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X-ray diffraction analysis of lactose crystallization in freeze-dried lactose–whey protein systems

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ABSTRACT

Water sorption, time-dependent crystallization and XRD patterns of lactose and lactose–WPI mixtures were studied with glass transition data. The results indicated that the sorbed water of lactose–WPI mixtures was fractional and water content of individual amorphous components in lactose–WPI mixtures at each a_w from 25 °C to 45 °C could be calculated. Crystallization occurred in pure lactose whereas partial crystallization was typical of lactose–WPI mixtures (protein content ≤50%) at intermediate and high a_w (>0.44 a_w) from 25 °C to 45 °C. The extents of crystallization were significantly delayed by WPI. The T_g values of lactose–WPI systems showed the composition-dependent property in systems and might indicate the occurrence of phase separation phenomena during 240 h storage. XRD showed no anhydrous β-lactose and mixed α-/β-lactose with molar ratios of 4:1 crystals in crystallized lactose–WPI systems (70:30 and 50:50 solids ratios). Reduced crystallization in the presence of WPI was more pronounced possibly because of reduced nucleation and diffusion during crystal-growth. The present study showed that WPI could present an important role in preventing sugar crystallization.

1. Introduction

Crystallization of amorphous sugars has a significant effect on the quality and shelf life of a number of products of biological, pharmaceutical and food industries (Das & Langrish, 2012a, 2012b; Edrisi Sormoli, Das, & Langrish, 2013; Roos, 1995, 1996). In amorphous systems, crystallization occurs as a result of increased molecular mobility above the glass transition (Jouppila, Kansikas, & Roos, 1997; Roos, 1995; Roos & Karel, 1992; Slade & Levine, 1991). The rates of crystallization of amorphous sugars are governed by water content, relative humidity (RH) and the temperature of storage above the glass transition temperature (T_g), T–T_g (Roos & Karel, 1991a). As water affects molecular mobility shown by a lowered T_g due to water plasticization, amorphous sugars may exhibit a high tendency for crystallization and a series of consequent problems during food processing and storage (Ibach & Kind, 2007; Roos, 1995; Slade & Levine, 1991). Below the T_g, however, mobility of sugar molecules is limited to vibrations and rotations, which kinetically limits crystallization and reduces rapid loss of product stability (Omar & Roos, 2007; Slade & Levine, 1991). Previous studies showed that crystallization of amorphous sugars could be delayed by the presence of other sugars or impurities, i.e. starch (Iglesias & Chirife, 1978), corn syrup solids (Gabarra & Hartel, 1998), trehalose (Mazzobre, Soto, Aguilera, & Buera, 2001), proteins (Sillick & Gregson, 2009), and maltodextrins (Potes, Kerry, & Roos, 2012).

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 a high storage RH (Choi, Tatter, & O'Malley, 1951; Herrington, 1934; Nickerson, 1979). X-ray diffraction (XRD) patterns have shown that lactose may crystallize in a complex manner into a number of crystalline forms, mainly α-lactose monohydrate, anhydrous β-lactose, stable and unstable α-lactose and anhydrous α-/β-lactose mixtures in molar ratios of 5:3 and 4:1 (Haque & Roos, 2005; Jouppila, Kansikas, & Roos, 1998). The rate of crystallization depends on several factors, such as the rate of nucleation, the time required to remove water, storage temperature and molecular anomerization during crystallization (Aguilar, Hollender, & Ziegler, 1994; Drapier-Beche, Fanni, Parmentier, & Vilasi, 1997; Haque & Roos, 2005; Jouppila et al., 1998). Crystalline forms of lactose also differ in melting behavior, solubility, density, crystal morphology, and relative sweetness (Lai & Schmidt, 1990; Nickerson, 1979). The crystalline forms of lactose in foods vary as their formation depends on the presence of other components, which may be related to interactions between lactose, supersaturation in systems, diffusion of lactose molecules or delayed mutarotation of molecules during nucleation and crystal-growth stages (Berlin, Anderson, & Pallansch, 1968; Fitzpatrick et al., 2007; Jouppila & Roos, 1994a, 1994b; Jouppila et al., 1997).

