Food Hydrocolloids 60 (2016) 85–97

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Crystallization and structural relaxation times in structural strength analysis of amorphous sugar/whey protein systems

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ARTICLE INFO

Article history:
Received 10 January 2016
Received in revised form
9 March 2016
Accepted 10 March 2016
Available online 15 March 2016

Keywords:
Crystallization
X-ray diffraction
Structural relaxations
Strength parameter
WLF model
Water

ABSTRACT

Water sorption, crystallization, calorimetric glass transition temperature (T_g), structural relaxations and relaxation times (τ) of freeze-dried sugars (lactose and trehalose) and whey protein isolates (WPI) in mixed systems were measured at various water activities ($a_w \leq 0.76$, and 25 °C). The results indicated that sugar/WPI mixtures showed fractional water sorption behavior. The crystallization and crystalline forms of lactose were affected by trehalose and WPI based on XRD analysis. DMA analysis showed that the α-relaxation temperatures (T_α) at loss modulus peak at frequency of 0.5 Hz for sugar/protein systems were affected by the presence of water and WPI. The τ and $T_\alpha - T_g$ relationships in sugar/WPI systems were successfully modeled by using the WLF model. The WLF-type *structural strength* (S), indicating decrease of τ from 10^2 to 10^{-2} s, of trehalose decreased with a_w up to 0.44 (from 15.8 to 12.1 °C) but WPI increased S. Moreover, a relationship for S and water content for glass forming sugar systems at $a_w \geq 0.56$ was established. The S concept gave a quantitative measure to estimate compositional effects on τ and could be used to estimate crystallization of food solids components.

1. Introduction

Sugars can exist in crystalline or amorphous states in food solids. It is important that the microstructure of a food material is maintained in a noncrystalline state; otherwise the flavor, color and taste of the food may be altered during storage (Hartel & Shastry, 1991; Roos & Drusch, 2015). In general, amorphous structures of sugars are fairly stable in the glassy state (Roos, 2008; Slade & Levine, 1991). However, at temperatures close to or above glass transition temperature (T_g) molecular mobility increases and solids are converted to viscous liquids showing time-dependent flow (Roudaut, Simatos, Champion, Contreras-Lopez, & Le Meste, 2004; Roos & Drusch, 2015; White & Cakebread, 1966). As water affects molecular mobility shown by a lowered T_g due to plasticization, amorphous sugars may exhibit a tendency of crystallization causing physical and chemical deterioration in food ingredients and dairy powders at high storage humidities or temperatures (Aguilar, Hollender, & Ziegler, 1994; Hartel & Shastry, 1991; Ibach & Kind, 2007; Miao & Roos, 2005; Roos & Karel, 1991; Slade & Levine, 1991). The rates of crystallization of amorphous sugars are governed by water content, relative humidity (RH) and the temperature of storage above T_g, $T-T_g$ (Fan & Roos, 2015). Previous studies showed that crystallization of amorphous sugars could be delayed by the presence of other components, i.e. starch (Iglesias & Chirife, 1978), corn syrup solids (Gabarra & Hartel, 1998), proteins (Fan & Roos, 2015; Silalai & Roos, 2010) and carbohydrates (Mazzobre, Soto, Aguilera, & Buera, 2001; Potes, Kerry, & Roos, 2012; Sillick & Gregson, 2010).

α, α-Trehalose (α-D-glucopyrano-syl-α-D-glucopyranoside) is a non-reducing and naturally occurring disaccharide of glucose monomers with high T_g (De Gusseme, Carpentier, Willart, & Descamps, 2003; Lammert, Schmidt, & Day, 1998). Indeed, trehalose can be added to biologically active solutions to overcome the limited stability range of proteins (pH, temperature, salt concentration, etc.) or effectively prevent the partial or even total degradation of biomolecules due to the lethal thermal or dehydration stresses encountered during industrial conservation methods (lyophilization) (Crowe, Crowe, & Jackson, 1983; Iglesias & Chirife, 1978; Miller, DePablo, & Corti, 1997; Roser, 1991; Schiraldi, Di Lernia, & De Rosa, 2002; Uritani, Takai, & Yoshinaga, 1995). Trehalose also has preservation capabilities of bio-systems such as cells, vaccines or therapeutic proteins employed in the food, pharmaceutical or cosmetics industry, whereas amorphous trehalose may undergo crystallization when exposed to a high humidity

