Nonenzymatic Browning Kinetics in Low-moisture Food Systems as Affected by Matrix Composition and Crystallization

SONG MIAO AND YRIJO H. ROOS

ABSTRACT: Nonenzymatic browning (NEB) in lactose, trehalose, and lactose/trehalose food model systems containing L-lysine and D-xylose (5% w/w) as reactants was studied at 4 different relative vapor pressure (RVP) (33.2%, 44.1%, 54.5%, 65.6%) environments at room temperature. Sorption isotherms of model systems were determined gravimetrically, and data were modeled using the Brunauer-Emmett-Teller (BET) and Guggenheim-Anderson-deBoer (GAB) models. Glass transition, \( T_g \), was measured by differential scanning calorimetry (DSC). NEB was followed spectrophotometrically. Although the 3 model systems showed similar glass transition behavior, different crystallization properties were observed from loss of sorbed water. Mixtures of trehalose and lactose showed delayed crystallization of component sugars. The NEB rate was affected by sugar composition. At a low RVP (33.1%) environment, the NEB rate in trehalose system was higher than in the lactose/trehalose system, and the NEB rate in lactose system was the lowest. The NEB rate in different models seemed to be affected by component crystallization. The highest extent of browning in the trehalose matrix system seemed to be related to the formation of trehalose crystals in the system after crystallization at high RVP. The results indicated that the composition of the carbohydrate-based low-moisture real food systems should be considered in controlling NEB reaction.

Keywords: crystallization, lactose, nonenzymatic browning, water content, trehalose

Introduction

Stability of food and other biological systems containing sugars and/or polymers is often related to the properties of their amorphous phases (O’Brien 1996). The physical state of amorphous matrix greatly influences their stability and affects both physical and chemical changes during processing and storage (Eichner 1981; Slade and Levine 1991; Karwas and others 1992; Buera and Karel 1995). The glass transition controls the physical state and physical characteristics of amorphous food materials and has been related to their chemical stability (Sperling 1986; Roos and Karel 1991; Slade and Levine 1991; Roos 1995; Roos and others 1996b; Champion and others 2000). The glass transition has been recognized as a possible factor affecting kinetics of enzymatic and nonenzymatic changes in low-moisture foods (Slade and Levine 1991; Karmas and others 1992; Karmas and Karel 1994; Schebor and others 1999; Kouassi and Roos 2000, 2001).

Nonenzymatic browning (NEB) through the Maillard reaction is a major deteriorative factor in the storage of dehydrated dairy food products (Labuza and Saltmarch 1981; Friedman 1996; Roos and others 1996b; van Boekel 2001). NEB may cause unacceptable nutritional and sensory effects in some stored food products and may be a limiting factor in the shelf life of some products (O’Brien and Morrissey 1989; O’Brien 1996). NEB as a model of a possible diffusion-controlled binary reaction (Buera and Karel 1995) between an amino acid and a reducing sugar has probably been given the most attention in studies of relationships between reaction rates and the physical state controlled by the glass transition (Karmas and others 1992; Karmas and Karel 1994; Roos and Himberg 1994; Bell 1996; Bell and others 1998; Lievonen and others 1998; Schebor and others 1999; Lievonen and Roos 2002b). The kinetics of NEB have been studied as a function of many parameters (water activity, temperature, reactant concentration pH, and so forth) and even related to physical aspects such as crystallization (Saltmarch and others 1981; Vuataz 1988; Buera and Karel 1995) and viscosity (Eichner 1981). The glass transition together with material composition, water activity, temperature, and other factors affect the rate of the reaction (Bell 1996; Roos and others 1996b; Lievonen and others 1998, 2002; White and Bell 1999; Lievonen and Roos 2002b; Mia and Roos 2004b).

Time-dependent crystallization of amorphous carbohydrates is known to result in serious adverse quality losses in dairy food powders during storage (Saltmarch and others 1981; Roos and Karel 1992; Roos and others 1996b; Biliaderis and others 2002). Crystallization of component sugars may enhance the rate of an enzymatic reaction as a result of release of sorbed water during the crystallization process (Cardona and others 1997; Kouassi and Roos 2000). The crystallization of amorphous lactose in dehydrated milk products has also been observed to result in acceleration of NEB reactions, loss of lysine as well as other deteriorative changes and caking (Labuza and Saltmarch 1981; Saltmarch and others 1981; Roos 2001). The rate of crystallization depends on the composition of food materials (Arvanitoyannis and Blandshard 1996; Shambolin and others 1996; Biliaderis and others 2002). Addition of a 2nd polymeric additive, such as maltodextrin, to an amorphous matrix delayed crystallization (Roos and Karel 1991; O’Brien 1996; Shambolin and others 1996; Gabarra and Hartel 1998; Biliaderis and others 2002); but in the food industry, because of quality and sensorial considerations, addition of polymeric materials is often replaced by addition of a material with similar characteristics, such as sugars (Mazzobre and others 2001).
Trehalose has been reported to be the most effective cryopreservation sugar for stabilizing proteins and membranes against damage caused by desiccation or freezing (Crowe and others 1984; Leslie and others 1995; Uritani and others 1995). Several studies have reported stabilization of enzymes by incorporation of trehalose in matrices (Cardona and others 1997; Mazzobro and others 1997a, 1997b; Schebor and others 1997). As a nonreducing sugar, trehalose has also been used as an ingredient in the food industry (Roser 1991; Schiraldi and others 2002). In dairy products, lactose is a very important component dominating the physical and chemical properties of dairy powders. The physical state of lactose is important in maintaining food quality (Simatos and Karel 1988; Hartel and Shastry 1991; Roos and Karel 1992). Crystallization of lactose has been reported to be delayed by trehalose (Mazzobro and others 2001). We presumed the physical properties of sugar-containing dairy products were affected by adding trehalose as a new ingredient, and thus the chemical stability might also be affected during storage.

