Glass Transitions and Crystallization in Milk Powders

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ABSTRACT

Glass transition temperatures were determined for dehydrated milk products with various water contents and water activities, and state diagrams were established. Crystallization behavior was studied with milk powders stored at various relative humidities. Glass transition temperatures of milk powders containing lactose were close to those of pure lactose. Skim milk powder with hydrolyzed lactose had considerably lower glass transition temperatures. Glass transition temperatures decreased as water content increased. The critical water content and water activity for stability at 24°C were predicted using data on glass transition temperature and water sorption. Lactose and milk powders containing lactose had the same critical water activity or storage relative humidity, but slightly different critical water contents. The critical values for skim milk with hydrolyzed lactose were much lower than those for regular milk powders. Loss of adsorbed water, indicating lactose crystallization, was observed in milk powders stored above the critical relative humidity. Glass transition temperatures and state diagrams can be used to evaluate changes during processing and storage of dairy foods.

(Key words: glass transition, milk powder, stability)

Abbreviation key: \( a_w \) = water activity, DSC = differential scanning calorimetry, RH = relative humidity, \( T_{cr} \) = crystallization temperature, \( T_g \) = glass transition temperature, \( T_g' \) = \( T_g \) of the maximally freeze-concentrated solutes, \( T_m \) = onset temperature of ice melting within the maximally freeze-concentrated solutes.

INTRODUCTION

Storage stability and quality of milk powders are significantly affected by the physical state of lactose, one of the main components in regular milk powders. Lactose exists often as an amorphous glass (7, 11, 27, 29) that is stable at temperatures below its glass transition temperature \( T_g \) (20, 22, 25, 31). Lactose glass is hygroscopic and may adsorb water, which results in plasticization and decreasing of the \( T_g \) (20, 25). Storage of amorphous lactose at a temperature above \( T_g \) increases molecular mobility and decreases viscosity, causing stickiness, caking, and crystallization (3, 7, 11, 20, 25, 27, 29, 31). Lactose crystallizes at temperatures above \( T_g \) in the rubbery state; the rate increases as the temperature difference between ambient temperature and the \( T_g \) increases (20, 22, 25). Excessive temperature difference between ambient temperature and the \( T_g \) may also facilitate nonenzymatic browning and lipid oxidation (23, 24, 31). The water content and corresponding water activity \( (a_w) \), which decrease \( T_g \) to below ambient temperature, were considered to be the critical values for water content and \( a_w \) for stability (19).

The \( T_g \) of carbohydrates decrease as molecular weight decreases [e.g., (15, 18, 25)]. Hydrolysis of lactose in milk to glucose and galactose presumably decreases the \( T_g \) in the dehydrated product and the critical values for water content and \( a_w \) because the \( T_g \) for anhydrous galactose and glucose are 30 and 31°C, respectively (18), but the \( T_g \) for lactose is 101°C (20). Even a slight increase in water content of milk powder with hydrolyzed lactose may decrease the \( T_g \) to below the usual storage temperature of milk powders. The low \( T_g \) of the galactose and glucose mixture is possibly the main cause of problems typical of...
production and storage of milk powders with hydrolyzed lactose.

The objectives of the present study were to determine \( T_g \) for dehydrated milk products with various water contents and \( a_w \), and to determine the effect of relative humidity (RH) during storage on lactose crystallization. The effect of fat and the effect of lactose hydrolysis on crystallization behavior were also studied. State diagrams were established by modeling of data on \( T_g \) and water sorption, which allowed prediction of the critical values for water contents and \( a_w \) for milk powders.

**MATERIALS AND METHODS**

**Sample Preparation**

The materials used were skim milk, skim milk with enzymatically hydrolyzed lactose, lowfat milks with 1 or 1.9% fat, whole milk with 3.9% fat (Valio Ltd., Helsinki, Finland), and lactose solution (10%, wt/wt, in distilled water, Sigma Chemical Co., St. Louis, MO). Samples (10 g of each material in Petri dishes or glass beakers for the determination of transition temperatures and 5.00 ± .02 ml in glass vials for the determination of crystallization behavior) were frozen (24 h at 4°C) and freeze-dried (Lyovac GT2 freeze dryer; Amsco Finn-Aqua GmbH, Hürth, Germany) for 48 h (pressure <.1 mbar), as reported by Jouppila and Roos (9). The residual moisture was removed by storage of the samples in evacuated desiccators over \( \text{P}_2\text{O}_5 \) at 24°C until weight was constant (at least 2 d). All vials were closed with caps immediately after the vacuum was released in the desiccators to avoid adsorption of water from the air before determination of the anhydrous sample weight.

