentina, C.P. 09340 Mexico, D.F., Mexico
⁴ Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa, San Rafael Atlixco No. 186, Col. Vicentina, C.P. 09340 Mexico, D.F., Mexico

Thermodynamic functions such as enthalpy and entropy are indices that provide valuable information to understand the properties of water, calculate the energetical requirements associated with the adsorption phenomena, and describe how the water interacts with the powder. Specifically, the minimum integral entropy (ΔS_{int}) is the most suitable criterion to predict the maximum storage stability in powder foodstuffs (Pérez-Alonso et al. 2006). Under minimum integral entropy, the powder food product has the highest stability with the suitable ratio of moisture content/water activity. On the other hand, changes in enthalpy are associated with bonding of repulsive forces between water and the food powder. The process related with enthalpy involves the strength of the intermolecular interaction or bonding, while that related with entropy reflects the randomness and disorder of the system. The glass transition temperature (T_g) has used as parameter for establishing stability conditions in a food powder from a physical or structural point of view (Basu et al. 2013). This parameter allows knowing if the powdered foods exhibit changes in their physical properties during storage such as collapsibility, stickiness, caking, agglomeration and re-crystallization phenomena as effect of its water content.

The aim of this manuscript was determined sorption thermodynamic properties and glass transition temperature of faba bean (*Vicia faba* L.) protein to establish optimal conditions of storage.

Materials and methods

Faba bean (*Vicia faba* L.) seeds were purchased in a local market (Toluca, Mexico). All reagents were analytical grade and were purchased from Sigma Aldrich S.A. de C.V. (Toluca, Mexico). Deionized water was used in all the experiments.

Protein extraction

Faba bean (*Vicia faba* L.) seeds were sun-dried until reached to 5% wt. and crushed with a mill (Pulvex 200, Mexico City, Mexico). Faba bean seed flour was sieved using a 70 mesh (Tyler Standard Sieve Series, Mentor, Ohio, USA). Extraction of FBP was carried out according to Vioque et al. (2012). Flour was defatted with hexane using soxhlet equipment. Hexane was evaporated, before carrying out protein extraction. Defatted flour (10 g) was added to 100 mL of Na_2SO_3 solution (0.25% w/v) and pH was adjusted to 10.5 with NaOH solution. The solution was kept under stirring 24 h at 4 °C. Further coarse alkaline solids were separated by centrifugation (5415R Eppendorf AG, Hamburg, Germany) at $15,000\times g$ during 10 min. The soluble fraction was transferred to a beaker and pH

adjusted to 4.0 (isoelectric pH of the FBP). The resulting solution was centrifuged again at $15,000\times g$ by 10 min. FBP was washed twice with deionized water and stored for subsequent freeze drying process.

Freeze drying process

Freeze drying was carried out using a laboratory freeze dryer (Freezone 6, Labconco, MO, USA). The solution of FBP was poured into a glass recipient to form a layer of 15 mm. The samples were placed at -40 °C for 24 h before transferring to the freeze dryer using a Labconco Bench Lyph-lock 6 laboratory freeze-dryer (Labconco, MO, USA). The vacuum pressure of the dryer was set at 5 mmHg, and the condenser was at -60 °C for 48 h. Dehydrated product was stored in desiccators above P_2O_5 , in order to prevent any increase in adsorbed moisture, until being required for experiments.