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http://dx.doi.org/10.1016/j.foodhyd.2016.03.019
0268-005X/

(Elbein, Pan, Pastuszak, & Carroll, 2003; Green & Angell, 1989; Miller & De Pablo, 2000). Lactose (β-D-galactopyranosyl (1–4)-D-glucopyranose) is often used in the food and pharmaceutical industries and it exhibits strong tendency to crystallize from its amorphous states, especially at high storage humidities (Nickerson, 1979). X-ray diffraction (XRD) patterns have shown that amorphous lactose crystallizes into a number of crystalline forms, which differ in melting behavior, solubility, density, crystal morphology, and relative sweetness (Jouppila, Kansikas, & Roos, 1998; Lai & Schmidt, 1990; Nickerson, 1979). Generally, the crystalline forms vary as their formation depends on the presence of other components, which may be related to interactions among sugars, supersaturation in systems, diffusion of molecules or the mutarotation of molecules during either nucleation or crystal-growth stages in crystallization (Fitzpatrick et al., 2007; Jouppila & Roos, 1994a,b).

Crystallization results in a dramatic change in structure of glass forming sugars with complete changes of mechanical properties and time-dependent flow characteristics (Chung, Chang, & Lim, 2004; Chung, Lee, & Lim, 2002; Chung, Woo, & Lim, 2004). Mechanical properties around the glass transition show an α-relaxation, which could be investigated by dynamic mechanical analysis (DMA) and
detected from changes in loss modulus (E'', mechanical energy dissipation), storage modulus
(E', mechanical energy storage) and *tan δ* ($tan\ \delta = E''/E'$) (Angell, Ngai, McKenna, McMillan, & Martin, 2000). A characteristic feature of mechanical properties of amorphous sugars, such as the T_g-dependence, could affect food quality and shelf life during storage (Slade & Levine, 1991; Silalai & Roos, 2010 & 2011). Mechanical properties of amorphous sugars could be affected by the presence of other components affecting their glass transition, e.g. water (Downton, Flores-Luna, & King, 1982; Slade & Levine, 1991), carbohydrates (Cruz, Oliveira, & MacInnes, 2001; Miao & Roos, 2005) and proteins (Fan & Roos, 2016; Silalai & Roos, 2010). As the measurements of solid flow characteristics or viscosity in the T_g region are extremely; difficult, Roos et al. (2015) defined a measure for flow characteristics given by "*strength, S*" and introduced a WLF model-based analysis of structural relaxation times (τ) within solids affecting flow characteristics in mixes of sugars and polymeric food components.

Whey protein may act as stabilizer in sugars/protein systems during spray drying and freeze-drying (Carullo & Vallan, 2012; Oetjen & Haseley, 2004; Ratti, 2001; Wang, Langrish, & Leszczynski, 2010). Previous studies showed that whey protein could delay crystallization of amorphous sugars in powder systems at high RH storage conditions due to reduced nucleation and diffusion during crystal-growth stage. However, characterization of the glass-forming properties is important for amorphous sugars and polymeric materials made to powders. The objectives of the present study were to investigate the influence of whey protein on crystallization kinetics and crystalline forms of amorphous trehalose and lactose/trehalose systems at high water activities (>0.56 a_w) as well as calorimetric glass transition, α-relaxation, and structural strength at low water activities (≤0.44 a_w) at isothermal storage conditions (25 °C). We expect that strength parameter provides a measure of relaxation rates and describes solids properties, i.e., crystallization, in sugars/protein systems, which could contribute to time-dependent powder characteristics in food and pharmaceutical materials.