**Differential Scanning Calorimetry**

Dehydrated materials (3 to 10 mg) were transferred in preweighed differential scanning calorimetry (DSC) aluminum pans (40 μl; Mettler-Toledo AG, Greifensee, Switzerland). The \( T_g \) and instant crystallization temperatures (\( T_{cr} \)) for the anhydrous materials were determined in triplicate, using samples that were stored in open pans for 24 h at 24°C over \( \text{P}_2\text{O}_5 \) in evacuated desiccators. Three samples of each material were equilibrated in open pans for 24 h at 24°C in evacuated desiccators over saturated salt solutions (17); \( \text{LiCl}, \text{CH}_3\text{COOK}, \text{MgCl}_2, \text{K}_2\text{CO}_3, \text{Mg(NO}_3)_2, \text{NaNO}_2, \text{and NaCl} \) (pro analysis; E. Merck, Darmstadt, Germany), with respective RH of 11.5, 23.9, 33.0, 44.4, 53.8, 66.2, and 76.4%, giving \( a_w \) of .01 × percentage of RH at equilibrium (13). After equilibration, the pans were hermetically sealed. The data on water sorption and sorption prediction, according to the Guggenheim-Anderson-de Boer model (19, 28), were from Jouppila and Roos (9).

Transition temperatures (onset values) were determined using DSC (Mettler TA 4000 analysis system with a DSC-30 low temperature cell, a TC10A TA processor, and GraphWare TA72AT.2 thermal analysis software; Mettler-Toledo AG). The samples were scanned at 5°C/min from 30°C below a predetermined \( T_g \) to well above \( T_{cr} \) (Figure 1). The DSC was calibrated for temperature using \( n\)-hexane (melting point, -95.0°C), distilled water (melting point, 0°C), and indium (melting point, 156.6°C), and for heat flow using indium (latent heat of melting, 28.5 J/g), as reported by Roos and Karel (21).

Thermal behavior of freeze-concentrated materials was studied using dispersions with
20, 30, and 40% (wt/wt) of TS in distilled water. Dispersions were prepared from the regular and lactose-hydrolyzed skim milk powders. Aliquots (15 to 20 mg) were transferred into DSC pans, which were hermetically sealed. Samples with 20% TS were scanned from -100 to 20°C at 5°C/min to locate the Tg of the maximally freeze-concentrated solutes (Tg) and onset temperature of ice melting within the maximally freeze-concentrated solutes (Tm) (21). Triplicate samples of each dispersion were cooled to -100°C, heated at 10°C/min to -33°C (Tm - 1°C of regular skim milk) or -45°C (Tm - 5°C of lactose-hydrolyzed skim milk), annealed for 15 min to allow ice formation, cooled at 10°C/min to -100°C, and scanned from -100 to 20°C at 5°C/min to determine Tg and Tm (onset values). Annealing allowed maximum freeze concentration and eliminated devitrification exotherms, which are typical of unannealed carbohydrate solutions (18, 21).

State Diagrams

The Gordon and Taylor (6) equation [1] was used to model data on Tg of various milk products. The predicted Tg curves were used to establish state diagrams.

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

where w1 and w2 are the respective weight fractions of the solute and water, Tg1 is the Tg of the anhydrous solute, Tg2 is the Tg of amorphous water, and k is a constant. The Tg of -135°C (8) was used for water. The value for k was the mean derived from the experimental data on Tg and water content (18). Concentration of the solids in the maximally freeze-concentrated solution was calculated using Equation [1] with the experimental Tg.

Crystallization Behavior of Lactose

Lactose crystallization was observed from the loss of adsorbed water [e.g., (1, 9, 14, 26, 29, 30)]. Crystallization behavior was studied using duplicate samples of dehydrated skim milk, lowfat milks (1 and 1.9% fat), and whole milk. The materials were stored at 24°C in evacuated desiccators over saturated solutions of MgCl2, K2CO3, Mg(NO3)2, NaNO2, and NaCl at 33.0, 44.4, 53.8, 66.2, and 76.4% RH, respectively. The samples were weighed at intervals shown in Figure 7. Water content for each material as a function of storage time was obtained from the mean weight of at least duplicate samples. All vials were kept closed with caps after the vacuum was released in the desiccators before weighing (9). After the first opening, each desiccator was kept evacuated for at least 5 d before the samples were reweighed to eliminate a possible error from frequent opening and closing of desiccators.