Sorption isotherms

Adsorption isotherms at 25, 35 and 40 °C (± 0.1 °C) were determined through the static gravimetric described by Lang et al. (1981) using eight saturated salt solutions (lithium chloride, potassium acetate, magnesium chloride, potassium carbonate, magnesium nitrate, sodium nitrite, sodium chloride and potassium chloride) with water activities (a_w) ranging from 0.11 to 0.85 (Mousa et al. 2014). Samples of approximately 0.5 g of freeze-dried powder faba bean protein (FD-FBP) were placed into small glass desiccators of 10 cm diameter on filter paper (Whatman No. 1) that was placed above the saturated salt solutions in a perforated plate used as a support for the samples to allow moisture transmission. Five desiccators with each sample of FD-FBP were placed into three forced convection drying ovens (Riossa, model E-51, Mexico City, Mexico) maintained the specified temperature until equilibrium was reached (between 20 and 25 days). Equilibrium was assumed when the difference between two consecutive measurements was less than 1 mg/g of solids. To prevent microbial spoilage of samples, 0.2 mL of toluene was placed in the desiccators where high water activities occurred ($a_w > 0.5$). Moisture content of the freeze-dried protein was determined by difference in weight after drying in a vacuum drying (Felisa model FE 100, Mexico City, Mexico) at 60 °C for 24 h in the presence of magnesium perchlorate desiccant. The water activity was measured with an Aqualab water activity meter with temperature compensation (model series 3 TE, Decagon Devices, Inc., Pullman, WA, USA). Longer drying times did not produce a sample weight decrease of more than 0.1 mg. The GAB (Guggenheim-Anderson-De Boer) equation is mathematically expressed as (Eq. 1):

$$M = \frac{M_0 C K a_w}{(1 - K a_w)(1 - K a_w + C K a_w)} \quad (1)$$

where M is the equilibrium moisture content [kg/100 kg of dry solids (d.s.)], M_0 is the moisture content of the monolayer (kg/100 kg d.s.), C and K are constants related to the energies of interaction between the first and distant adsorbed molecules at the individual sorption sites. The parameters were estimated by fitting the mathematical model to the experimental data, using non-linear regression with Origin Pro version 8.5 Scientific Software (Origin Lab Corp., Northampton, MA, USA). The mean relative deviation modulus (MRD) was used to evaluate the goodness of fit. The MRD value is given as a percentage and may be estimated as follows

$$MRD = \frac{100}{n} \sum \frac{|M_i - M_{Ei}|}{M_i} \quad (2)$$

where M_i refers to the experimental value of moisture content at i , M_{Ei} is the predicted moisture content at that observation and N is the number of observations. It is generally assumed that a good fit is obtained when $MRD < 5\%$.

Sorption properties

The sorption surface area (S_0) was evaluated using the following equation (Moraes and Pinto 2012):

$$S_0 = M_0 \frac{1}{M_w} N_0 A_{H_2O} = 3.5 \times 10^3 M_0 \quad (3)$$

where M_w is the molecular weight of water (kg/mol), N_0 is the Avogadro number (6.0×10^{23} molecules/mol) and A_{H_2O} is the area of a water molecule (1.06×10^{-19} m²). The moisture content of the monolayer to calculate the area was obtained from the GAB model.

The critical radius was calculated using Kelvin equation:

$$r_c = \frac{2\sigma V_M}{RT \ln(a_w)} \quad (4)$$

where r_c is the critical radius (m), σ is the surface tension (N/m), V_M is the molar volume of sorbate (m³/mol), R is the universal gas constant (8.314×10^{-3} kJ/mol K), T is the temperature (K) and a_w is the water activity. This equation applies primarily in the condensation region of the isotherm (Moraes and Pinto 2012). On the other hand, the Halsey equation:

$$t = 0.354 \left(\frac{-5}{\ln a_w} \right)^{1/3} \quad (5)$$

where t is the thickness of the adsorbed water multilayer (nm), was used to evaluate the thickness of the adsorbed water multilayer (Singh et al. 2001). Finally, pore radius