Whey protein isolate (WPI) may act as stabilizer in sugar–protein systems during spray drying and freeze-drying (Carullo & Vallan, 2012; Oetjen & Haseley, 2004; Ratti, 2001; Roos, 1995; Wang, Langrish, & Leszczynski, 2010). Roos and Karel (1991a) showed that rates of lactose crystallization were controlled by T_g values and whey

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protein could delay crystallization of lactose and stabilize lactose in skim milk powder at high RH storage conditions. However, such inhibition of lactose crystallization may not entirely result from the T_g-dependant state of lactose in binary systems (Mazzobre et al., 2001; Silalai & Roos, 2010). The crystallization and glass transition properties of lactose in foods have been well documented and also several technologies are used in investigating the mechanism of protein inhibition of crystallization, i.e. Scanning electron microscope (SEM) and protein characterization technology (Jin et al., 2000; Shawqi Barham, Kamrul Haque, Roos, & Kieran Hodnett, 2006; Wang, 2005). Common hypotheses used in attempts to explain the mechanism of inhibition on lactose crystallization by proteins include the bond-hinder theory (Lopez-Diez & Bone, 2000), stereo-hindrance theory (Adhikari, Howes, Bhandari, & Langrish, 2009; Garti & Leser, 2001) and diffusion-limitation theory (Das, Lin, Sormoli, & Langrish, 2013).

The objectives of the present study were to investigate the relationships between the quantity of protein, T–T_g, lactose crystallization kinetics and crystalline forms of lactose as derived from water sorption and XRD data. This study is useful for understanding lactose–whey protein systems and crystallization of lactose in food and pharmaceutical materials as whey protein may present an important role in preventing sugar crystallization.

2. Materials and methods

2.1. Preparation of amorphous materials

α-lactose monohydrate (Sigma-Aldrich, St. Louis, Mo., U.S.) and whey protein isolate (WPI; Isolac®, Carbery Food Ingredients, Co., Ballineen, Ireland; impurities including carbohydrates or lipids <3%) were used (O'Loughlin et al., 2013). Lactose was dissolved in distilled water to obtain 20% (w/w) solution and then cooled to room temperature (20 ± 3 °C). WPI solution with 20% (w/w) solids was prepared using continuous stirring for 4 h at room temperature. Lactose and WPI solutions at room temperature were used to obtain solids ratios of 100:0, 70:30, 50:50, 30:70 and 0:100 of lactose: WPI, respectively. Samples of mixed solutions (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 lactose crystallization

Water sorption by freeze-dried lactose, WPI and lactose–WPI at 70:30, 50:50 and 30:70 ratios were monitored for 96 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 depending on storage temperature (25–45 °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 each temperature is given in Table 1. Evacuated desiccators in incubators (Series 6000, Termaks, Bergea, Norway) were stored at 25 °C, 35 °C and 45 °C, respectively. 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, 1994a; Potes et al., 2012). Lactose crystallization was monitored from loss of sorbed water during storage over $Mg(NO_3)_2$, $NaNO_2$ and NaCl at various storage temperatures (Potes et al., 2012). All vials were closed with septum when transferred out of desiccators and septum was moved when vials were removed for weighing. Water content of the materials was measured as a function of time, and the average weight of triplicate samples was used in calculations. The Guggenheim–Anderson–de Boer (GAB) equation was fitted to experimental data to model water sorption at 25 °C, 35 °C and 45 °C, respectively (Eq. (1)) (Jouppila & Roos, 1997; Lievonen & Roos, 2002; Timmermann, Chirife, & Iglesias, 2001; Torres, Bastos, Gonçalves, Teixeira, & Rodrigues, 2011).

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

Table 1
Water content and water activity (a_w) for freeze-dried non-crystalline lactose, amorphous WPI and lactose–WPI mixtures at fraction ratios of 70:30, 50:50 and 30:70 stored for 96 h at 25 °C, 35 °C and 45 °C. The water content of non-crystalline lactose at 0.53 to 0.76 a_w (25 °C and 35 °C) and 0.42 to 0.74 a_w (45 °C) was derived from experimental non-crystalline lactose in lactose: WPI 30:70 ratio. Water content of non-crystalline lactose: WPI systems were obtained from experimental data at 0.11 to 0.53 a_w and fractional water content calculated for non-crystalline and measured for lactose: WPI 70:30 and 50:50 to predict sorbed water content for lactose: WPI mixtures at 0.61 and 0.76 a_w.