The objective of the present work was to study NEB kinetics in lactose, trehalose, and lactose/trehalose matrices as affected by glass transition and water content changes, and to evaluate the effects of matrix composition and component crystallization on NEB behavior.

Materials and Methods

Preparation of food models

Three amorphous food model systems were prepared. The models consisted of lactose (Sigma Chemical Co., St. Louis, Mo., U.S.A.), trehalose (British Sugar Co., Peterborough, U.K.), and lactose/trehalose (1:1) as matrix materials, and they contained L-lysine and D-xylose (Sigma Chemical Co.). 1:1 as reactants. According to the results of previous studies (Miao and Roos 2004a) and pre-experiments carried out in the present study, the exact amounts of the reactants were adjusted to 5% (w/w) of the total solids. Lactose, lactose/trehalose (1:1), and trehalose were dissolved in distilled water under mild heating to obtain clear solutions. The reactants were then added after cooling to about 20 °C and mixed (cooling was essential to minimize browning during mixing), final 20% (w/w solids) clear solutions of the matrix materials, the reactants and distilled water were thus prepared. Solutions in 20- to 22-mL aliquots were frozen on Petri dishes immediately after preparation (24 h at –20 °C, followed by 24 h at –80 °C) and freeze-dried (>48 h, P < 0.1 mbar) (Lyovac GT 2; Amsco Finn-Aqua GmbH, Hürth, Germany) to produce amorphous glassy food models (Roos and Karel 1991). The freeze-dried materials were ground using a mortar and pestle immediately after freeze-drying. Ground powders were subsequently stored in evacuated desiccators over P2O5 for a period of no less than 1 wk. These samples were taken as “zero” moisture content (Buera and others 1992) before experiments.

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The Brunauer-Emmett-Teller (BET) (Brunauer and others 1938; Roos 1993) and Guggenheim-Anderson-deBoer (GAB) (van den Berg and Bruin 1981; Roos 1993) sorption isotherm models were fitted into water sorption data using linear regression analysis and quadratic analysis, as described by Jouppila and Roos (1994). The BET model is described as Eq. 1:

\[ \frac{m}{m_w} = \frac{a_w}{m(1-a_w)} = \frac{a_w(K-1)}{m_w K} + \frac{1}{m_w K} \]  

where \( m_w \) is the BET monolayer value (g water/100 g solids), and \( K \) is a constant. Equation 1 can be simplified to a linear form as shown by Eq. 2:

\[ \frac{a_w}{m(1-a_w)} = b a_w + c \]  

The GAB isotherm model is described by Eq. 3:

\[ \frac{m}{m_w} = \frac{C K a_w}{(1-K a_w)(1-a_w+C K a_w)} \]  

The GAB model can also be written to the form of the second-order polynomial:

\[ \frac{a_w}{m} = c a_w^2 + b a_w + \gamma \]  

from which the constants \( C, K' \) and \( m_w \) can be calculated as described by Roos (1995).

The relative percentage Root Mean Square (RMS-value) was calculated as an indication of the fit of the BET and GAB models (Bizot 1983; Lievonen and Roos 2002a).

\[ \text{RMS} = \frac{\sum \left( W_i - W_i^* \right)^2}{N} \times 100 \]  

where \( W_i \) is the experimental water content; \( W_i^* \) is the calculated water content; and \( N \) is the number of experimental points.