(r_p) is the sum of the critical radius when the capillary condensation or evaporation occurs (r_c) and the multilayer thickness (t):

$$r_p = r_c + t \quad (6)$$

Thermodynamic functions

The integral (enthalpy and entropy) as thermodynamic functions were determined as indicated by Velázquez-Gutiérrez et al. (2015); these authors have provided a thorough description of the following procedures and equations used for this purpose. The pressure of diffusion (Φ) can be determined by Nunes and Rotstein (1991):

$$\Phi = \mu_{ap} - \mu_a = RT \frac{W_{ap}}{W_v} \int_0^{a_w} M d \ln a_w \quad (7)$$

$$\Phi = \alpha_1 T \int_0^{a_w} M d \ln a_w \quad (8)$$

where μ_{ap} is the chemical potential of the pure adsorbent; μ_a is the chemical potential of the adsorbent in the condensed phase; W_{ap} is adsorbent molecular weight; W_v is the water molecular weight; Φ/α_1 constant is similar to a process at constant Φ . Equation (8) was stepwise evaluated. For $a_w < 0.05$, the computed values of the constant pressure of diffusion at any temperature were determined assuming a linear relationship (Henry's law) (Nunes and Rotstein 1991):

$$M = k_w a_w \quad (9)$$

where k_w is the slope of the Eq. (9). When $a_w > 0.05$, the GAB model [Eq. (1)] was used. Therefore, Eqs. (1) and (9) are substituted in Eq. (8) to determine Φ/α_1 . This parameter was calculated with the Runge–Kutta method using MatLab 2013b Software (Math Works Inc., Natick, MA, USA).

The integral enthalpy was calculated using the Clausius–Clapeyron equation at constant diffusion pressure or surface potential (Φ) (Nunes and Rotstein 1991):

$$\left(\frac{\partial \ln a_w}{\partial (1/T)} \right)_\Phi = \frac{H_s - H_l}{R} = \frac{\Delta H_{int}}{R} \quad (10)$$

where H_s is the integral molar enthalpy of water adsorbed of the mucilage (kJ/mol), H_l is the partial molar enthalpy of adsorbed water at constant temperature and pressure (kJ/mol), R is universal gas constant (8.314×10^{-3} kJ/mol K) and ΔH_{int} is the integral enthalpy at a constant temperature (kJ/mol). A plot in the form $\ln a_w$ vs $1/T$, at a specific constant pressure of diffusion, ΔH_{int} is determined from the slope $\Delta H_{int}/R$.

The use of the Clausius–Clapeyron equation implies that the moisture content of the system under consideration remains constant and that the enthalpy of vaporization of pure water (as well as the excess heat of sorption) does not change with temperature (Rizvi 1986).

The integral enthalpy is needed to determine the integral entropy associated with the sorption process. The integral entropy can be calculated as follows:

$$\Delta S_{\text{int}} = S_s - S_l = \frac{\Delta H_{\text{int}}}{T} - R \ln a_w \quad (11)$$

where $S_s = S/N_1$ is the integral entropy of water adsorbed in the protein; S_l is the partial molar entropy of adsorbed water at constant temperature and pressure (kJ/mol) and ΔS_{int} is the integral entropy at a constant temperature.

Glass transition temperature

A differential scanning calorimetry (Q1000, TA-Instruments, New Castle, DE, USA) equipped with a mechanical cooling system (RCS-refrigerated cooling accessory) was used to perform experiments. The calorimeter was calibrated according to the instructions provided by TA instruments user manual by checking temperature and enthalpy of fusion of indium and sapphire as standards. An empty aluminum hermetic pan was used as a reference. The instrument was purged with nitrogen at a flow rate of 100 cm³/min. Freeze-dried faba bean protein samples (4–5 mg) equilibrated at different water activities were in the range of the 0.11–0.85 and temperatures (25, 35 and 40 °C) were placed in aluminum hermetic pans and were cooled to −50 °C at 2.5°C/min, and equilibrated for 2 min. After equilibrated it was scanned from −50 to 150 °C at a rate of 2.5°C/min. Each thermogram was analyzed for the glass transition temperature (T_g), and midpoint values were used. The data were analyzed using Universal Analysis 2000 software, version 4.7a (TA Instruments, New Castle, USA).

