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Stability and Plasticizing and Crystallization Effects of Vitamins in Amorphous Sugar Systems

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ABSTRACT: Increased molecular mobility and structural changes resulting from water plasticization of glassy solids may lead to loss of the entrapped compounds from encapsulant systems. In the present study, the stability of water-soluble vitamins, vitamin B_1 (vB_1, thiamin hydrochloride) and vitamin C (vC, ascorbic acid), in freeze-dried lactose and trehalose at various water activities was studied. Water sorption of lactose−vB_1, lactose−vC, trehalose−vB_1, and trehalose−vC systems was determined gravimetrically. Glass transition and crystallization of anhydrous and plasticized sugar−vitamin systems were determined using thermal analysis. Critical water activity was calculated using water sorption and glass transition data. The retention of the vitamins was measured spectrophotometrically. The results showed that the amorphous structure protected the entrapped vitamins at low a_w. Crystallization of lactose accelerated vitamin degradation, whereas trehalose retained much higher amounts of the vitamins. Glass transition and critical water activity of solids and crystallization of component sugars should be considered in the stabilization of sensitive components.

KEYWORDS: *ascorbic acid, crystallization, glass transition, lactose, thiamin, trehalose, water activity*

INTRODUCTION

Ascorbic acid retention correlates well with retention of other nutrients in foods. Its retention is often used as an index for nutrient quality in processed and stored foods.[1,2] Thiamin is one of the least heat-stable vitamins,[3] and it is sensitive to pH,[4] oxygen,[5] and trace metals[6] similarly to other water-soluble vitamins. Their stability is also dependent on water content and water activity (a_w) in low-water systems.[5,7−9] The degradation of sensitive compounds increased with increasing a_w, which decreased viscosity and increased the level of dissolved oxygen.[10]

Carbohydrates often exist as amorphous solids (glasses), which makes them potential food components for nutrient entrapment and use as encapsulant materials in nutrient stabilization. The amorphous encapsulants are well-known to provide rapid release of active substances in pharmaceuticals, and they are desirable in therapeutic uses.[11] However, the amorphous glasses are nonequilibrium materials. When plasticized by temperature or water, they may transform to the supercooled liquid state; that is, the glass transition takes place, leading to physical changes and loss of shelf life.[12,13] The glass transition also affects the stability of food ingredients, which may show collapse and crystallization of components.[14−17] Chen and others[18] and Bell and White[19] reported that the stability of tyrosinase and thiamin in model systems was more correlated to the glass transition than to water activity. The glass transition of the matrices increased the molecular mobility of the encapsulants and also the entrapped particles, resulting in an increased rate of diffusion-controlled chemical reactions.[18−20] Therefore, the glass transition behavior of the solids should be considered in addition to water activity in the stabilization of sensitive components.

Lactose and trehalose are glass-forming disaccharides that are commonly used in the food industry. They have similar hygroscopic and glass transition properties, but very different solubilities in water and crystallization behavior.[17,21] Numerous studies have addressed the amorphous state and crystallization behavior of common encapsulant sugars, but the effects of water and sugar crystallization on the retention of sensitive components, such as vitamins, have not been intensively studied. The purpose of the present study was to entrap and stabilize model water-soluble vitamins (B_1 and C) in amorphous lactose and trehalose matrices by freezing and freeze-drying. The water sorption and glass transition properties of the systems in the presence of the vitamins were analyzed, and the stability of the vitamins in frozen and freeze-dried lactose and trehalose systems, as affected by composition, process, storage conditions (temperature and water activity), and physical changes (glass transition and sugar crystallization), was determined.

MATERIALS AND METHODS

Materials. α-Lactose monohydrate (Sigma-Aldrich, St. Louis, MO), trehalose dihydrate (Cargill Inc., Minneapolis, MN), L-ascorbic acid (vC, Sigma-Aldrich), and thiamin hydrochloride (vB_1, Sigma-Aldrich) were purchased to prepare lactose−vB_1, lactose−vC, trehalose−vB_1, and trehalose−vC (250 mL; 20% sugar, w/v; 0.5% vitamin, w/v) systems. Sugars were dissolved in distilled water at 45 °C on a hot plate to obtain clear solutions and then cooled to room temperature (24 ± 1 °C). The vitamins were first dissolved in water and then added to sugar solutions, and the volume was adjusted to 250 mL in a volumetric flask.

Aliquots of the lactose−vB_1, lactose−vC, trehalose−vB_1, and trehalose−-vC solutions (5 mL) at pH 3.0 ± 0.1 were transferred using a volumetric pipet (Pipetman P5000, Gilson Inc., Middleton, WI) into glass vials (Clear glass ND18, 10 mL, VWR, U.K.). The samples in the vials were frozen at −35 °C ($T_g' < T < T_m'$)[12,13] for 24 h

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and subsequently transferred to a −80 °C freezer for 5 h to lower the sample temperature to avoid ice melting during transfer to a freeze-dryer (Lyovac GT2, STERIS, Hürth, Germany), which was prerun for 2 h for precooling of the condenser prior to sample loading. Frozen samples in glass vials with semiclosed rubber septa were freeze-dried at <0.1 mbar (T < −40 °C) for ≥72 h. All vials were hermetically closed in the freeze-dryer using the rubber septa prior to the vacuum being broken with ambient air. The freeze-dried materials theoretically contained 2.44% (w/w) of either ascorbic acid (vitamin C) or thiamin hydrochloride (vitamin B_1).

Water Sorption and Sorption Isotherms. Triplicate samples of lactose−vB_1, lactose−vC, trehalose−vB_1, and trehalose−vC in opened vials were equilibrated to reach constant water contents (up to 120 h) at room temperature (24 ± 1 °C) in evacuated desiccators over saturated salt solutions (LiCl, CH_3COOK, $MgCl_2$, and K_2CO_3), which at experimental conditions gave water activities (a_w) of 0.11, 0.23, 0.33, and 0.44, respectively, at equilibrium.[22] Desiccators were evacuated (approximately 1 min) until the onset of boiling of water to establish desired water vapor pressure conditions. The samples in vials were weighed at 0, 3, 6, 9, 12, 24, 48, 72, 96, and 120 h. Time-dependent sugar crystallization was monitored during storage of samples in vials over saturated salt solutions of $Mg(NO_3)_2$, $NaNO_2$, and NaCl, which at experimental conditions gave a_w of 0.54, 0.65, and 0.76, respectively, at equilibrium.[22] The vials with samples were weighed every hour up to 6 h and then at 8, 10, 12, 24, 48, 72, 96, and 120 h. The a_w of the saturated salt solutions was confirmed with an a_w meter (Aqua Lab 4TE, Decagon Devices, Inc., Pullman, WA). The initial sample weights and the weights after storage were used to derive water contents. The mean water content ± standard deviation (SD) of triplicate samples for each material and a_w was plotted against time to assess water sorption kinetics and crystallization.

The Guggenheim−Anderson−de Boer (GAB) relationship was used to model water sorption as suggested by Roos.[23] The GAB isotherm parameters were obtained by plotting a_w/m against a_w.[23,24] The constants α, β, and γ were calculated by applying the second-order polynomial regression for GAB over the a_w range of 0.11−0.44, as higher a_w values could not be used because of the crystallization of the sugars (steady-state water contents for fully amorphous solids could not be obtained). The m_m (monolayer value), K, and C were derived from α, β, and γ.[24]

Thermal Analysis. The thermal behavior of water and solids was determined using differential scanning calorimetry (DSC; Mettler Toledo 821e with liquid N_2 cooling, Schwerzenbach, Switzerland). The DSC was calibrated as described by Haque and Roos.[25] The thermograms were analyzed using STAR thermal analysis software, version 6.0 (Mettler Toledo).