Differential scanning calorimetry

The glass transition temperatures for the 3 different models stored at different RVPs were determined using Differential Scanning Calorimetry (DSC) (Mettler Toledo 821e system with 821e TA processor, DCS 821e measuring cell, and STAR® Thermal Analysis System version 6.0 software; Mettler-Toledo AG, Schwerzenbach, Switzerland). The instrument was calibrated for temperature and
heat flow as reported by Miao and Roos (2004a). Samples (9 to 15 mg) were prepared in preweighed DSC aluminum pans (40 μL; Mettler Toledo-2731, Switzerland) and stored in open pans for 120 h at room temperature in evacuated desiccators over different saturated salt solutions: LiCl, CH₃COOK, MgCl₂, K₂CO₃ (Sigma Chemical Co.) with respective RVPs of 11.4%, 23.1%, 33.2%, and 44.1%, giving a water activity, a_w, of 0.01 × RVP at equilibrium (Labuza and others 1985). After equilibration, the pans were hermetically sealed. Triplicate samples of each material were analyzed. An empty pan was used as a reference. The samples were scanned 1st at 5 °C/min over the glass transition range, then cooled at 10 °C/min to at least 30 °C below the glass transition, and an immediate 2nd heating scan at 5 °C/min was run to at least 30 °C above the glass transition for each sample to eliminate relaxations and improve interpretation of the thermograms. The glass transition temperature, T_g, was taken as the onset temperature of the glass transition temperature range. An average of 3 replicate samples was used as the glass transition temperature.

The Gordon–Taylor (Gordon and Taylor 1952) Eq. 6 was fitted to the experimental glass transition temperature data:

\[ T_g = \frac{w_1 T_{g1} + w_2 T_{g2}}{w_1 + w_2} \]

where \( w_1 \) and \( w_2 \) are the weight fractions of the solute and water respectively, \( T_{g1} \) is the \( T_g \) of anhydrous solute, \( T_{g2} \) is the \( T_g \) of amorphous water, –135 °C (Johari and others 1987) and \( k \) is a constant. The value for \( k \) was the mean derived from the experimental \( T_g \) and water content data as described by Jouppila and Roos (1994). Equation 9 was applied in the present study for predicting water plasticization of the multicomponent model systems, considering that all solid components contributing to the observed glass transition were miscible and formed a single phase (Roos 1995). Because substantial browning occurred at high temperatures above 100 °C, water produced in the reaction plasticized the samples (Roos and others 1996b). Hence, no reliable glass transition temperature (\( T_g \)) could be measured for the anhydrous model systems. We assumed that the low concentration of reactants only slightly affected the \( T_g \) and we used the \( T_g \)'s of the lactose, lactose/trehalose, and trehalose systems as the \( T_g \)'s for the anhydrous lactose/reactant, lactose/trehalose/reactant, and trehalose/reactant systems.

Nonenzymatic browning

Aliquots of 1 g of the powdered freeze-dried model materials were transferred into 20-mL glass vials, and the glass vials were stored in the desiccators at 4 RVP values ranging from 33% to 65% (22 °C to 23 °C). Four saturated salt solutions (MgCl₂, K₂CO₃, Mg(NO₃)₂, and NaN₃) were used to achieve RVP values of 33.2%, 44.1%, 54.5%, and 65.6%, respectively (Greenspan 1977; Labuza and others 1985). Triplicate samples were removed at 10-h to 24-h intervals depending on the RVP, and subsequently stored at ~80 °C to avoid further browning before analysis. The extent of browning was determined spectrophotometrically (Perkin-Elmer Lambda 2 UV-VIS spectrometer; Norwalk, Conn., U.S.A.) from the optical density (OD) at 280 nm, which is considered to indicate formation of furfural compounds (Hodge and Osman 1976; Resnik and Chirife 1979) and is used to detect products of early stage of browning (Flink 1983), and at 420 nm for yellow and brown pigments (Whistler and Daniel 1985). Samples were dissolved with 40 mL of distilled water at 5 °C to avoid further browning and dilution when necessary to obtain reliable absorbance readings. Rate constants of the browning, \( k \), and their 95% confidence limits, and the coefficients of determination (\( R^2 \)) were calculated from zero-order kinetics using a linear regression analysis (Labuza and Baïsier 1992).