RESULTS AND DISCUSSION

Tg

The Tg with corresponding water contents for milk powders are given in Table 1. The Tg decreased as water content increased, as shown in the state diagrams (Figures 2 and 3). The decrease was nearly linear as a, increased (Figures 4 and 5), which is typical of various amorphous foods (17, 19). The Tg could be successfully predicted using the Gordon and Taylor (6) equation [1]. The state diagram of amorphous lactose, with experimental data for lactose and milk powders containing lactose, is shown in Figure 2. The constant, k, for lactose was 6.7. Roos and Karel (20) reported Tg for amorphous lactose that were almost the same as those in the present study. The Tg of milk powders containing lactose were almost equal to those of pure lactose. Thus, the physical state of SNF in milk powders seems to be governed mostly by lactose. Shimada et al. (24), Karmas et al. (10), and Labrousse et al. (12) also reported Tg for model systems based on lactose that were close to those in the present study for lactose and milk powders. Milk fat did not affect the Tg of milk powders. However, Tg could not be determined from the DSC curves for powders containing fat at the RH range of 23.9 to 44.4% because of the dominant fat melting endotherm in the same temperature range. Obviously, milk fat is immiscible with lactose and other water-soluble substances that are responsible for the physical state of SNF. Milk fat was also not a water-adsorbing component (9).
TABLE 1. Water contents\(^1\) (w) and glass transition temperatures (\(T_g\)) of dehydrated milk products stored for 24 h at various relative humidities (RH) at 24°C.

<table>
<thead>
<tr>
<th>Salt</th>
<th>RH(^2) (w)</th>
<th>Lactose</th>
<th>Skim milk (0% fat)(^4)</th>
<th>Lowfat milk (10.7% fat)</th>
<th>Lowfat milk (18.6% fat)</th>
<th>Whole milk (32.4% fat)</th>
<th>Skim milk with hydrolyzed lactose (0% fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%)</td>
<td>(T_g)</td>
<td>w (\bar{X}) (\pm SD)</td>
<td>w (\bar{X}) (\pm SD)</td>
<td>w (\bar{X}) (\pm SD)</td>
<td>w (\bar{X}) (\pm SD)</td>
<td>w (\bar{X}) (\pm SD)</td>
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<tr>
<td>(\text{P}_2\text{O}_5)</td>
<td>0.00</td>
<td>97.1</td>
<td>0.00</td>
<td>92.2</td>
<td>0.00</td>
<td>88.2</td>
<td>0.00</td>
</tr>
<tr>
<td>(\text{LiCl})</td>
<td>11.5</td>
<td>2.4</td>
<td>64.1</td>
<td>3.7</td>
<td>58.1</td>
<td>4.0</td>
<td>62.1</td>
</tr>
<tr>
<td>(\text{CH}_3\text{COOK})</td>
<td>23.9</td>
<td>4.3</td>
<td>43.1</td>
<td>5.6</td>
<td>34.4</td>
<td>5.8</td>
<td>5.9</td>
</tr>
<tr>
<td>(\text{MgCl}_2)</td>
<td>33.0</td>
<td>5.9</td>
<td>33.2</td>
<td>7.1</td>
<td>33.2</td>
<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td>(\text{K}_2\text{CO}_3)</td>
<td>44.4</td>
<td>8.6</td>
<td>15.1</td>
<td>8.8</td>
<td>9.1</td>
<td>8.9</td>
<td>9.0</td>
</tr>
<tr>
<td>(\text{Mg(NO}_3)_2)</td>
<td>53.8</td>
<td>10.7</td>
<td>11.6</td>
<td>12.2</td>
<td>12.2</td>
<td>12.2</td>
<td>12.2</td>
</tr>
<tr>
<td>(\text{NaNO}_2)</td>
<td>66.2</td>
<td>18.1</td>
<td>16.9</td>
<td>17.8</td>
<td>17.9</td>
<td>17.9</td>
<td>17.9</td>
</tr>
<tr>
<td>(\text{NaCl})</td>
<td>76.4</td>
<td>5.5</td>
<td>16.7</td>
<td>20.1</td>
<td>22.9</td>
<td>22.4</td>
<td>22.4</td>
</tr>
</tbody>
</table>

\(^1\)Jouppila and Roos (9).
\(^2\)Labuza et al. (13).
\(^3\)Grams of \(\text{H}_2\text{O}\) per 100 g of SNF.
\(^4\)Percentage of fat of TS calculated from the amount of milk fat in the fresh product given by manufacturer.
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Figure 2. State diagram of amorphous lactose with experimental data for lactose (●), skim milk (○), lowfat milks with 1% (△) and 1.9% (▲) fat, and whole milk (x) showing concentration dependence of the glass transition temperature (Tg). The Tg curve indicates the estimated Tg of lactose calculated with the Gordon and Taylor (6) equation [1] (constant, k = 6.7). The Tg of the maximally freeze-concentrated solutes (Tg) (●) and onset temperature of ice melting within the maximally freeze-concentrated solutes (Tm) (△) were determined. Concentration of the solids in the maximally freeze-concentrated solution (Cg) was estimated using the experimental Tg.