For frozen-state transitions, freshly prepared solutions of lactose−vB_1, lactose−vC, trehalose−vB_1, and trehalose−vC (15−25 mg) were transferred using a volumetric pipet (Pipetman P200, Gilson Inc., Middleton, WI) into preweighed DSC aluminum pans (40 μL, Mettler Toledo), and the pans with samples were hermetically sealed and weighed (Mettler Toledo AG245 balance). A sealed empty pan was used as a reference in all measurements. Measurements were carried out according to the method of Zhou and Roos[17] for the onset temperature of glass transition of the maximally freeze-concentrated solutes, T_g', and the onset temperature of ice melting in the maximally freeze-concentrated systems, T_m'.

For transitions in low-water systems, the onset, T_g (onset), and endset, T_g (endset), temperatures of the glass transition of anhydrous lactose−vB_1, lactose−vC, trehalose−vB_1, and trehalose−vC and those equilibrated to various water activities (a_w), and the instant crystallization temperature (the onset temperature of crystallization), T_{ic}, were measured using DSC. To determine the T_g and T_{ic}, 10−15 mg of the powdered freeze-dried materials was prepared in preweighed DSC aluminum pans and equilibrated over P_2O_5 or saturated salt solutions for 72 h to a_w of 0−0.44 at room temperature (24 ± 1 °C). After equilibration, the pans were hermetically sealed and weighed. A sealed empty pan was used as a reference in all measurements. The T_g and T_{ic} were taken from the second heating scans, and anhydrous samples were analyzed using punctured pans.[17] All measurements were carried out in triplicate. The mean values of triplicate samples were used in modeling water plasticization.

Prediction of the T_g and Critical Water Contents. The Gordon−Taylor[26] equation (eq 1) was used to model water plasticization

$$T_g = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2} \quad (1)$$

where w_1 and w_2 are the respective weight fractions of the solid and water, T_{g1} is the T_g of the anhydrous solids, T_{g2} is the T_g of amorphous water (−135 °C was used[27]), and k is a constant. The constant k was derived from the experimental T_g data. The critical water content and the corresponding critical a_w at 24 °C were obtained using eq 1 and the GAB water sorption isotherms.[28] Diagrams of the relationships between water plasticization and the critical water content and the corresponding critical a_w at 24 °C were plotted.

Determination of Ascorbic Acid and Thiamin Hydrochloride Contents. The retention of vitamins was measured spectrophotometrically (Varian Cary 300, Bio UV−visible spectrophotometer, Agilent Technologies, Santa Clara, CA). To determine the retention of thiamin hydrochloride, the anhydrous and humidified lactose−vB_1 and trehalose−vB_1 were reconstituted to the original weight and diluted using 0.1 M hydrochloric acid (1/400). The absorbance was measured at 246 nm using a spectrophotometer,[29] and no effect of sugars on the absorbance was observed. This wavelength was found to correspond to the maximum absorbance of thiamin hydrochloride in 0.1 M hydrochloric acid over the spectrum of wavelengths from 200 to 300 nm. The standard curve was obtained by measuring the absorbance of thiamin hydrochloride at various concentrations (0−25 μg/mL) in 0.1 M hydrochloric acid. A blank sample of 0.1 M hydrochloric acid was used in the double-beam spectrophotometer along with the samples as reference. A pair of quartz cuvettes (CEL1600, Hellma Cuvette UV Quartz, Scientific Laboratory Supplies Ltd., Nottingham, U.K.) were used in this study. The sample cuvette was washed using 0.1 M hydrochloric acid after each measurement and prerinsed with sample solutions before each measurement.

To determine the retention of ascorbic acid, the anhydrous and humidified lactose−vC and trehalose−vC were reconstituted to the original weight and diluted using corresponding sugar solutions (20%, w/v). The diluted solution (4 mL) was vigorously mixed with 0.2 M Folin−Ciocalteu reagent (FCR, 0.8 mL, 1/10 dilution from 2 M, Sigma-Aldrich) and left at room temperature for 30 min before the color was measured at 760 nm[30] using a spectrophotometer. The standard curve was obtained by measuring the absorbance of the color intensity produced by reaction between the FCR and ascorbic acid at various concentrations (0−100 μg/mL) in corresponding sugar solutions. A blank sample of the FCR with corresponding sugar solutions was used in the spectrophotometer along with the samples as reference.

The stability of thiamin hydrochloride in lactose and trehalose during freezing was studied at various temperatures (−10, −20, −35, and −80 °C) for 60 days. The retention of thiamin hydrochloride was measured every 5 days through 30 days of storage then every 10 days through 60 days of storage. Triplicate samples were removed from the freezers and thawed at room temperature for 1 h in dark. The retention of thiamin hydrochloride was diluted using 0.1 M hydrochloric acid and measured as described above.

The retention of thiamin hydrochloride in freshly freeze-dried systems was measured, and the resultant amount of thiamin hydrochloride was considered as 100% during the storage study. The freeze-dried lactose−vB_1 and trehalose−vB_1 in open glass vials were stored over P_2O_5 and various saturated salt solutions giving a_w values of 0, 0.23, 0.44, and 0.65 in evacuated desiccators at room temperature (24 ± 1 °C). The retention of thiamin hydrochloride was measured at days 0, 1, 3, 6, 9, and 15 of storage. Triplicate samples were taken out at each time point, and the salt solutions were stirred using a glass rod to avoid the formation of film on the surface of salt solutions and dehydration of samples. The weight of each sample was

recorded before and after storage to monitor water sorption during storage.

The retention of ascorbic acid in freshly freeze-dried systems was measured, and the resultant amount of ascorbic acid was considered as 100% during the storage study. The freeze-dried lactose−vC and trehalose−vC in open glass vials were stored over P_2O_5 and various saturated salt solutions giving water activities of 0, 0.11, 0.23, 0.33, 0.44, and 0.65 in evacuated desiccators at room temperature (24 ± 1 °C). The retention of ascorbic acid was measured at days 0, 1, 5, 10, 15, 20, 30, and 60 of storage. Triplicate samples were taken out at each time point, and the salt solutions were stirred using a glass rod. The weight of each sample was recorded before and after storage to monitor water sorption during storage.

Statistics. All measurements were carried out in triplicate. The water sorption data, transition temperatures, and retention of vitamins are reported as the mean value ± 1 SD. All predictions were based on the triplicate data and mean values.

RESULTS AND DISCUSSION

Frozen-State Transitions of Sugar−Vitamin Systems and the Stability of Thiamin Hydrochloride in Frozen Systems. The onset temperatures of glass transition and ice melting in maximally freeze-concentrated lactose−vB_1, lactose−vC, trehalose−vB_1, and trehalose−vC systems (T_g' and T_m') are given in Table 1. Lactose−vitamin and trehalose−vitamin systems showed transition temperatures corresponding to those of pure lactose and trehalose systems, respectively,[17] showing that at the level of 0.5% (w/v) addition of vitamins, differences in ice-melting or glass transition properties could not be found using DSC.