**Results and Discussion**

**Water sorption**

The freeze-dried lactose/reactant, lactose/trehalose/reactant, and trehalose/reactant model systems were very hygroscopic. When exposed to the RVPs ≤ 44.1% environments, steady-state water contents were reached after 24 h storage at room temperature (22 °C to 23 °C), as showed in Figure 1. When models were exposed to RVPs ≥ 54.5%, the 3 systems showed different sorption behavior (Figure 2). Increasing sorption of water followed by a decrease in sorbed water content was observed in 3 systems at RVPs ≥ 65.6%. The loss of sorbed water at higher RVPs resulted from component crystallization (Berlin and others 1968; Vuataz 1988; Lai and Schmidt 1990; Roos and Karel 1992; Jouppila and Roos 1994; Haque and Roos 2004). Compared with the lactose/reactant and trehaloseAYOUTER/REACTANT systems, crystallization of component sugars in the lactose/trehalose/reactant system occurred more slowly (Mazzobbe and others 2001; Miao and Roos 2004a). At 54.5% RVP, lactose/trehalose/reactant system did not show decreasing water contents within 250 h, whereas loss of sorbed water in the lactose/reactant system and trehalose/reactant system was observed after 48 h and 43 h, respectively. As showed in Figure 2, at RVP 65.6% and 76.1%, loss of sorbed water in the lactose/reactant system and trehalose/reactant system was observed after 21 h and 8 h of storage, in the trehalose/reactant system after 5 h and 3 h, respectively, and in the lactose/trehalose/reactant system, after 79 and 33 h, respectively. The loss of water during sorption study was used by several researchers as the indicator for studying the lactose crystallization in different systems (Berlin and others 1968; Linko and others 1982; Vuataz 1988; Lai and Schmidt 1990; Roos and Karel 1992; Jouppila and Roos 1994; Saleki-Gerhardt and Zografì 1994; Haque and Roos 2004). Although the main component of the lactose/reactant system is lactose (90%), the sorption results in the present study showed that time-dependent crystallization of lactose in the lactose/reactant system was delayed compared with the pure lactose reported by other authors (Haque and Roos 2004; Jouppila and Roos 1994; Miao and Roos 2004a). In our previous study (Miao and Roos 2004a), crystallization of freeze-dried pure lactose was observed from the loss of sorbed water after 3 h, 4 h, and 11 h of storage at 76.1%, 65.6%, and 54.5% RVPs, respectively. Jouppila and Roos (1994) reported pure lactose crystallized within 24 h at RVPs > 40%, whereas Haque and Roos (2004) observed that the loss of the sorbed water in spray-dried lactose powder appeared after 2 h, 3 h, and 20 h of storage at 76.1%, 65.6%, and 54.5% RVPs, respectively. This was probably because of the addition of the reactants and the difference in materials and sorption conditions. Iglesias and others (1997) studied the sorption isotherm of amorphous trehalose; time-dependent trehalose crystallization observed in their study was similar to the present study for the trehalose/reactant system. In the present study, 3 model systems sorbed different amounts of water before showing the loss of water when exposed to high RVP environments, especially at 65.6% and 76.1% RVPs. The lactose/trehalose/reactant system sorbed 19.3 and 25.2 g water/100 g dry solids, respectively; the trehalose/reactant system sorbed 14.4 and 16.8 g water/100 g solids; and the lactose/reactant system sorbed 12.6 and 13.1 g/100 g solids before the lactose crystallization was observed from the loss of sorbed water after 24 h at RVPs > 40%, whereas Haque and Roos (2004) observed that the loss of the sorbed water in spray-dried lactose powder appeared after 2 h, 3 h, and 20 h of storage at 76.1%, 65.6%, and 54.5% RVPs, respectively. 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The lactose/trehalose/reactant system sorbed 19.3 and 25.2 g water/100 g dry solids, respectively; the trehalose/reactant system sorbed 14.4 and 16.8 g water/100 g solids; and the lactose/reactant system sorbed 12.6 and 13.1 g/100 g solids before the loss of water occurred at 65.6% and 76.1% RVPs. This higher water content in the lactose/trehalose/reactant system probably related to the interaction between the lactose and trehalose in the mix. In fact, the lactose/trehalose/reactant system also took longer time to...
release the sorbed water during crystallization. At RVPs ≥ 65.6%, loss of water after initial crystallization in 3 model systems leveled off after 150 h of storage (Figure 2b and 2c), crystallization of component sugars in the model systems was considered to proceed only to a given extent.

The experimental sorbed water contents of the 3 model systems stored for 144 h at various RVPs at room temperature are given in Table 1. At lower RVPs (≤ 44.1%), leveled-off water contents in the 3 systems were close to each other. At higher RVPs (≥ 54.5%), after crystallization of components, 3 model systems had different water contents. The effect of crystallization on water content in a system containing crystalline and amorphous components was dependent on the type of crystals (Karmas 1994; Roos 1995; Cardona and others 1997). Formation of anhydrous crystals (such as lactose and sucrose) released water (Iglesias and Chirife 1978; Roos 1995), but during formation of hydrated crystals (such as monohydrate lactose and dihydrate trehalose), water was sorbed (Karmas 1994; Iglesias and others 1997). The amount of water in the trehalose dihydrate was 10.5% (dry trehalose basis). This was close to the water content corresponding to the plateau in the sorption isotherm (Figure 2) of the trehalose/reactant model. These water contents for trehalose/reactant systems in the present study agreed with the results of Karmas (1994), Iglesias and others (1997), and Cardona and others (1997). The water contents in the lactose/reactant system after crystallization ranged from 1.7 to 5.7 g/100 g of dry solids depending on RVP, which were slightly higher than those for pure lactose reported in previous studies (Jouppila and Roos 1994, Miao and Roos 2004a), indicating that reactant in the system might have sorbed more water after lactose crystallization or there could be incomplete crystallization of lactose in presence of reactants.