Scanning Electron Microscopy

Scanning Electron Microscope (JSM-7600F, Jeol Co. Ltd., Tokyo, Japan) of high vacuum with the GB-H mode at 1 kV accelerating voltage, was used to investigate the microstructural properties of FBP samples previously kept under controlled atmospheres such as described in “Thermodynamic functions” section. The samples were mounted on carbon sample holders using double-side sticky tape. Micrographs at 2000× magnification are presented.

Results and discussion

Sorption isotherms

The experimental sorption isotherms at 25, 35 and 40 °C of FBP are shown in Fig. 1. The amount of sorbed water increased as temperature increased, at a constant value of water activity. This could be explained by the degree of exposure of hydrophobic regions of protein. It is possible that when the protein is elongated, the water adsorption on the specific sites is being promoted and, when the protein is short, the water adsorption on the specific sites becomes more difficult. Therefore, when temperature increases, the degree of exposure of hydrophobic regions also increases. It improves the amount of water strongly adsorbed to specific sites. According to Brunauer’s classification (Brunauer et al. 1938), FBP isotherms had typical type II sigmoid shape. This type of isotherms was observed for vegetal and animal derived proteins as cottonseed protein isolate (Tunç and Duman 2007), soy protein (Cassini et al. 2006), acid casein from buffalo skim milk (Sawhney et al. 2011). The experimental sorption data were fitted to GAB model. The model parameters M_0 , C and K were determined by non-linear regression procedure (Table 1). The mean relative deviation modulus (MRD) values were less than 2% and the coefficients of determination (R^2) were over 0.998 for all temperatures. The value of the monolayer (M_0) is of particular interest. It shows the amount of water that is strongly adsorbed to specific sites and is considered as the optimum value at which a food is more stable. The values of the monolayer of FBP were in the range of 4.32–5.52 kg H₂O/100 kg d.s. and decreased as temperature increased from 25 to 40 °C. These results can be attributed to reductions in the number of available sites for

Fig. 1 Sorption isotherms of FD-FBP

Table 1 Estimated GAB parameters of FD-FBP

T (°C)	M_0 (kg H ₂ O/100 kg of dry solids)	C	K	R^2	MRD (%)
25	5.52 ± 0.18^b	9.300 ± 0.111^b	0.714 ± 0.012^a	0.999	1.65
35	4.55 ± 0.15^a	6.320 ± 0.178^a	0.742 ± 0.019^a	0.999	1.82
40	4.32 ± 0.13^a	5.989 ± 0.234^a	0.723 ± 0.021^a	0.998	1.59

Values are mean $\pm$ standard error, of three replicates. Superscripts with different letters in same column indicate significant differences ($P \leq 0.05$)

water binding due to excitation state of molecules, an increase in kinetic energy leading to an increase in the distance between them. Therefore, water molecules with a low motion at low temperatures bound more easily to suitable binding sites on surfaces (McLaughlin and Magee 1998). The water adsorption in other proteins extracted from legumes such as soy protein and cowpea, has been reported in the literature, where the monolayer moisture content values has been found in a range between 1.39 and 7.4 kg H₂O/100 kg d.s. (Cassini et al. 2006; Ayranci and Duman 2005). These values are in the range where the monolayer moisture content is found for the FBP (Table 1). There is not a clear tendency in M_0 values depending on the chemical nature of the protein (vegetal or animal) and its chemical structure (globular, fibrillary and others). It has been stated that the amount of sorbed water in proteins depends essentially on the number and availability of two kinds of hydrophilic groups that enhance the binding water through hydrogen bond formation. These are the polar side chains and the carbonyl and amid groups of peptide bonds. Water sorption by proteins occurs into polar side chains at low humidities, spreads to peptide linkages and then leads to multilayer formation at higher humidities (Singh et al. 2001). On the other hand, it can be seen that the maximum moisture content reached for the FD-FBP was about 12 kg H₂O/100 kg d.s., while for others hydrocolloids and biopolymers the moisture contents values are higher, in the same range of studied water activities (Włodarczyk-Stasiak and Jamroz 2008). This may be due to the hydrophobic groups in protein structure, which did not favor the adsorption of moisture in the surface of the hydrocolloid. The parameter C obtained from the GAB model was related to the heat of adsorption of water by the FD-FBP. It was assumed that strong adsorbent-adsorbate interactions were favored at lower temperatures, resulting in an increase in C with increasing temperature (Diosady et al. 1996). In this work the value of C decreased with increasing temperature suggesting that interactions between the freeze-dried FBP and water vapor were lower at 25 °C than at 35 and 40 °C. Alternatively, it is possible that C lacks any physical meaning being the result of mathematical compensation among parameters during the curve-fitting process.