Table 1. Onset Temperatures of Glass Transition (T_g') and Ice Melting (T_m') of Maximally Freeze-Concentrated Lactose−vB_1, Lactose−vC, Trehalose−vB_1, and Trehalose−vC systems

system	T_g' (°C)	T_m' (°C)
lactose−vB_1	−41 ± 1	−31 ± 1
lactose−vC	−42 ± 1	−31 ± 1
trehalose−vB_1	−42 ± 1	−32 ± 1
trehalose−vC	−42 ± 1	−32 ± 1

The cryostabilization technology proposed by Levine and Slade[12] assumed frozen food stability during storage at temperatures below the T_g'. Above the T_g', the glass transition of the freeze-concentrated solute matrix may control the rates of deteriorative changes in frozen foods.[33] In the present study, >95% and about 100% of thiamin hydrochloride were retained in lactose−vB_1 and trehalose−vB_1 systems, respectively, after 60 days of storage at −80 °C. This high retention was possibly a result of (1) the low rate of chemical reactions at low temperatures and (2) the low diffusion rates in the viscous glassy structures of the freeze-concentrated solutes.[12,33]

At −35 °C, which was above the T_g' but below the T_m', the temperature dependence of rates of diffusion and viscosity-related changes in frozen foods may follow the Williams−Landel−Ferry (WLF) relationship using $T - T_g'$ as the temperature difference to the T_g'.[12,33] The $T - T_g'$ value of 7 °C was applied to the present study during storage at −35 °C. Such a small $T - T_g$ in the storage of frozen systems is within the glass transition temperature range. It may result in a decrease of the viscosity from 10^{12} to 10^{10} Pa s, but coupled with the low temperature, the rates of deteriorative changes of thiamin, such as nonenzymatic browning, may not be affected. The results showed that in storage below T_m', thiamin hydrochloride stability was temperature independent, and retentions of thiamin hydrochloride were similar at −35 and −80 °C. A thin layer of sediments was observed in the thawed lactose−vB_1 system after 5 days of storage and onward, indicating crystallization of lactose during thawing of the system. No trehalose crystallization was found in thawing of the trehalose−vB_1 system.

Storage at −20 and −10 °C increased substantially the $T - T_g$ for storage, and the $T - T_g$ values were affected by the melting of ice above the T_m'.[33] Degradation of thiamin hydrochloride was, however, <5% in both lactose−vB_1 and trehalose−vB_1 systems at −20 and −10 °C. Sedimentation was pronounced in the thawed lactose−vB_1 system, suggesting an increased extent of lactose crystallization above T_m', because of dilution and decreased viscosity of the supersaturated unfrozen solute phase.[33] Clear solutions were obtained after thawing of trehalose−vB_1, because trehalose had a higher solubility than lactose and it was less likely to crystallize from the freeze-concentrated solute phase (Figure 1). The solubility of lactose was significantly influenced by the α/β form of lactose. The α:β ratio of lactose changes depending on the temperature. At the low temperature, the solubility of lactose is mainly controlled by the α form. Due to the high amount of α form, lactose showed very low solubility at −20 and −10 °C. It should be remembered that rates of deteriorative changes, such as nonenzymatic browning, are significantly decreased at low temperatures,[34] which may aid to retain the thiamin hydrochloride in frozen systems. The stability of thiamin hydrochloride in the unfrozen phase was possibly also promoted by the low pH.[4]

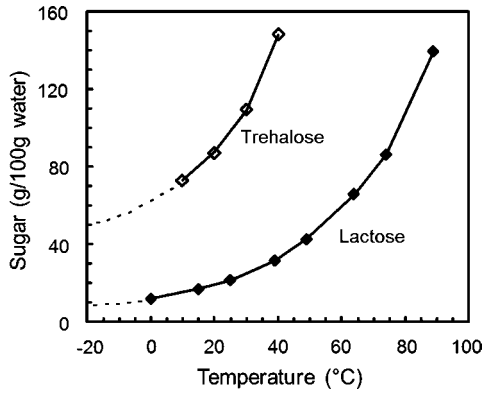

Figure 1. Solubility (g/100 g water) of lactose[31] and trehalose[32] as a function of temperature.

Water Sorption and Sorption Isotherms. Water sorption of freeze-dried lactose−vB_1, lactose−vC, trehalose−vB_1, and trehalose−vC systems over various water activities as a function of time was plotted, as shown in Figures 2 and 3, respectively. The final water contents are given in Table 2. At a_w values of 0.11−0.44, all systems showed rapid water sorption initially and reached constant water contents. At low a_w, lactose−vB_1 and lactose−vC and trehalose−vB_1 and trehalose−vC showed more rapid water sorption but reached lower final water contents than pure lactose and trehalose, respectively (Figures 2A and 3A). The final water contents were found to be higher in lactose−vB_1 and trehalose−vB_1 systems than in lactose−vC and trehalose−vC systems, respectively (Table 2).

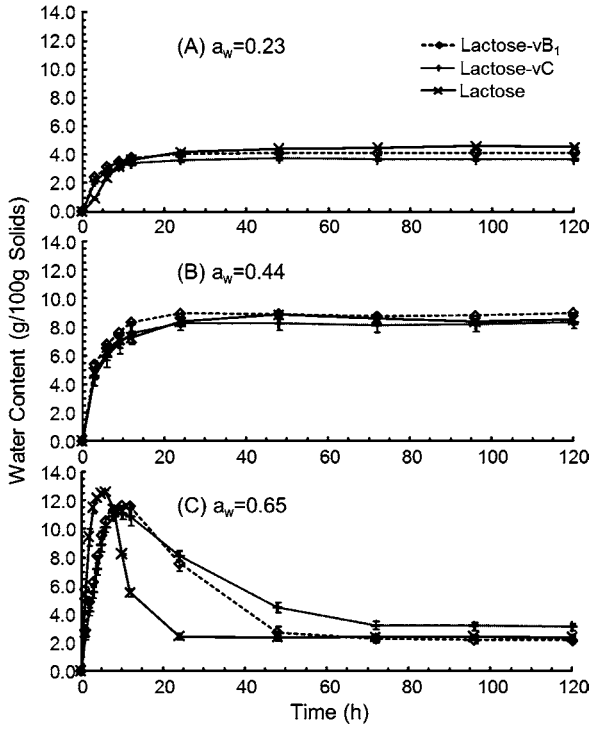

Figure 2. Water sorption of freeze-dried lactose,[17] lactose−vB$_1$, and lactose−vC systems at a_w = (A) 0.23, (B) 0.44, and (C) 0.65 at room temperature (24 ± 1 °C). Loss of water at a_w = 0.65 indicates lactose crystallization. Vertical bars represent ±1 SD of data for triplicate samples.