2. Materials and methods

2.1. Preparation of amorphous materials

α, α-Trehalose dihydrate (Hayashibara Co. Ltd, Okayama, Japan), α-lactose monohydrates (Sigma–Aldrich, St. Louis, Mo., U.S.) and whey protein isolates (WPI; Isolac®, Carbery Food Ingredients, Co., Ballineen, Ireland; impurities including carbohydrates and lipids <3%) were used. De-ionized water (KB Scientific, Cork, Ireland) was used for all
experimental work. Lactose and trehalose was dissolved in de-ionized water to obtain 20% (mass) solution. WPI solution with 20% (mass) solution was prepared using continuous stirring for 4 h at room temperature (~23 °C). Lactose and trehalose solutions at room temperature were used to obtain solids ratios of 2:3, 1:1 and 3:2 of lactose/trehalose mixtures by mass. Trehalose and WPI solutions at room temperature were used to obtain solids ratios of 7:3, 1:1, 3:7 of trehalose/WPI by mass, respectively. Similarly, lactose, trehalose and WPI solutions were used to obtain solid ratios of 2:3:5, 1:1:5 and 3:2:5 of lactose/trehalose/WPI by mass, respectively. Samples (5 mL in total) were prepared in pre-weighted 20 ml glass vials (10 mL, diameter 24.3 mm × height 46 mm; Schott Müllheim, Germany). All samples in the vials (semi-closed with septum) were frozen in a still air freezer at −20 °C for 20 h and then subsequently tempered at −80 °C for 3 h prior to freeze-drying using a laboratory freeze-dryer (Lyovac GT2 Freeze Dryer, Amsco Finn-Aqua GmbH, Steris®, Hürth, Germany). After freeze-drying at pressure <0.1 mbar, triplicate samples of each material were stored in evacuated vacuum desiccators over P_2O_5 (Sigma–Aldrich, St. Louis, Mo., U.S.) prior to subsequent analysis.

2.2. Water sorption and time-dependent crystallization

Water sorption by freeze-dried lactose, trehalose, lactose/trehalose, trehalose/WPI, lactose/trehalose/WPI and WPI at each ratios was monitored for 120 h (non-crystallizing samples) and 240 h (crystallizing samples) over saturated solutions of *LiCl*, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $NaNO_2$ and *NaCl* (Sigma Chemical Co., St. Louis, Mo., U.S.A.) at respective water activities (a_w) of 0.11, 0.23, 0.33, 0.44, 0.54, 0.65 and 0.76 a_w at 25 °C (Greenspan, 1977; Labuza, Kaanane, & Chen, 1985), in vacuum desiccators. The a_w measured (Dew Point Water Activity Meter 4TE, Aqualab, WA, USA) for the systems at 25 °C is given in Table 1. Evacuated desiccators in incubators (Series 6000, Termaks, Bergea, Norway) were stored at 25 °C. Vials with samples were weighted to monitor water sorption at 0, 3, 6, 9, 12 and 24 h followed by 24 h intervals up to 240 h, respectively (Jouppila & Roos, 1994b). Lactose and trehalose crystallization was monitored from loss of sorbed water during storage over $Mg(NO_3)_2$, $NaNO_2$ and *NaCl* at 25 °C. All vials were closed with septums when removed desiccators and septums were opened for weighing. Water contents of the materials were measured as a function of time, and the average weights of triplicate samples were used in calculation. The Guggenheim-Anderson-de Boer (GAB) equation (Eq. (1)) was used to fit the experimental data to model water sorption at 25 °C (Lievonen & Roos, 2002; Timmermann, Chirife, & Iglesias, 2001; Torres, Bastos, Gonçalves, Teixeira, & Rodrigues, 2011).

$$\frac{m}{m_0} = \frac{CKa_w}{(1 - Ka_w)(1 - Ka_w + Cka_w)} \quad (1)$$

where, m is water content, m_0 is the monolayer value and C and K were respectively calculated from m_0.

2.3. XRD analysis

Vials with trehalose, lactose/trehalose, trehalose/WPI, and lactose/trehalose/WPI mixtures stored over $NaNO_2$ and *NaCl* were filled with liquid nitrogen and stored at −80 °C for 3 h followed by freeze-drying for 1 day. Such treatment ceased crystallization as a