**Sorption isotherm**

The BET and the GAB sorption isotherms could be used to model experimental sorption data of the 3 model systems over the a_w range from 0.114 to 0.441, as shown in Figure 3. The isotherms had the sigmoid shape typical of most food materials (Biliaderis and others 1999; Bell and Labuza 2000). The BET and GAB monolayer values and constants for lactose/reactant, lactose/trehalose/reactant, and trehalose/reactant systems are given in Table 2. The GAB model showed better fit to the sorption data than the BET model,
as observed from a smaller RMS value (Bizot 1983; Lievonen and Roos 2002a; Miao and Roos 2004b). The GAB monolayer values were 5.20, 3.95, and 4.56 g water/100 g solids, whereas the BET monolayer values were 6.57, 6.89, and 6.68 g water/100 g solids for lactose/reactant, lactose/trehalose/reactant, and trehalose/reactant systems, respectively. The corresponding \( a_{w} \) for the GAB monolayer values were 0.24, 0.16, and 0.19 for lactose/reactant, lactose/trehalose/reactant, and trehalose/reactant systems, respectively, and the corresponding \( a_{w} \) for the BET monolayer values were 0.31, 0.31, and 0.30, respectively.

**Glass transition temperature**

The glass transition temperatures, \( T_g \), of 3 model systems stored at various RVPs for 144 h are given in Table 3. At RVPs \( > 54.5\% \), lactose and trehalose in lactose/reactant and trehalose/reactant systems crystallized during equilibration; no glass transition could be observed. The glass transition of the lactose/reactant system was close to that of pure lactose observed by Miao and Roos (2004a) and slightly lower than that of lactose found by Jouppila and Roos (1994). The glass transition temperatures of the trehalose/reactant system at different RVPs were lower than those measured by Cordona and others (1997) for trehalose; this was probably due to the additional plasticization of the trehalose by xylose and lycine (Roos and Karel 1991; Buera and others 1992). The Gordon-Taylor (Gordon and Taylor 1952) Eq. 9 was successfully fitted to the experimental glass transition temperatures, \( T_g \), of the 3 model systems, as shown in Figure 4. Water plasticized the food models and caused a substantial decrease of the glass transition (Roos 1987; Slade and Levine 1991; Roos 1993). The constant, \( K \), for the Gordon-Taylor equation was found to be 7.6 ± 0.8 for lactose/reactant systems, 7.2 ± 0.7 for lactose/trehalose/reactant systems, and 7.9 ± 0.9 for trehalose/reactant systems. The small difference between the \( K \) values and the close glass transition temperature (Table 3) indicated that the 3 model systems had similar glass transition behavior, which was typical of lactose-based dairy products (Miao and Roos 2004a). Glass transition is often related to the stability of amorphous foods (White and Cakebread 1966; Roos and Karel 1990; Slade and Levine 1991). The critical water content was defined as the water content that depressed \( T_g \) to ambient temperature (Roos 1993). The critical water contents at 23°C in the present study obtained from \( T_g \) data for lactose/reactant, lactose/trehalose/reactant, and trehalose/reactant systems were 7.0, 7.4, and 7.1 g/100 g of dry solids, respectively, which were higher than the GAB monolayer water contents given in Table 3. Consequently, the critical water activity calculated from GAB model for the lactose/reactant, lactose/trehalose/reactant, and trehalose/reactant systems were 0.34, 0.35, 0.33 accordingly with the results for dehydrated milk products reported by Lai and Schmidt (1990), Roos (1993), and Jouppila and Roos (1994). Most deteriorative reactions, such as NEB, are suggested to be more related to molecular mobility above \( T_g \) rather than the monolayer value (Slade and Levine 1991; Kar-

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**Table 1—Water contents (mean ± SD) of model systems stored for 144 h at various relative vapor pressure (RVP) at room temperature (22°C to 23°C) \( (n = 3, SD = \text{standard deviation})^{a} \)**

<table>
<thead>
<tr>
<th>Salt</th>
<th>RVP (%) of water</th>
<th>L/R</th>
<th>L/T/R</th>
<th>T/R</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiCl</td>
<td>11.4</td>
<td>2.89 ± 0.08</td>
<td>3.31 ± 0.09</td>
<td>3.26 ± 0.15</td>
</tr>
<tr>
<td>CH₃COOK</td>
<td>23.1</td>
<td>5.03 ± 0.03</td>
<td>4.96 ± 0.14</td>
<td>5.37 ± 0.12</td>
</tr>
<tr>
<td>MgO₂</td>
<td>33.3</td>
<td>6.71 ± 0.03</td>
<td>7.04 ± 0.02</td>
<td>6.88 ± 0.06</td>
</tr>
<tr>
<td>K₂CO₃</td>
<td>44.1</td>
<td>9.48 ± 0.11</td>
<td>10.24 ± 0.17</td>
<td>10.10 ± 0.08</td>
</tr>
<tr>
<td>Mg(NO₃)₂</td>
<td>54.5</td>
<td>3.57 ± 0.16</td>
<td>13.00 ± 0.08</td>
<td>9.77 ± 0.03</td>
</tr>
<tr>
<td>NaNO₂</td>
<td>65.6</td>
<td>4.29 ± 0.05</td>
<td>9.61 ± 0.11</td>
<td>9.46 ± 0.15</td>
</tr>
<tr>
<td>NaCl</td>
<td>76.1</td>
<td>5.97 ± 0.07</td>
<td>10.58 ± 0.04</td>
<td>10.22 ± 0.11</td>
</tr>
</tbody>
</table>