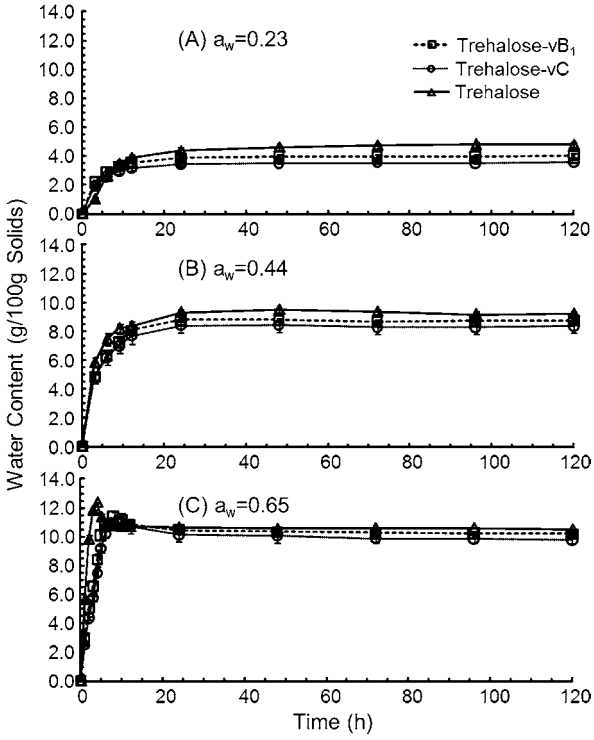

Figure 3. Water sorption of freeze-dried trehalose,[17] trehalose−vB$_1$, and trehalose−vC systems at a_w = (A) 0.23, (B) 0.44, and (C) 0.65 at room temperature (24 ± 1 °C). Loss of water at a_w = 0.65 indicates trehalose crystallization. Vertical bars represent ±1 SD of data for triplicate samples.

At a_w = 0.44, a minor loss of sorbed water was observed for pure lactose and trehalose systems after 72 h of storage; however, loss of water was not found for vB$_1$-containing and vC-containing systems. Loss of water was pronounced at $a_w \geq$ 0.54; all systems showed time-dependent loss of sorbed water, indicating component sugar crystallization during storage. Sugar crystallization was delayed by the component vitamins. At a_w = 0.65, different rates of water sorption and crystallization were found for lactose and trehalose in the presence of component vitamins. Lactose−vB$_1$ and trehalose−vB$_1$ showed slightly more rapid and higher water sorption than lactose−vC and trehalose−vC systems, respectively; however, the water sorption of the above systems was less rapid compared to the corresponding pure sugar systems. A delayed lactose crystallization was observed during storage for lactose−vB$_1$ and lactose−vC systems (after 12 h) than for the lactose system (after 6 h), which is shown in Figure 2C. The desorption of water from the systems indicated the rate of lactose crystallization. The rate was most rapid for lactose, followed by lactose−vB$_1$, and lactose−vC showed the most delayed lactose crystallization. Similar effects of crystallization delay by the vitamins were found for trehalose-containing systems. Trehalose crystallization was observed after 4 and 12 h of storage for trehalose and trehalose−vitamin systems, respectively (Figure 3C). All systems reached constant but different final water contents after crystallization (Table 2). However, the molar ratio of the sugar:water indicated similar crystalline forms for lactose and trehalose at different a_w values in the absence/presence of vitamins, which were a mixture of anhydrate and monohydrate for lactose and dihydrate for trehalose (Table 3). With regard to the rate of sugar crystallization, the molar ratios of sugar:vB$_1$ and sugar:vC were considered. The molar ratio was approximately 40:1 for lactose:vB$_1$ and trehalose:vB$_1$ and 20:1 for lactose:vC and trehalose:vC. That means there were twice as many ascorbic acid molecules in comparison to thiamin hydrochloride molecules per mole of sugar. This explained the more delayed sugar crystallization in the presence of ascorbic acid than in the presence of thiamin hydrochloride.

The GAB model was fitted to the water sorption data. Different m_m values were obtained for all systems. Lactose−vB$_1$ and trehalose−vB$_1$ systems showed higher m_m values than lactose−vC and trehalose−vC systems, respectively (Table 4). The monolayer value is important because above that value there would be water, which could be available for chemical reactions.[9]

Glass Transition and Crystallization. The glass transition temperatures for anhydrous and water-plasticized lactose−vB$_1$, lactose−vC, trehalose−vB$_1$, and trehalose−vC systems are given in Table 5. Anhydrous lactose and trehalose systems with ascorbic acid and thiamin hydrochloride showed lower T_g (onset) values than those for pure lactose (105 °C) and trehalose (111 °C), respectively.[17] The anhydrous systems contained about 2.44% of either ascorbic acid or thiamin hydrochloride (molar ratios of 1:20 and 1:40, respectively, Table 3), at which level the component vitamins showed plasticizing effects on the component sugars. At increasing a_w, the T_g of all systems decreased (Figure 4). The water-plasticized systems showed T_g values similar to those of the pure sugars. This was probably because the plasticizing effect of water as a small molecular weight plasticizer was substantially stronger[13] than that of the vitamins. Crystallization of lactose and trehalose in the DSC measurements was found only for the

Table 2. Water Content (Mean Values ± SD) in Freeze-Dried Lactose, Trehalose, Lactose−vB$_1$, Lactose−vC, Trehalose−vB$_1$, and Trehalose−vC Systems after Equilibration at Various Water Activities (a_w) for 120 h at Room Temperature (24 ± 1 °C)

	water content (g/100 g of solids) at a_w =							
system	0.00	0.11	0.23	0.33	0.44	0.54	0.65	0.76
lactose[a]	0 ± 0.0	2.5 ± 0.1	4.5 ± 0.1	5.4 ± 0.2	8.4 ± 0.3	3.4 ± 0.1	2.4 ± 0.2	2.0 ± 0.3
trehalose[a]	0 ± 0.0	2.8 ± 0.1	4.8 ± 0.2	6.0 ± 0.1	9.2 ± 0.2	9.6 ± 0.7	10.5 ± 0.0	10.4 ± 0.2
lactose−vB$_1$	0 ± 0.0	2.3 ± 0.1	4.1 ± 0.1	5.5 ± 0.3	9.0 ± 0.1	2.5 ± 0.3	2.2 ± 0.3	2.9 ± 0.1
lactose−vC	0 ± 0.0	2.0 ± 0.1	3.7 ± 0.1	5.0 ± 0.3	8.4 ± 0.4	3.5 ± 0.2	3.1 ± 0.3	3.3 ± 0.6
trehalose−vB$_1$	0 ± 0.0	2.3 ± 0.0	4.0 ± 0.2	5.7 ± 0.1	8.7 ± 0.1	10.0 ± 0.2	10.2 ± 0.2	9.8 ± 0.1
trehalose−vC	0 ± 0.0	2.0 ± 0.1	3.6 ± 0.1	5.4 ± 0.1	8.4 ± 0.5	9.7 ± 0.1	9.8 ± 0.3	9.9 ± 0.2

[a]Data from Zhou and Roos.[17]

Table 3. Molar Ratios between Components (Lactose, L; Trehalose, T; Water, W; vB$_1$; vC) in Freeze-Dried Lactose, Trehalose, Lactose−vB$_1$, Lactose−vC, Trehalose−vB$_1$, and Trehalose−vC Systems at High Water Activities (a_w) after Crystallization

		molar ratio at a_w =		
system	components	0.54	0.65	0.76
lactose	L:W	5:1	2:1	3:1
trehalose	T:W	1:2	1:2	1:2
lactose−vB$_1$	L:W:vB$_1$	39:20:1	39:17:1	39:20:1
lactose−vC	L:W:vC	21:14:1	21:13:1	21:13:1
trehalose−vB$_1$	T:W:vB$_1$	39:77:1	39:77:1	39:75:1
trehalose−-vC	T:W:vC	21:40:1	21:40:1	21:40:1

humidified systems (Table 6). Pure lactose showed T_{ic} at 174 °C.[17] The T_{ic} values of anhydrous lactose−vB$_1$ and lactose−vC systems were not found in DSC thermograms in scans up to 200 °C. It is hypothesized that, although the vitamins did appear to show a plasticizing effect on the systems, diffusion of lactose molecules to nucleation and crystal growth sites was restricted due to the interactions between lactose and other molecules. The T_{ic} of lactose−vB$_1$ and lactose−vC decreased with increasing a_w as a result of water plasticization (Figure 4). Lactose showed crystallization in lactose−vB$_1$ and lactose−vC systems in the presence of water, which was possibly due to (1) the increased molecular mobility in the water-plasticized solids, (2) the interaction between lactose and vitamins being diminished by the interaction between lactose and water, and (3) the predominant plasticizing effects of water compared with that of vitamins at increasing a_w. No instant crystallization was found for trehalose in anhydrous or in humidified trehalose−vB$_1$ and trehalose−vC systems with a_w < 0.44, possibly due to the insufficient amount of water for trehalose to crystallize as dihydrate during a dynamic measurement.