Figure 3. State diagram of lactose-hydrolyzed skim milk powder with experimental data (●) showing concentration dependence of the glass transition temperature (Tg). The Tg curve indicates the estimated Tg calculated with the Gordon and Taylor (6) equation [1] (constant, k = 8.0). The Tg of the maximally freeze-concentrated solutes (Tg) (●) and onset temperature of ice melting within the maximally freeze-concentrated solutes (Tm) (△) were also determined. Concentration of the solids in the maximally freeze-concentrated solution (Cg) was estimated using the experimental Tg.

Skim milk powder with hydrolyzed lactose had substantially lower Tg than those of the regular milk powders. The Tg decreased as water content increased, as shown in the state diagram (Figure 3). The Tg curve was calculated using Equation [1] with the constant, k = 8.0. The low Tg were most probably due to hydrolysis of lactose to glucose and galactose. However, the Tg of the anhydrous skim milk powder with hydrolyzed lactose was higher than those of anhydrous galactose and glucose (30 and 31°C, respectively), reported by Roos (18). A higher Tg suggested incomplete hydrolysis of lactose because a high Tg component should increase the Tg of a carbohydrate mixture (25).

The Tg and Tm, which were independent of the initial solids concentration (25), were −50 and −32°C for skim milk, respectively, and −65 and −40°C for skim milk with hydrolyzed lactose, respectively. The Tg were lower than the −41, −56, and −57°C for lactose, galactose, and glucose, respectively (18), probably because of other compounds and salts present in milk solids. The Tm in the present study were close to the −30, −45, and −46°C for lactose, galactose, and glucose, respectively (18). The estimated concentration of the solids in the maximally freeze-concentrated solution was 79.5% for skim milk and 83.1% for skim milk with hydrolyzed lactose. These concentrations of the solids in the maximally freeze-concentrated solutions were close to 80%, which is typical of various carbohydrates (18). The thermal behavior can be related to freezing behavior, storage stability, and the behavior of a frozen product during freeze-drying [e.g., (15, 25)]. Frozen dairy foods (e.g., ice cream) contain high amounts of freeze-concentrated sugars, which may have a significant influence on stability and product quality. Goff et al. (5) found that the stability is affected by viscosity above Tg. Polysaccharide stabilizers, which increase viscosity, were useful in the control of ice crystal growth in the freeze-concentrated, viscoelastic, but not glassy, liquid.
Figure 4. Relationships between glass transition temperature ($T_g$), water content, and relative humidity (RH) at equilibrium for lactose and milk powders containing lactose. The sorption isotherms of lactose and skim milk powder were predicted with the Guggenheim-Anderson-de Boer model, in which the water content was expressed on the basis of SNF. The critical $T_g$ of 24°C for storage, corresponding critical RH, and water content are indicated with arrows.

Instant $T_{cr}$

The $T_{cr}$, which indicate instant crystallization during a dynamic increase of temperature at 5°C/min, as a function of storage RH and water content, are shown in Figure 6. The $T_{cr}$ of pure lactose were lower than those of lactose in milk powders. The $T_{cr}$ of lactose decreased as RH and water content increased, as reported by Roos and Karel (20), who found that water plasticization of lactose caused about an equal decrease in $T_g$ and $T_{cr}$. We observed a greater temperature difference at intermediate water contents. The decrease of $T_{cr}$ of milk powders did not follow the decrease of $T_g$. Lactose crystallized in milk powders at a fairly constant temperature until the...
water content increased >9 g of H₂O/100 g of SNF. Lactose crystallization was probably delayed by other milk solids, e.g., protein. The presence of high molecular weight compounds retards lactose crystallization (e.g., 2, 10, 12, 25). Lactose crystallized in milk powders containing fat at slightly higher temperatures than in skim milk powder as α increased, suggesting delayed lactose crystallization in the presence of fat. The crystallization exotherm in skim milk powder with hydrolyzed lactose, which contains a mixture of two to three sugars, occurred at a constant temperature that was independent of water content (Figure 6). Obviously, the instant T<sub>cr</sub> shows the effect of composition on the relaxation time of crystallization of amorphous components in milk powders.

