Proton mobility for the description of dynamic aspects of freeze-dried fruits

Lina M. Agudelo-Laverde, Carolina Schebor, María del Pilar Buera *

Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (FCEyN-UBA), Buenos Aires, Argentina

ABSTRACT

The partitioning of water sorption isotherms into different zones, according to the strength of water interactions with solids has very important practical applications. However, the dynamic properties of water play an important role in complementing the information provided by water sorption isotherms. One of the most successful techniques used to prove the dynamic behavior of water in foods systems is pulsed NMR. The aim of this study was to apply the concept of proton mobility in order to better define the water-related dynamic aspects of freeze-dried fruits. Different water mobility populations were defined through ^{1}H NMR transversal relaxation times, obtained after the application of several pulses sequences. The water content limits at which proton populations with different mobility appeared, allowed a more complete and precise description of water behavior at the different sorption stages than the parameters obtained by the application of sorption mathematical models.

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1. Introduction

Water presence and its interactions with other food compounds are important in defining process variables, stability and food quality aspects. The water sorption behavior in fruits is affected by many variables, such as sugar content, ripening, process and storage conditions (Bolin, 1980; Kaya et al., 2002).

Mathematical interpretation of water sorption isotherms has received much attention due to its relationship with food shelf life (Labuza et al., 1970; Labuza, 1980; Simatos and Karel, 1988). Brunauer–Emmett–Teller (BET) (Brunauer et al., 1938) and Guggenheim–Anderson–de Boer (GAB) (Van Den Berg and Bruin, 1981) are models extensively used. BET model applicability is limited to relative vapor pressure (*RVP*) range between 0.1 and 0.5 (Labuza, 1968) while GAB sorption equation has a wider *RVP* ranging from 0.1 up to around 0.9 (Timmermann, 2003).

Some works on the advantage of the generalized D'Arcy & Watt equation (GDW) (D'Arcy and Watt, 1970) application in foods have been published (Furmaniak et al., 2007a,b, 2009). GDW maintains all the considerations of the GAB model, but also assumes that only a proportion of water molecules bound to primary centers of adsorption can act as secondary centers. Also, in some cases one primary center can absorb more than one water molecule (Furmaniak et al., 2009).

The sorption isotherms have been used for stability estimations, and their partitioning into different zones according to the *RVP*

value, as proposed by Labuza and coworkers in 1970, had very important practical applications, and has been referenced and reproduced thousands of times (Schmidt, 2004). The limits between the different zones are, however, difficult to define only with the water sorption data.

It has been recognized that the molecular mobility of water and solids is related to kinetic aspects and its analysis has broad applications in food stability predictions (Le Meste et al., 1991). These studies include different materials as starch (Richardson et al., 1987), sucrose and starch systems (Kou et al., 1999), milk protein concentrate (Haque et al., 2010), apples (Hills and Remigereau, 1997), and pintado fish (Pitombo and Lima, 2003). Thus, the dynamic properties play an important role in complementing the information provided by water sorption isotherms and can be employed for establishing the limits between the different sorption zones.

One of the most successful techniques used to prove the dynamic behavior of water in food systems is pulsed NMR (Le Meste et al., 1991; Hills, 1999; Haque et al., 2010). A relationship between NMR relaxation times and *RVP* can thus allow to have a complete scheme of the water behavior in foods (Schmidt, 2004). The limits of the different water sorption stages have been empirically described but are not well defined. Even more, the references to "bound", "monolayer", "multilayer" and "free" water for indicating the different degree and strength of water–solid interactions are questioned (Fennema, 1999). The definition of zones according to proton mobility could give a more appropriated description. Nuclear magnetic resonance (NMR) technique is employed to characterize the mobility of water and solids in food systems (Schmidt,

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* Corresponding author. Tel./fax: +54 11 4576 3366.
  E-mail address: pilar@di.fcen.uba.ar (M.d.P. Buera).

2004) and could serve as a reference replacing or complementing the water sorption isotherm concept.

The aim of this study was to apply the concept of proton mobility in order to better define the water-related dynamic aspects of freeze-dried fruits.

2. Materials and methods

2.1. Fruits

Fully ripe fresh apple (Granny Smith), melon (Honeydew) and pear (Packam's Triumph) were obtained from the local market and stored at 4 °C prior to experimentation. The fruits were washed, peeled and were cut transversally into cylinders (2.5 cm diameter and 0.5 cm thickness). The cut material was immediately frozen with liquid nitrogen and stored at −20 °C.