Prediction of the Critical Water Content and Water Activity. The T_g (onset) curves as a function of water content (g/100 g solid) were predicted using eq 1.[26] The k constants, critical water contents (g/100 g solid), and critical a_w at 24 °C for lactose−vB$_1$, lactose−vC, trehalose−vB$_1$, and trehalose−vC were calculated using eq 1 and the GAB water sorption isotherms and are given in Table 7. Lactose−vB$_1$ and trehalose−vB$_1$ systems showed slightly higher critical water contents than lactose−vC and trehalose−vC systems, respectively, but corresponding critical a_w were observed for all systems. The critical water content as well as the corresponding a_w is important for the stability of low-moisture foods[13,17] when changes are controlled by the glass transition of the solids.

Stability of Ascorbic Acid and Thiamin Hydrochloride in Freeze-Dried Systems. About 100% of thiamin hydrochloride was retained in both lactose and trehalose systems after freeze-drying. This was in good agreement with the high retention of the compounds in frozen storage below T_m'. The retention of thiamin hydrochloride in freeze-dried lactose and trehalose systems during 15 days of storage at various a_w was plotted against time (Figure 5). The first-order kinetics was applied to the degradation data. At a_w = 0 and 0.23 (Figure 5A), the rate constants for thiamin hydrochloride loss were very small (<0.001 day^{-1}) and the correlation coefficient was <0.60. Other kinetic functions were also applied to the degradation data, but none of them revealed a better fit. Therefore, thiamin hydrochloride was considered to be stable at a_w = 0 and 0.23 in lactose−vB$_1$ and trehalose−vB$_1$ systems during 15 days of storage. At a_w = 0.44 and 0.65 (Figure 5B), thiamin hydrochloride degraded slowly. Sugar crystallization, as indicated by loss of water, caused simultaneous loss of thiamin hydrochloride (about 30%) in the lactose−vB$_1$ system, whereas in trehalose−vB$_1$ systems, no significant loss of thiamin hydrochloride was observed.

About 95 and 100% of ascorbic acid were retained in lactose−vC and trehalose−vC systems, respectively, after freeze-drying. The resultant amount of ascorbic acid was considered as 100% retention in the storage study. The retention of ascorbic acid in freeze-dried lactose and trehalose systems at various a_w values was plotted against time as shown in Figure 6. At a_w = 0, degradation of ascorbic acid was very slow and followed first-order kinetics. Rate constants of 0.0012 day^{-1} (R^2 = 0.9439) and 0.0007 day^{-1} (R^2 = 0.9724) were

Table 4. Guggenheim−Anderson−de Boer[a] Constants (α, β, γ, K, and C), Monolayer Values (m_m), and R^2 for Freeze-Dried Lactose−vB$_1$, Lactose−vC, Trehalose−vB$_1$, and Trehalose−vC Systems

system	α	β	γ	K	C	m_m (g/100 g solid)	R^{2b}
lactose−vB$_1$	−0.3484	0.1943	0.0318	1.45	6.22	3.49	0.8872
lactose−vC	−0.4081	0.2194	0.0361	1.49	6.07	3.06	0.9051
trehalose−vB$_1$	−0.3176	0.1793	0.0335	1.40	5.81	3.66	0.9788
trehalose−vC	−0.3615	0.1883	0.0398	1.47	5.23	3.28	0.9898

[a]Experimental sorption data at a_w from 0.11 to 0.44 were used to fit equations. [b]R^2 for quadratic regression $a_w/m = \alpha a_w^2 + \beta a_w + \gamma$.

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Table 5. Onset and Endset Temperatures of Glass Transition (T_g) at Various Water Activities for Freeze-Dried Lactose−vB$_1$, Lactose−vC, Trehalose−vB$_1$, and Trehalose−vC Systems

		T_g (°C) at a_w =				
system		0.00	0.11	0.23	0.33	0.44
lactose−vB$_1$	onset	99 ± 1	50 ± 1	39 ± 1	29 ± 1	13 ± 1
	endset	117 ± 1	69 ± 1	54 ± 1	44 ± 1	27 ± 1
lactose−vC	onset	101 ± 1	47 ± 1	39 ± 1	29 ± 1	12 ± 1
	endset	117 ± 1	62 ± 1	53 ± 1	43 ± 1	26 ± 1
trehalose−vB$_1$	onset	103 ± 1	51 ± 1	39 ± 1	28 ± 1	13 ± 1
	endset	119 ± 1	68 ± 1	53 ± 1	43 ± 1	27 ± 1
trehalose−vC	onset	106 ± 1	49 ± 1	38 ± 1	27 ± 1	13 ± 1
	endset	117 ± 1	63 ± 1	53 ± 1	42 ± 1	26 ± 1

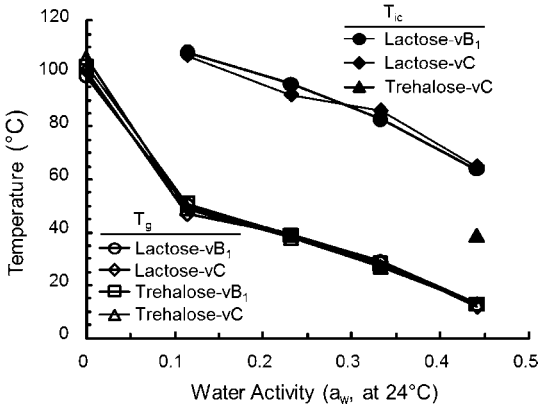

Figure 4. Effect of water activity (a_w) on the onset temperature of glass transition (T_g) and instant crystallization (T_{ic}) in freeze-dried lactose−vB$_1$, lactose−vC, trehalose−vB$_1$, and trehalose−vC systems.

Table 6. Instant Crystallization Temperature (T_{ic}) of Freeze-Dried Lactose−vB$_1$, Lactose−vC, Trehalose−vB$_1$, and Trehalose−vC Systems at Various Water Activities (a_w)

	T_{ic} (°C) at a_w =				
system	0.00	0.11	0.23	0.33	0.44
lactose−vB$_1$	N/O[a]	108 ± 1	96 ± 1	83 ± 1	64 ± 1
lactose−vC	N/O	107 ± 1	92 ± 1	86 ± 1	65 ± 1
trehalose−vB$_1$	N/O	N/O	N/O	N/O	N/O
trehalose−vC	N/O	N/O	N/O	N/O	39 ± 1

[a]N/O, not observed.