Storage temperature	a_w	Lactose (non-crystalline)	Water content (gH_2O/100 g of solids)				
			Lactose	Lactose:WPI 70:30	Lactose:WPI 50:50	Lactose:WPI 30:70	WPI
25 °C	0.11 ± 0.00[a]	2.0	2.0 ± 0.2	2.7 ± 0.2	3.0 ± 0.1	3.4 ± 0.1	4.3 ± 0.1
	0.23 ± 0.00	4.0	4.0 ± 0.3	4.9 ± 0.1	5.3 ± 0.1	5.8 ± 0.1	6.7 ± 0.1
	0.33 ± 0.00	6.1	6.1 ± 0.3	6.6 ± 0.1	7.0 ± 0.1	8.0 ± 0.3	8.9 ± 0.2
	0.43 ± 0.00	8.4	8.4 ± 0.4	9.0 ± 0.2	9.2 ± 0.0	9.6 ± 0.1	10.5 ± 0.1
	0.53 ± 0.00	10.9	2.4 ± 0.2	11.0 ± 0.2	9.8 ± 0.1	12.0 ± 0.1	12.4 ± 0.1
	0.65 ± 0.01	15.0	2.8 ± 0.4	4.7 ± 0.1	11.5 ± 0.1	14.5 ± 0.3	14.4 ± 0.1
	0.76 ± 0.01	19.6	3.0 ± 0.4	5.8 ± 0.1	12.4 ± 0.6	17.7 ± 0.2	18.8 ± 0.1
35 °C	0.11 ± 0.00	1.5	1.5 ± 0.3	2.0 ± 0.1	2.2 ± 0.2	2.9 ± 0.2	3.1 ± 0.1
	0.22 ± 0.01	3.0	3.0 ± 0.1	3.4 ± 0.1	3.8 ± 0.2	4.2 ± 0.1	5.4 ± 0.2
	0.32 ± 0.00	4.5	4.5 ± 0.1	4.9 ± 0.4	5.1 ± 0.1	5.5 ± 0.2	7.0 ± 0.1
	0.43 ± 0.01	6.4	6.4 ± 0.3	7.6 ± 0.3	6.7 ± 0.2	7.7 ± 0.1	8.8 ± 0.2
	0.50 ± 0.00	7.8	1.5 ± 0.5	9.8 ± 0.3	8.8 ± 0.3	9.1 ± 0.2	10.2 ± 0.2
	0.63 ± 0.00	11.1	1.9 ± 0.6	3.9 ± 0.5	10.1 ± 0.2	12.0 ± 0.2	12.8 ± 0.1
	0.75 ± 0.01	16.0	2.4 ± 0.4	5.5 ± 0.3	9.6 ± 0.2	16.5 ± 0.2	17.2 ± 0.1
45 °C	0.11 ± 0.00	1.0	1.0 ± 0.2	1.8 ± 0.3	1.9 ± 0.4	2.6 ± 0.2	2.8 ± 0.1
	0.20 ± 0.00	2.1	2.1 ± 0.1	2.5 ± 0.3	3.0 ± 0.3	3.4 ± 0.1	4.3 ± 0.2
	0.31 ± 0.01	3.5	3.5 ± 0.1	3.9 ± 0.2	4.4 ± 0.1	5.0 ± 0.3	6.5 ± 0.1
	0.42 ± 0.00	5.6	1.2 ± 0.4	5.8 ± 0.2	6.0 ± 0.1	6.5 ± 0.1	7.9 ± 0.1
	0.47 ± 0.01	6.7	0.8 ± 0.5	7.8 ± 0.3	6.9 ± 0.2	7.3 ± 0.2	9.2 ± 0.4
	0.61 ± 0.01	10.5	1.3 ± 0.8	3.3 ± 0.3	9.4 ± 0.3	9.7 ± 0.2	11.7 ± 0.2
	0.74 ± 0.00	14.7	1.6 ± 0.5	4.2 ± 0.3	8.0 ± 0.4	13.7 ± 0.1	14.5 ± 0.1

[a] Values are mean ± SD (n = 3).