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**Table 2—Monolayer water contents (g/100 g solids), Brunauer-Emmett-Teller (BET) constant \( K \), Guggenheim-Anderson-deBoer (GAB) constant \( K' \) and \( C \), and \( R^2 \) for lactose/reactant, lactose/trehalose/reactant, and trehalose/reactant model systems \(^{a} \)**

**BET**

<table>
<thead>
<tr>
<th>Water content (g/100 g solids)</th>
<th>( m_a )</th>
<th>( K' )</th>
<th>( C )</th>
<th>( R^2 )</th>
<th>%RMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose/reactant</td>
<td>6.57</td>
<td>4.783</td>
<td>0.990</td>
<td>0.990</td>
<td>1.471</td>
</tr>
<tr>
<td>Lactose/trehalose/reactant</td>
<td>6.89</td>
<td>4.939</td>
<td>0.937</td>
<td>0.937</td>
<td>3.121</td>
</tr>
<tr>
<td>Trehalose/reactant</td>
<td>6.68</td>
<td>5.480</td>
<td>0.965</td>
<td>0.965</td>
<td>0.804</td>
</tr>
</tbody>
</table>

**GAB**

<table>
<thead>
<tr>
<th>Water content (g/100 g solids)</th>
<th>( m_a )</th>
<th>( K' )</th>
<th>( C )</th>
<th>( R^2 )</th>
<th>%RMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose/reactant</td>
<td>5.20</td>
<td>1.184</td>
<td>9.969</td>
<td>0.981</td>
<td>0.633</td>
</tr>
<tr>
<td>Lactose/trehalose/reactant</td>
<td>3.95</td>
<td>1.439</td>
<td>11.649</td>
<td>0.986</td>
<td>0.598</td>
</tr>
<tr>
<td>Trehalose/reactant</td>
<td>4.56</td>
<td>1.309</td>
<td>9.048</td>
<td>0.962</td>
<td>0.457</td>
</tr>
</tbody>
</table>

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**Figure 3—Water sorption isotherm for the model systems (L/R = lactose/reactant system; L/T/R = lactose/trehalose/reactant system; T/R = trehalose/reactant system). Guggenheim-Anderson-deBoer (GAB) isotherm (a) and Brunauer-Emmett-Teller (BET) isotherm (b) are shown.**

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mas and others 1992; Roos 1993). Critical water contents are useful and important as criteria for processing and storage of amorphous, low-moisture foods.

Nonenzymatic browning

NEB was observed to occur in the 3 model systems at all RVP environments studied during storage. Plot of OD at 280 nm and 420 nm against storage time is shown in Figure 5. The browning kinetics observed by 280 nm and 420 nm were very similar. The 3 model systems showed different NEB kinetics during storage at different RVPs. At RVPs 33.2% and 44.1% (Figure 5a and 5b), optical densities for the 3 food models increased linearly, indicating apparent zero-order kinetics (Labuza and Baisier 1992), but leveled at a plateau as the reaction proceeded, and agreed with the results of Lievonen and others (1998) and Lievonen and Roos (2002b). The rate constants of NEB were determined by linear regression of the OD versus storage time over the linear region of the plots and are given in Table 4. The NEB rate at 44.1% RVP was much higher than at 33.2%. Higher RVPs increased the NEB rate. The NEB rates in the trehalose/reactant system were higher than those in lactose/trehalose/reactant systems, and the rate in lactose/reactant system was the lowest of 3 model systems studied at 33.2% and 44.1% RVP.