Table 7. Critical Water Content, Critical Water Activity (a_w) at 24 °C, k for Gordon−Taylor Equation, and Monolayer Water Activity of Freeze-Dried Lactose−vB$_1$, Lactose−vC, Trehalose−vB$_1$, and Trehalose−vC

system	critical water content (g/100 g solid)	critical a_w	k	monolayer a_w[a]
lactose−vB$_1$	5.5	0.32	8.1 ± 2.3	0.20
lactose−vC	4.8	0.31	9.7 ± 3.3	0.20
trehalose−vB$_1$	5.4	0.32	8.7 ± 2.4	0.21
trehalose−vC	4.8	0.31	10.3 ± 3.5	0.21

[a]a_w corresponding to the monolayer water content as predicted from the GAB model.

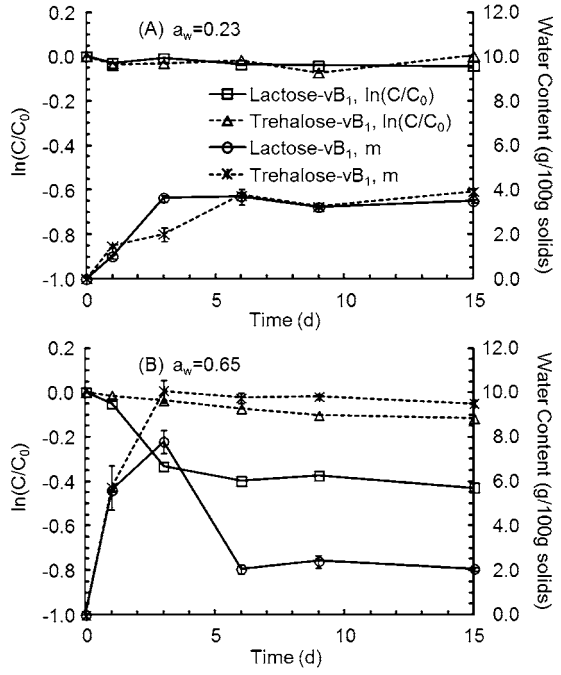

Figure 5. $\ln(C/C_0)$ of thiamin hydrochloride in lactose−vB$_1$ and trehalose−vB$_1$ systems as a function of water content (m, g/100 g solid) during 15 days of storage at a_w = (A) 0.23 and (B) 0.65.

determined for lactose−vC and trehalose−vC, respectively. At low a_w, loss of ascorbic acid was observed in lactose−vC system within 1 day of storage, but the degradation was still very slow (Figure 6A). At a_w = 0.44, concomitant loss of ascorbic acid (about 30%) and loss of water from lactose crystallization were observed at day 15 (Figure 6B). Trehalose crystallization did not cause this sharp loss of ascorbic acid in trehalose−vC system. The effect of lactose crystallization on loss of ascorbic acid was confirmed in the lactose−vC system after 1 day of storage at a_w = 0.65 (Figure 6C).

The degradation k constants were plotted as a function of water activity (a_w), as shown in Figure 7. Thiamin hydrochloride (Figure 7A) and ascorbic acid (Figure 7B) showed very low degradation rates in lactose−vB$_1$, lactose−vC, trehalose−vB$_1$, and trehalose−vC systems at a_w up to 0.44 (<0.004 and <0.006 day^{-1} for thiamin hydrochloride and ascorbic acid, respectively). The greatest stabilities of thiamin hydrochloride and ascorbic acid were observed for anhydrous systems, showing that the degradation of thiamin hydrochloride

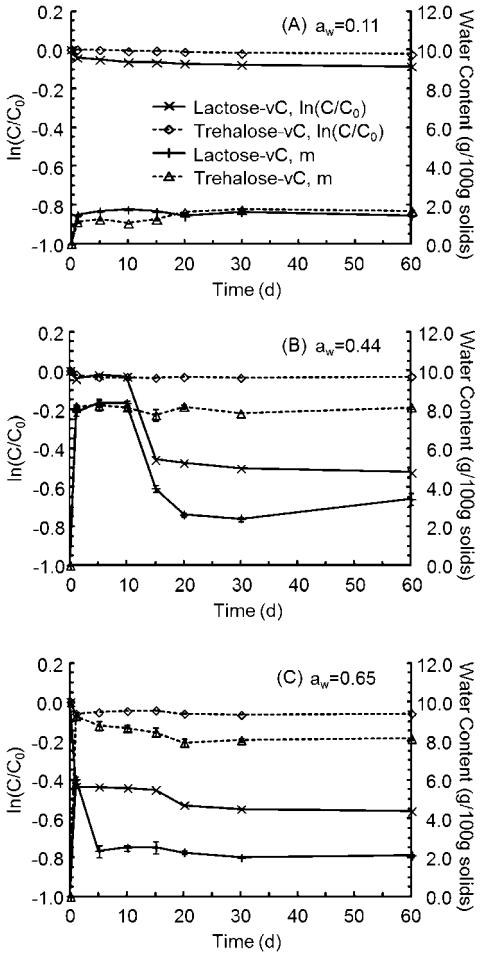

Figure 6. $\ln(C/C_0)$ of ascorbic acid in lactose–vC and trehalose–vC systems as a function of water content (m, g/100 g solid) during 60 days of storage at a_w = (A) 0.11, (B) 0.44, and (C) 0.65.

and ascorbic acid in lactose and trehalose systems was strongly influenced by water.[5,7,8] Thiamin hydrochloride exhibited less sensitivity to water as compared with ascorbic acid. At $a_w \geq 0.54$, increase of the k constants for thiamin hydrochloride and ascorbic acid in lactose and trehalose was observed. This corresponded to the phenomenon of lactose and trehalose crystallization. The effect of lactose crystallization was more severe than that of trehalose crystallization on the stability of the vitamins.

Stabilization of Vitamins: Process and Storage Stability. Diagrams of the structure formation during freezing and freeze-drying, as well as the structural changes during storage for model systems, are shown in Figure 8. Sugar–vitamin solutions that were frozen and held at different temperatures had different structures.[12] At −80 °C, a number of small ice crystals were formed as a result of fast freezing. The solute sugar was freeze-concentrated due to the crystallization of water, and a continuous amorphous glass was formed. The vitamin molecules were entrapped as dispersed particles in the structure (Figure 8A). At −35 °C, which was above the T_g' but below the T_m', the ice crystals were expected to be larger than at −80 °C due to the slower freezing rate and less rapid nucleation (Figure 8B). At −20 and −10 °C, which were above the T_m', the systems may have a large amount of unfrozen water, slow nucleation, and large ice crystals (Figure 8C). In the unfrozen sugar solutions, lactose may crystallize out due to its

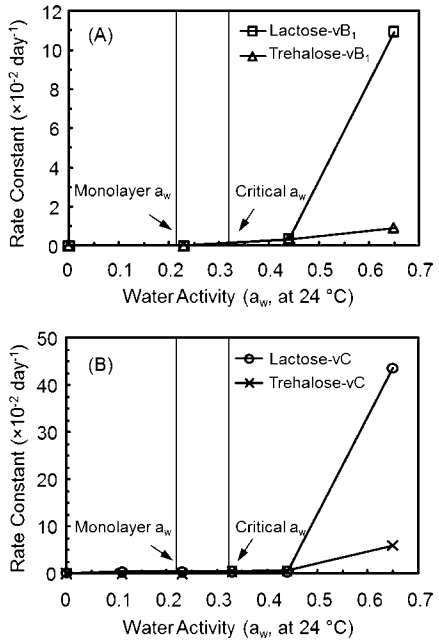

Figure 7. First-order rate constant ($\times 10^{-2}$ day^{-1}) of (A) thiamin hydrochloride and (B) ascorbic acid in freeze-dried lactose–vB_1, lactose–vC, trehalose–vB_1, and trehalose–vC systems as a function of water activity (a_w). The a_w values corresponding to monolayer water content and critical a_w at 24 °C are indicated with arrows.