2.2. Materials preparation

Previously frozen fruit cylinders were taken from the −20 °C storage were covered with liquid nitrogen before freeze-drying. A freeze-drier (ALPHA 1–4 LD2 Martin Christ Gefriertrocknungsanlagen GMB, Germany) was used. The freeze-drier was operated at −55 °C, at a chamber pressure of 4 Pa, and the process lasted 48 h. After freeze-drying, samples were humidified at 25 °C for 14 days over saturated salt solutions in vacuum desiccators. Saturated salt solutions of lithium chloride (LiCl), potassium acetate (CH_3CO_2K), magnesium chloride ($MgCl_2$), potassium carbonate (K_2CO_3), magnesium nitrate ($MgNO_3$), sodium chloride (NaCl), and potassium chloride (KCl) were used for 0.11, 0.22, 0.33, 0.43, 0.52, 0.75 and 0.84 RVP, respectively (Greenspan, 1977). The selected equilibration time was based on several experiments in different fruits, and the equilibrium was confirmed at the end of the humidification time by measuring the a_w values of the samples using a dew-point water activity meter Aqualab Series 3 (Decagon Devices, Pullman, WA, USA).

2.3. Sugar content

Approximately 1 g of freeze-dried fruit was suspended in 10 mL of ethanol (80%) by mechanical stirring. The resulting suspensions were maintained at 4 °C overnight. Sugar analysis was performed by HPLC (Kontron Eching Germany) isocratically, employing a Thermo Hypersil Amino column (5 μm, 250 mm × 4.6 mm) and a refractive index detector. Acetonitrile:water mixture (70:30) was employed as the mobile phase. An average value of two replicates was reported along with the standard deviation.

2.4. Water content

The water content (wc) of the previously humidified fruits was determined gravimetrically by difference in weight before and after vacuum drying over magnesium perchlorate at 80 °C for 48 h (Agudelo-Laverde et al., 2011), and it was expressed as g H_2O/100 g solids. An average value of at least two replicates was reported along with the standard deviation.

2.5. Mathematic models

The mathematical form of the GAB model is:

$$M = \frac{m_o CKRVP}{(1 - KRVP)(1 - KRVP + CKRVP)}$$

where m_o is the monolayer value (hydration water content), C is the kinetic constant related to the sorption in the first layer, K is the kinetic constant related to multilayer sorption. M is the water content at each RVP.

The mathematical form of the GDW model is:

$$M = \frac{M_e KRVP}{(1 + KRVP)} \times \frac{1 - k(1 - w)RVP}{1 - kRVP}$$

where M_e is the maximum sorption value on primary centers, K and k are the kinetic constants related with sorption on primary and secondary centers, and w is the parameter that determines the ratio of molecules bonded to primary centers and converted into the secondary ones. M is the water content at each RVP.

The experimental values were mathematically described by GAB and GDW equations by nonlinear regression analysis minimizing the root mean square deviation, through GraphPad Prism 5 software.

2.6. Thermal transitions

Glass transitions were determined by differential scanning calorimetry (DSC; onset values) using a DSC 822e Mettler Toledo calorimeter (Schwerzenbach, Switzerland). The instrument was calibrated with indium (156.6 °C), lead (327.5 °C), zinc (419.6 °C), and water (0 °C). All measurements were performed at a heating rate of 10 °C/min. Hermetically sealed 40 mL medium pressure aluminum pans were used, (an empty pan served as a reference). Thermograms were evaluated using Mettler Stare program.

2.7. Proton mobility

Transversal or spin–spin relaxation times (T_2) were measured by time resolved proton nuclear magnetic resonance (^{1}H NMR) in a Bruker Minispec mq20 (Bruker Biospin Gmbh, Rheinstetten, Germany) with a 0.47 T magnetic field operating at a resonance frequency of 20 MHz. Humidified fruits were removed from the desiccators placed into 10 mm diameter glass tubes and returned to the desiccators for additional 24 h prior to analysis. All determinations were performed in triplicate at 25 °C. The average and standard deviation are reported.

Proton populations of different mobility were measured using the following three methods.

2.7.1. Measurements using a single pulse method

The spin–spin relaxation time (T_2) associated with the fast relaxing protons (related to the protons of the solid matrix and to the water molecules interacting tightly with solids) was measured using a free induction decay analysis (FID) after a single 90° pulse (Schmidt, 2004). The following settings were used: scans = 4, recycle delay = 2 s, gain = 68 dB and number of points = 100. The decay envelopes were fitted to mono-exponential behavior with the following equation:

$$I = A \exp(-t/T_{2-FID})$$

where I represents the protons' signal intensity, $T_{2\text{-FID}}$ corresponds to the relaxation time of protons in the polymeric chains of the material and of tightly bound water and A is a constant. Since no 180° refocus pulse was used in the experiments, the spin–spin relaxation time constants are apparent relaxation time constants, i.e., T^*_{2-FID}. However for solid materials (like ours), we can consider that the intrinsic $T_{2\text{-FID}}$ is very close to the T^*_{2-FID} as reported previously by Fullerton and Cameron (1988). Therefore, $T_{2\text{-FID}}$ was used for convenience. Dehydrated fruits humidified between 0.11 and 0.84 RVP were analyzed using this method. The device was calibrated using three certified standards composed of plastics in oil, provided by Bruker.