The values of K involve interactions between water molecules and the adsorbent (protein) in the multilayer. $K \ll 1$ indicates a structured state of the adsorbate in the adjacent layers to the monolayer (Lewicki 1997). In this study, K values were in range of 0.71–0.74 for the FD-FBP, which implies that there were fewer interactions between water molecules and the protein in the multilayer, and a tendency was not found as the temperatures increase or decrease.

Sorption properties

The sorption properties are physical properties described as pore radius, critical radius pore, surface area of sorption and multilayer thickness, which determine the water adsorption rate on solids. They have been studied using the GAB model. The rate and extent of hydration of the food materials are decided by the surface properties of pores. Furthermore, the temperature at each pore is likely to affect the rate of entry and exit of water molecules (Kapsalis 1981). The specific surface area values for the FBP were 193.30, 159.41, and 151.29 m²/g for 25, 35 and 40 °C, respectively. The sorption surface area values decrease as temperature increases. This behavior is attributed to the availability of active sites for hydrophilic binding energies, which decreased as consequence of induced changes by temperature, modifying physical and chemical properties of the adsorbent (Rakshit et al. 2014). Labuza (1968) indicated that S_0 values of food products are within the range of 100–250 m²/g. Some authors reported similar values of S_0 for vegetable proteins. For instance, Tunç and Duman (2007) reported values between 115.3 and 137.4 m²/g at 15 and 45 °C for cottonseed protein isolate. Moreover Cassini et al. (2006) analyzed texturized soy protein finding values between 162.75 and 260.05 m²/g at 10 and 40 °C.

Pore radius (r_p) of FBP was determined at different moisture contents and temperatures (Table 2). The pore radius increased as moisture content and temperature increased and ranged in values between 0.75 and 6.68 nm. In agreement with the International Union of Pure and Applied Chemistry (IUPAC) pore radius less than 2 nm are classified as micropores, and pore radius between 2 and 50 nm are denoted as mesopores (Moraes and Pinto 2012).

Table 2 Pore radius of FD-FBP at different moisture contents and temperatures

Pore radius (nm)			
<i>M</i> (kg H ₂ O/100 kg of dry solids)	25°C	35°C	40°C
1	0.75 ± 0.05 ^{a,x}	1.02 ± 0.03 ^{a,y}	0.78 ± 0.02 ^{a,x}
2	0.85 ± 0.06 ^{a,b,x}	0.92 ± 0.04 ^{a,x,y}	0.98 ± 0.05 ^{a,b,y}
3	0.99 ± 0.01 ^{b,c,x}	1.10 ± 0.07 ^{a,b,x,y}	1.23 ± 0.07 ^{b,c,y}
4	1.18 ± 0.04 ^{c,x}	1.40 ± 0.06 ^{b,y}	1.55 ± 0.09 ^{c,y}
5	1.43 ± 0.05 ^{d,x}	1.76 ± 0.11 ^{c,y}	1.96 ± 0.10 ^{d,y}
6	1.74 ± 0.08 ^{e,x}	2.17 ± 0.09 ^{d,y}	2.47 ± 0.11 ^{e,z}
7	2.12 ± 0.11 ^{f,x}	2.66 ± 0.17 ^{e,y}	3.12 ± 0.14 ^{f,z}
8	2.58 ± 0.14 ^{g,x}	3.29 ± 0.18 ^{f,y}	3.97 ± 0.19 ^{g,z}
9	3.14 ± 0.11 ^{h,x}	4.13 ± 0.15 ^{g,y}	5.10 ± 0.17 ^{h,z}
10	3.84 ± 0.08 ^{i,x}	5.19 ± 0.16 ^{h,y}	6.68 ± 0.20 ^{i,z}