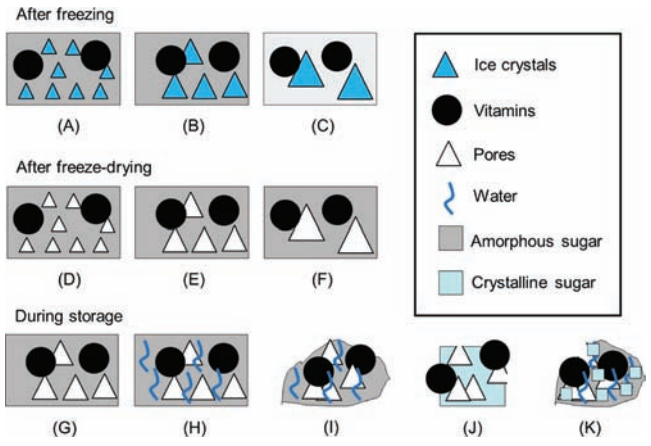

Figure 8. Diagrams of the model systems frozen at temperatures below T_g' (A) and then freeze-dried (D), frozen between T_m' and T_g' (B) and then freeze-dried (E), and frozen above T_m' (C) and then freeze-dried (F). During storage, the freeze-dried systems could be anhydrous (G), water-plasticized (H), and collapsed (I). Crystallization of sugars may lead to compact lactose crystals (J) or an amorphous sugar–water phase with dispersed vitamins and trehalose crystals (K). The symbols do not represent the real sizes of components.

low solubility (Figure 1). Freezing is important for the structure of the freeze-dried systems. Pores were left after sublimation of ice crystals, and the size of ice crystals determined the porosity of the freeze-dried matrices. The vitamins were either well entrapped in the thick glassy membranes or partially exposed to the pores (Figure 8D–F).

In the present study, the solutions were frozen at −35 °C and freeze-dried. The anhydrous glasses were very hydroscopic due to the high surface areas and porosity (Figure 8G). Water was sorbed when the materials were exposed to the humidity. Both

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the sugar and the vitamin sites may be hydrogen-bonded to water molecules until the monolayer water content is reached (Figure 8H). The corresponding a_w for monolayer water content was about 0.2 (Table 7). At this a_w, the systems showed T_g around 40 °C, which was higher than room temperature, indicating the systems were in the glassy state during storage. No significant change of k constants was observed at a_w = 0.23. The stability of vitamins was possibly retained due to the low molecular mobility in the viscous solids,[33] although above that monolayer value, water became increasingly available for chemical reactions.[9] Therefore, thiamin hydrochloride and ascorbic acid were generally considered to be stable in glassy lactose and trehalose matrices. However, further water plasticization caused the depression of the glass transition temperature to the room temperature (storage temperature in the present study, 24 ± 1 °C), and the materials could undergo glass transition. The molecular mobility may be increased significantly above the T_g.[13,33] A slight increase of k constants was found in lactose−vB_1, lactose−vC, trehalose−vB_1, and trehalose−vC systems at a_w = 0.33 and 0.44, which were higher than the critical a_w (Table 5). Therefore, the critical a_w had a larger correlation to the degradation of thiamin hydrochloride and ascorbic acid compared with the a_w corresponding to the monolayer water content, and the glass transition properties should be considered in the stabilization of water-soluble active components,[13] in agreement with Chen and others[18] for tyrosinase and with Bell and White[19] for thiamin.

The effects of glass transition on the stability of the entrapped sensitive compounds were pronounced when time-dependent physical changes occurred above the T_g. The materials may no longer retain the structure and collapse may happen during storage due to the softening (Figure 8I). When surface saturation of water sorption was reached, as well as the increased molecular mobility, the sugar molecules gained translational mobility and approached other sugar molecules and formed sugar crystals. The presence of vitamin molecules disturbed the sugar−sugar interactions and delayed the lactose and trehalose crystallization. Water sorption-induced sugar crystallization occurred in all the systems, as shown in Figures 5B, 6B, and 6C. Sharp increase of rate constants was found for lactose-vB_1 (Figure 7A) and lactose-vC (Figure 7B) systems, while much smaller changes of rate constants were found for trehalose-vB_1 (Figure 7A) and trehalose-vC (Figure 7B) systems. The vitamins may be excluded from the continuous phase and exposed to the environment (Figure 8J), which could be the main cause of the loss of vitamins. However, trehalose-containing systems retained most of the vitamins after crystallization. This could possibly result from their protection by the viscous trehalose syrup (Figure 8K), because trehalose crystals showed higher solubility and the extent of trehalose crystallization was less than that of lactose (Figure 1).

In addition to the physical changes above the T_g, other factors dependent on the T_g may also affect the stability of vitamins. For example, the rate of nonenzymatic browning was extremely low below the T_g and increased sharply above the T_g due to the increased diffusion.[20,34] Browning was visually observed in lactose−vB_1 and lactose−vC systems at a_w = 0.65 during storage, but not in trehalose−vB_1 and trehalose−vC systems, suggesting that reactions between thiamin hydrochloride or ascorbic acid and reducing sugar occurred.[35−37] This could be responsible for the higher loss of vitamins in lactose-containing systems.

In conclusion, the storage stability of thiamin hydrochloride below the melting temperature of the maximally freeze-concentrated lactose−vB_1 and trehalose−vB_1 systems (T_m') was retained, possibly due to the low rate of chemical reactions at low temperatures and the low diffusion rates in the viscous, freeze-concentrated solutes. The stability of thiamin hydrochloride in the systems above T_m' may be enhanced by the low pH. Thiamin hydrochloride and ascorbic acid showed good stability in freeze-dried lactose−vB_1, lactose−vC, trehalose−vB_1, and trehalose−vC systems at $a_w \leq 0.44$ water activity, especially at a_w = 0. The critical a_w had a larger effect on the degradation of thiamin hydrochloride and ascorbic acid compared with the a_w corresponding to the monolayer water content. The critical a_w related the glass transition temperature, T_g, to the a_w of the systems; it interpreted the T_g of a water-plasticized solid at room temperature (24 °C) in terms of the extent of water plasticization (at which a_w the systems were equilibrated). Above the critical a_w (i.e., the room temperature was higher than the T_g), the molecular mobility in the solids was increased; time-dependent changes, particularly component sugar crystallization, caused the loss of the water-soluble vitamins. Therefore, the T_g should be considered in the stability assessment of vitamins. Vitamins appeared to show plasticizing effects on the sugars, especially in the anhydrous state, and appeared to delay sugar crystallization, which was more dependent on the molar ratio of sugar:vitamin than their weight ratios. Depending on the crystallization properties of the sugars, different protecting effects of the sugars on the stability of water-soluble vitamins would be expected. Trehalose, because of its higher solubility, showed better stabilizing properties on the water-soluble vitamins than lactose, even when trehalose crystallization occurred.