In the present study, the $T_g$ of the 3 model systems at 33.2% RVP ranged from 26.6 °C to 28.8 °C, which was higher than the storage temperature (22 °C to 23 °C). However, the NEB still occurred, although at a lower rate. This results gave further evidence for the statement by Simatos and others (1995), that glass transition could not be used alone to characterize a material in relation to temperature/water conditions prevailing during storage and could not be considered as an absolute threshold of stability. Although NEB in low-moisture food materials was often suggested to be a diffusion-controlled reaction (Karel and Saguy 1991; Slade and Levine 1991; Karel and Buera 1994; Buera and Karel 1995; Roos 1995, 2001), the formation of glassy polymeric matrix results in significantly "frozen" translational molecular motion, and translational mobility and diffusion are considered to be virtually nonexistent (Slade and others 1989; Slade and Levine 1991, 1992). The heterogeneities of materials and preexisting pores due to defects and porosity in the matrix structure (Kovarskii and others 1978; Hori and others 1986; Cardona and others 1997; Schebor and others 1999) probably could account for the occurring of NEB at temperature lower than $T_g$.

### Table 3—Glass transition temperatures (mean ± SD) of model systems stored for 144 h at various relative vapor pressure (RVP) at room temperature (22 °C to 23 °C) ($n = 3$, SD = standard deviation)*

<table>
<thead>
<tr>
<th>Salt</th>
<th>RVP (% of water)</th>
<th>Glass transition temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiCl</td>
<td>11.4</td>
<td>L/R 58.4 ± 0.3, L/T/R 59.7 ± 0.1, T/R 55.5 ± 0.1</td>
</tr>
<tr>
<td>CH₃COOK</td>
<td>23.1</td>
<td>L/R 39.4 ± 0.0, L/T/R 36.9 ± 0.2, T/R 37.1 ± 0.3</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>33.2</td>
<td>L/R 28.8 ± 0.2, L/T/R 28.8 ± 0.3, T/R 26.6 ± 0.1</td>
</tr>
<tr>
<td>K₂CO₃</td>
<td>44.1</td>
<td>L/R 11.9 ± 0.6, L/T/R 9.6 ± 0.1, T/R 9.9 ± 0.3</td>
</tr>
<tr>
<td>Mg(NO₃)₂</td>
<td>54.5</td>
<td>L/R 0.2 ± 0.4</td>
</tr>
</tbody>
</table>

* L/R = lactose/reactant; L/T/R = lactose/trehalose/reactant; T/R = trehalose/reactant.

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**Figure 4**—The relationship between the glass transition and water contents for the model systems (L/R = lactose/reactant system; L/T/R = lactose/trehalose/reactant system; T/R = trehalose/reactant system). The Gordon-Taylor equation was applied for predicting glass transition ($T_g$).

**Figure 5**—Optical density development of the model systems at 280 nm as a function of storage time at room temperature (22 °C to 23 °C). The inset figure shows the optical density development at 420 nm. (L/R = lactose/reactant; L/T/R = lactose/trehalose/reactant; T/R = trehalose/reactant). Error bars represent standard deviation ($n = 3$).
reaction between the xylose and lysine may have occurred primary in the pores that remained after freeze-drying. The pores and capillaries in food materials can hold moisture (Labuza 1968; Karel 1973; Labuza 1975), which may also explain the reactions in glassy systems (Bell and others 1998). In fact, from the result of the sorption study for the 3 model systems, water contents of the 3 model system at 33.2% RVP (shown in Table 1) were slightly higher than the BET and GAB monolayer water contents for corresponding model systems. This might also partially account for the browning at 33.2% RVP. It is also possible that materials contain local reactant-rich regions with lower $T_p$ and higher water contents. Such regions can brown substantially faster than the continuous glassy matrix. The NEB extent in trehalose model is highest at 33.2% RVP probably because of the local heterogeneities and the special molecular structure of trehalose. Trehalose has a special molecular structure: the disaccharide bond is formed between the C$_6$ atoms of the pyranose rings, and trehalose has no direct internal hydrogen bonds; this arrangement gives the molecule unusual flexibility above the disaccharide bond (Rosser 1991). This may allow trehalose to conform more closely to the irregular surface of macromolecules than more rigid, directly hydrogen-bonded disaccharides and probably leaves reactants closer in the trehalose/reactant system than in the lactose/reactant system. In fact, during our preparation of the freeze-dried samples in Petri dishes, we found that trehalose models showed different extents of browning between the top and bottom of the sample, and furthermore, that trehalose samples were more adhesive to the Petri dish surface. All samples in the present study were prepared by freeze-drying, and the freeze-dried samples had higher porosity and surface area. Hence, in the amorphous trehalose/reactant system, the trehalose molecules were arranged more heterogeneously and were adhesive, allowing more mobility and availability to react. Of course, the uptake of water at 33.2% and 44.1% RVP environments enhanced this mobility (Sherwin and others 2002; Sherwin and Labuza 2003). Although trehalose is a nonreducing sugar and lactose is a reducing sugar, which might also participate in the NEB reaction as reactant, in present study, the reactant concentration in the systems was designed to be 5% (w/w), which was very high. Participation of the matrix lactose in the NEB reaction could be very small in comparison with the previous effects. At 54.5% and 65.6% RVPs, interesting NEB kinetics were obtained, as shown in Figure 5c and 5d. When samples were stored at 54.5% RVP (Figure 5c), OD development was observed in the 3 food models in 3 stages. In the 1st stage, browning developed at a low rate, corresponding to the water sorption in Figure 2a, and loss of water contents was not observed, indicating no crystallization of component sugars at this stage. During our experiment, we found 3 model systems at this stage were in a transparent completely collapsed state, probably in the collapsed supercooled liquid state (rubbery state). On collapse of the matrix, because of the elimina-