Values are mean ± standard error, of three replicates. Superscripts with different letters in same column and line indicate significant differences ($P \leq 0.05$)

Therefore, at moisture contents between 1 and 6 kg H₂O/100 kg d.s., the FBP presented micropores and when the moisture content is ≥ 6 kg H₂O/100 kg d.s. mesopores were observed. The diffusion mechanisms depend on the properties within the local structure of the porous matrix. In micropores, diffusion is dominated by interactions between the water molecules and the pore walls, i.e., steric, and other effects associated with the proximity of the pore walls (entropic effects) become important, and barriers control the process. In mesopores, surface forces and capillary forces become important, whereas for the macropores, very little is contributed by the pore characteristics to the adsorption capacity (Viganó et al. 2012). According to this description, the adsorption mechanism is controlled by diffusion and capillarity phenomena in the FBP.

Thermodynamic functions

Integral enthalpy ΔH_{int} is the parameter that indicates the degree of water–solid interaction. The net equilibrium heat for the FBP was determined. It increased to maximum value of 31 kJ/mol at 3.5 kg H₂O/100 kg d.s. of moisture content. At this point the strongest binding sites and the greatest water–protein powder interaction occurs. From this point, integral enthalpy gradually decreased as the moisture content increased. An increase on the moisture content indicates the covering of fewer favorable sites promoting the formation of multi-layers for which the enthalpy is reduced. Besides, low enthalpies values (less than 60 kJ/mol) are associated with physical sorption (Smith et al. 2005). In this work, the integral enthalpy values in the range of moisture content studied were < 60 kJ/mol and therefore it can be assumed that a physisorption phenomenon is occurring.

The integral entropy is a thermodynamic function used to describe the degree of arrangement of water molecules during the sorption process. The ΔS_{int} is considered as the maximum stability point. It is here where water molecules have more ordered arrangement with the solid (FBP) and strong bonds between the adsorbate and the adsorbent occur. Thus, water is less available to carry out spoilage reactions (Bonilla et al. 2010; Viganó et al. 2012). Figure 2 shows the integral entropy as a function of moisture content at 25, 35 and 40 °C of FBP. The integral entropy decreased until reaching a minimum point and then increased as moisture content increased. The decrease in integral entropy represents a diminution on mobility of water molecules promoting the saturation of available sites. Higher energy is required to continue the adsorption phenomena. Thus the increase implies that water molecules are able to form multilayers. At higher moisture contents,

Fig. 2 Integral entropy at 25, 35 and 40 °C of FD-FBP

entropy will be approximately the same as liquid water (McMinn and Magee 2003). The conditions for maximum stability of FBP were 4.33 kg H₂O/100 kg d.s. ($a_w = 0.40$) at 25 °C, 4.37 kg H₂O/100 kg d.s. ($a_w = 0.36$) at 35 °C, and 4.44 kg H₂O/100 kg d.s. ($a_w = 0.24$) at 40 °C, as it can be observed in Fig. 2. As temperature increased, the moisture content and water activity in the FBP increased. Besides, the integral entropy can be directly related to the order–disorder of water molecules sorbed on protein and, therefore, it is a useful function on the stability food.