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ABBREVIATIONS USED

vB_1, vitamin B; vC, vitamin C; w/v, weight per volume; w/w, weight per weight; a_w, water activity; T_g', glass transition temperature of maximally freeze-concentrated solute; T_m', ice-melting temperature in the maximally freeze-concentrated systems; T_g, glass transition temperature; GAB, Guggenheim−Anderson−de Boer relationship; m, water content; m_m, monolayer water content; FCR, Folin−Ciocalteu reagent; WLF, Williams−Landel−Ferry equation.

REFERENCES

(1) Fennema, O. R. Loss of vitamins in fresh and frozen foods. *Food Technol.* **1977**, *31* (12), 32−36.

(2) Uddin, M. S.; Hawlader, M. N. A.; Ding, L.; Mujumdar, A. S. Degradation of ascorbic acid in dried guava during storage. *J. Food Eng.* **2002**, *51*, 21−26.

(3) Labuza, T. P.; Kamman, J. F. Comparison of stability of thiamine salts at high temperature and water activity. *J. Food Sci.* **1982**, *47*, 664−665.

(4) Pachapurkar, D.; Bell, L. N. Kinetics of thiamine degradation in solutions under ambient storage conditions. *J. Food Sci.* **2005**, *70* (7), 423−426.
(5) Dennison, D. B.; Kirk, J. R. Oxygen effect on the degradation of ascorbic acid in a dehydrated food system. *J. Food Sci.* **1978**, *43*, 609−612, 618.
(6) Dennison, D. B.; Kirk, J. R. Effect of trace mineral fortification on the storage stability of ascorbic acid in a dehydrated model food system. *J. Food Sci.* **1982**, *47*, 1198−1200.
(7) Lee, S. H.; Labuza, T. P. Destruction of ascorbic acid as a function of water activity. *J. Food Sci.* **1975**, *40*, 370−373.
(8) Laing, B. M.; Schlueter, D. L.; Labuza, T. P. Degradation kinetics of ascorbic acid at high temperature and water activity. *J. Food Sci.* **1978**, *43*, 1440−1443.
(9) Labuza, T. P. The effect of water activity on reaction kinetics in food deterioration. *Food Technol.* **1980**, *34* (4), 36−41.
(10) Labuza, T. P.; Tannenbaum, S. R.; Karel, M. Water content and stability of low moisture and intermediate moisture foods. *Food Technol.* **1970**, *24*, 543−550.
(11) Hancock, B. C.; Zografi, G. Characteristics and significance of the amorphous state in pharmaceutical systems. *J. Pharm. Sci.* **1997**, *86* (1), 1−12.
(12) Levine, H.; Slade, L. Principles of "cryostabilization" technology from structure/property relationships of carbohydrate/water systems − a review. *CryoLetters* **1988**, *9*, 21−63.
(13) Slade, L.; Levine, H. Beyond water activity: recent advances based on an alternative approach to the assessment of food quality and safety. *Crit. Rev. Food Sci. Nutr.* **1991**, *30* (2−3), 115−360.
(14) Roos, Y.; Karel, M. Differential scanning calorimetry study of phase transitions affecting the quality of dehydrated materials. *Biotechnol. Prog.* **1990**, *6* (2), 159−163.
(15) Chuy, L. E.; Labuza, T. P. Caking and stickiness of dairy-based food powders as related to glass transition. *J. Food Sci.* **1994**, *59*, 43−46.
(16) Bhandari, B. R.; Datta, N.; Howes, T. Problems associated with spray drying of sugar-rich foods. *Drying Technol.* **1997**, *15* (2), 671−684.
(17) Zhou, Y.; Roos, Y. H. Characterization of carbohydrate-protein matrices for nutrient delivery. *J. Food Sci.* **2011**, *76* (4), 368−376.
(18) Chen, Y.-H.; Aull, J. L.; Bell, L. N. Solid-state tyrosinase stability as affected by water activity and glass transition. *Food Res. Int.* **1999**, *32*, 469−472.
(19) Bell, L. N.; White, K. L. Thiamin stability in solids as affected by the glass transition. *J. Food Sci.* **2000**, *65* (3), 498−501.
(20) Miao, S.; Roos, Y. H. Nonenzymatic browning kinetics in low-moisture food systems as affected by matrix composition and crystallization. *J. Food Sci.* **2005**, *70* (2), 69−77.
(21) Miao, S.; Roos, Y. H. Crystallizationkinetics and X-ray diffraction of crystals formed in amorphous lactose, trehalose, and lactose/trehalose mixtures. *J. Food Sci.* **2005**, *70* (5), 350−358.
(22) Labuza, T. P.; Kaanane, A.; Chen, J. Y. Effect of temperature on the moisture sorption isotherms and water activity shift of two dehydrate foods. *J. Food Sci.* **1985**, *50*, 385−91.
(23) Roos, Y. H. Water activity and physical state effects on amorphous food stability. *J. Food Process. Preserv.* **1993**, *16*, 433−447.
(24) Bizot, H. Using the "G.A.B." model to construct sorption isotherms. In *Physical Properties of Foods*; Jowitt, C. R., Escher, F., Hallströom, B., Meffert, H. F. T., Spiess, W. E. L., Vos, G., Eds.; Applied Science Publishers: London, U.K., 1983; pp 43−54.
(25) Haque, K.; Roos, Y. H. Water plasticization and crystallization of lactose in spray dried lactose/protein mixtures. *J. Food Sci.* **2004**, *69* (1), 23−29.
(26) Gordon, M.; Taylor, J. S. Ideal copolymers and the second-order transitions of synthetic rubbers, I. Non-crystalline copolymers. *J. Appl. Chem.* **1952**, *2*, 493−500.
(27) Johari, G. P.; Hallbrucker, A.; Mayer, E. The glass-liquid transition of hyper-quenched water. *Nature* **1987**, *330*, 552−553.
(28) Jouppila, K.; Roos, Y. H. Glass transitions and crystallization in milk powders. *J. Dairy Sci.* **1994**, *77* (10), 2907−2915.
(29) Dinç, E.; Kökdil, G.; Onur, F. A comparison of matrix resolution method, ratio spectra derivative spectrophotometry and HPLC method for the determination of thiamine HCl and pyridoxine HCl in pharmaceutical preparation. *J. Pharm. Biomed. Anal.* **2000**, *22*, 915−923.
(30) Proot, P.; De Pourcq, L.; Raymarkers, A. A. Stability of ascorbic acid in a standard total parenteral nutrition mixture. *Clin. Nutr.* **1994**, *13*, 273−279.
(31) Hunziker, O. F.; Nissen, B. H. Lactose solubility and lactose crystal formation. *J. Dairy Sci.* **1926**, *9* (6), 517−537.
(32) Lammert, A. M.; Schmidt, S. J.; Day, G. A. Water activity and solubility of trehalose. *Food Chem.* **1998**, *61* (1/2), 139−144.
(33) Roos, Y. H. *Phase Transitions in Foods*; Academic Press: San Diego, CA, 1995.
(34) Roos, Y. H.; Himberg, M.-J. Nonenzymatic browning behavior, as related to glass transition, of a food model at chilling temperatures. *J. Agric. Food Chem.* **1994**, *42*, 893−898.
(35) Hodge, J. E. Chemistry of browning reactions in model systems. *J. Agric. Food Chem.* **1953**, *1* (15), 928−943.
(36) Brownley, C. A. Jr.; Lachman, L. Browning of spray-processed lactose. *J. Pharm. Sci.* **1964**, *53* (4), 452−454.
(37) Doyon, L.; Smyrl, T. G. Interaction of thiamine with reducing sugars. *Food Chem.* **1983**, *12*, 127−133.