| RVP (% of water) | L/R | $R^2$ | L/T/R | $R^2$ | T/R | $R^2$
<table>
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<tbody>
<tr>
<td>33.2</td>
<td>0.06 ± 0.03</td>
<td>0.9943</td>
<td>0.11 ± 0.09</td>
<td>0.9888</td>
<td>0.19 ± 0.21</td>
<td>0.9895</td>
</tr>
<tr>
<td>44.1</td>
<td>0.45 ± 0.08</td>
<td>0.9810</td>
<td>0.78 ± 0.12</td>
<td>0.9862</td>
<td>2.04 ± 0.09</td>
<td>0.9950</td>
</tr>
<tr>
<td>54.5b</td>
<td>1.02 ± 0.11</td>
<td>0.9880</td>
<td>1.29 ± 0.07</td>
<td>0.9915</td>
<td>4.68 ± 0.08</td>
<td>0.9993</td>
</tr>
<tr>
<td>65.6b</td>
<td>4.69 ± 0.04</td>
<td>0.9841</td>
<td>1.90 ± 0.36</td>
<td>0.9822</td>
<td>5.88 ± 0.11</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

$L/R =$ lactose/reactant; $L/T/R =$ lactose/trehalose/reactant; $T/R =$ trehalose/reactant.

$R^2$ Rate constants were calculated by linear regression from the linear region of OD development plot.
results for the model systems at 65.6% RVP (Figure 2b), crystallization of component sugars was observed from the loss of sorbed water at 5 h, 21 h, and 56 h for trehalose/reactant, lactose/reactant, and lactose/trehalose/reactant systems, respectively. The rapid linear increasing region of OD in the trehalose/reactant and lactose/reactant systems seemed to be directly related to the crystallization of the component sugars in the systems. The slow loss of the sorbed water in the lactose/trehalose/reactant system probably explained the continuously linear increase in the OD before the OD leveled off. It should be mentioned that the rate constant (shown in Table 4) in the trehalose/reactant system at 65.6% RVP was close to that in lactose/reactant system at 65.6% RVP, and also only slightly higher than that at 54.5% RVP. Typically, the maximum NEB rates in foods and model systems occur in a RVP range of 50% to 75% (Labuza and Saltmarch 1981; Buera and Karel 1993) depending on the composition of the systems; probably the rate constant in the trehalose/reactant system at 65.6% RVP seemed to approach the maximum value.

Trehalose has been proved in many studies to be a excellent protectant for stabilization of enzymes and other biomaterials (Crowe and others 1984; Leslie and others 1995; Scherbor and others 1996; Cardona and others 1997; Mazzobre and others 1997a, 1997b). Some studies (Mazzobre and others 1997a) suggested that the trehalose-protecting effect cannot be based solely on its glass-forming properties because other dried molecular weight polymers with higher $T_g$ (such as, PVP 400000) than trehalose exerted less protective effect than trehalose, and a specific protective mechanism, related to the maintenance of the tertiary structure, was suggested (Cardona and others 1997). From the results of the present study, the relatively higher NEB rate in trehalose/reactant system seemed to be more related to the composition, rather than the glass transition properties.

Conclusions

The present study compared the physical state, thermal behavior, and the NEB kinetics in freeze-dried lactose-, trehalose-, and lactose/trehalose-based food model systems at different RVP environments. The results have shown that the 3 model systems had similar glass transition behavior but different crystallization properties. Mixtures of lactose and trehalose showed delayed crystallization of components. Addition of trehalose in lactose-based dairy products may prevent crystallization during storage. The NEB rate was accelerated by crystallization and affected by the matrix composition. In the trehalose/reactant system, the NEB rate was higher than in lactose/trehalose/reactant and trehalose/reactant systems. As a nonreducing sugar, trehalose has been suggested to be used as a new ingredient in the food industry to prevent NEB, especially in the dairy industry. We found that the NEB rate was the highest in the trehalose/reactant system, and the reaction was accelerated by crystallization during storage. These results indicated that NEB was related to the composition of the matrix. When trehalose was used in dairy products, especially those containing proteins and reducing sugars, which might likely cause browning, much attention should be paid in controlling the NEB reaction during storage and processing. Because most of the dairy powders are produced by spray-drying, to evaluate the NEB kinetics in real food products, further research is needed to confirm the effects of matrix composition and crystallization on NEB kinetics in spray-dried systems.

Acknowledgments

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References