Glass transition temperature (T_g) and morphology

Moisture content, water activity (a_w) and glass transition temperature (T_g) are parameters used in order to predict the stability of a food during processing and storage, because its physical state is affected by these factors. The glass transition temperature (T_g) of the FBP at different water activities is shown in Fig. 3. It can be observed that T_g decreases as water activity increases due mainly to the plasticization effect of the water. Also it can be appreciated that at the same level of water activity, the T_g is the highest at the temperature of 40 °C of storage. The T_g values ranged from 9.78 to 47.10 °C.

Values of T_g for FBP were lower than those reported from other proteins. Protein isolated from barley with T_g values from 48 to 63 °C at a moisture content of 0.12% (van Donkelaar et al. 2015), gelatin with T_g of 100 °C in anhydride conditions whey protein isolate (WPI, with water content of 6–16%), and whey protein hydrolysates (WPH, with water content of 50–26%) with a T_g between 119–75 °C, and 99–15 °C, respectively and soy, casein and gluten protein (with moisture contents less than 30%) with T_g 's ranged approximately from 50 to 105, 20 to 105 and

10 to 140 °C, respectively (Bengoechea et al. 2007). It is possible that the variation of T_g among proteins may be due to the nature of them, such as source (vegetal or animal, or modification method such as hydrolysis, unfolding or denaturation). In addition, the values of glass transition temperature for the different kind of proteins also may be due to the existence of hydrophilic-hydrophobic sites in its molecular arrange, total apparent charge, the presence of sulfhydryl groups and disulphites-sulfhydryl bonds (Caurie 1981).

SEM micrographs of FBP at 25 °C and different water activities are shown in Fig. 4. The micrographs of the FBP showed that not structural changes were noticeable, all the samples showed dense, continues, compact and rigid structures regardless of their water activity. Thus, it can be inferred that the protein was in glassy state even at high water activities. SEM micrographs of the protein equilibrated at 35 and 40 °C, at a_w 's of 0.2, 0.4 and 0.6 respectively, showed a similar trend (not shown here). Therefore, FBP appeared to be stable in the range of tested temperature (25, 35 and 40 °C) even at high water activity values, and there was not evidence of stickiness, caking or collapsing. Within literature, it has been reported that different drying methods (freeze drying and convective drying) used for the preparation of protein concentrates in powders, influence its thermal and functional properties (Ghribi et al. 2015). It seems to be that the drying method by which the protein concentrates are obtained, has influence on the microstructure and the changes that it presents during storage. Other hydrocolloids obtained by freeze-drying have remained in glassy state in the water activity range of 0.11–0.85 such as the chia mucilage (Velázquez-Gutiérrez et al. 2015; Capitani et al. 2013), while those obtained by spray drying have presented coalescence, agglomeration, stickiness and collapsing phenomena, such as maltodextrin and skim milk (Malafronte et al. 2016).

Fig. 3 Glass transition temperature as function of water activity of FD-FBP at 25, 35 and 40 °C

Conclusion

Sorption isotherms for FBP presented a sigmoidal shape. Experimental data of moisture content and water activity of the FBP were well adjusted to the GAB model. The pore radius of the freeze-dried FBP presented increasing values as moisture content and temperature increased so that they were considered as micropores and mesopores, which have an effect in the adsorption mechanism. Not only micropores but also mesopores are able to allow the physisorption mechanism and form the monolayer. The minimum of integral entropy was found at low water activities (between 0.24 and 0.40) for the temperature range between 25 and 40 °C. It means that at these water activities there exist the most suitable conditions for storing the FBP. FBP showed

Fig. 4 Morphology of FD-FBP at **a** 0.2, **b** 0.4 and **c** 0.6 of water activity and 25 °C

dense, continuous, compact and rigid structures, and was inferred that it remained in glassy state in the range of water activity at which the protein was equilibrated. FBP

properties suggest that it could be stored under conditions of temperature, moisture content and water activity determined in this study and used as additive in powder as possible emulsifier or stabilizers.

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Compliance with ethical standards

Conflict of interest No conflict of interest is declared by the authors.

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