**Critical Water Content and Storage RH**

Stability of amorphous foods is related to the T<sub>g</sub> [e.g., (20, 25, 31)]. The critical α<sub>w</sub> was .37 for pure lactose (Figure 4). Milk powders with lactose seem to have the same critical α<sub>w</sub> and, therefore, the same critical storage RH. The critical water contents were 6.8 g of H₂O/100 g of TS for pure lactose and 7.6 g of H₂O/100 g of SNF for milk powders containing lactose (Figure 4). The higher value for milk powders was probably due to the water sorption of other compounds except fat. For pure lactose at 25°C, Roos (19) predicted that the critical α<sub>w</sub> was .33 and that the critical water content was 7.2 g of H₂O/100 g of TS, which were slightly different from the values in the present study. Several critical values for dehydrated milk products have been reported [e.g., (3, 14, 16, 30)]. The critical water contents for skim milk powder varied from 6 to 8.5 g of H₂O/100 g of TS (3, 14, 30), which also includes the critical water content in the present study. However, prior critical values have been based on the crystallization break in sorption isotherms, and those previous studies have not considered the T<sub>g</sub> of lactose. When T<sub>g</sub> is exceeded in spray drying, caking on drier surfaces may result, and a controlled exceeding of the T<sub>g</sub> is essential for agglomeration of fine particles in the manufacture of instant milk powders.

The critical values for skim milk powder with hydrolyzed lactose were much lower than those for regular milk powders (Figure 5); the critical α<sub>w</sub> was .16, and the corresponding critical water content was 2.0 g of H₂O/100 g of TS. These low critical values would be expected to have a substantial effect on processability and storage stability of milk powders with hydrolyzed lactose. We assume that milk with hydrolyzed lactose is more difficult to dehydrate because of the low T<sub>g</sub>. In storage, a slight increase in water content might drastically increase the rate of deteriorative changes and cause stickiness and caking. Hydrolysis of lactose also increases the reactivity of milk powder components, e.g., the rate of nonenzymatic browning, because hydrolysis doubles the number of reducing sugar molecules.

**Crystallization During Storage**

Loss of adsorbed water within 600 h occurred in samples stored at RH ≥44.4%, but not in samples stored at 33.0% RH (Figure 7). Loss of adsorbed water became evident in milk powders stored at 53.8% RH after 9 d. At 66.2% and 76.4% RH, the water contents decreased to constant values after 5 and 4 d of storage, respectively. The loss of adsorbed water, indicating lactose crystallization, often occurs in...
milk powders at corresponding RH [e.g., (10, 12, 14, 16, 26, 29, 30)]. Time dependency of lactose crystallization in milk powders at various RH has been reported in some studies (9, 14, 26, 29). Vuataz (29) found that lactose began to crystallize in skim milk powder at 39% RH at 25°C after 200 h of storage, which indicated more rapid lactose crystallization than that in the present study at corresponding RH. However, preconcentration of milk before drying may result in the formation of nuclei, which probably affect the crystallization behavior of lactose in powders.

Storage temperature affects lactose crystallization (1, 20, 22, 32). Lactose crystallizes at temperatures above $T_g$; the rate increases as the difference between ambient temperature and the $T_g$ increases (20, 22). Würsch et al. (32) reported that lactose crystallized very rapidly in whole milk powder with water content of 3.1 g/100 g of TS stored at >55°C, but, at 50°C, lactose crystallized much more slowly. Lactose did not crystallize at <45°C. According to the results of the present study, the estimated $T_g$ for whole milk powder with water content of 3.1 g/100 g of TS (32), which was assumed to correspond to water content of 4.4 g/100 g of SNF, was 44°C, indicating that lactose may crystallize with various rates in whole milk powder at temperatures above $T_g$. Other deteriorative changes in milk powders also can be related to $T_g$. Flink et al. (4) found that the rate of nonenzymatic browning in skim milk powder increased sharply above a critical temperature, which depended on storage RH. Shimada et al. (24) observed that lactose crystallization released encapsulated fat, which was subsequently oxidized rapidly. Similarly, lactose crystallization in milk powders may increase free fat content and, therefore, promote oxidation.

Water sorption of milk powders with various fat contents leveled off at certain water contents, depending on RH (Figure 7). The water contents after 400 h of storage were 7, 9, 11, 10, and 14 g of H$_2$O/100 g of SNF at 33.0, 44.4, 53.8, 66.2, and 76.4% RH, respectively, suggesting incomplete crystallization at <66.2% RH. Vuataz (29) and Lai and Schmidt (14) reported that water sorption by skim milk powder leveled off at water contents comparable with those in the present study. If lactose (about 57.9% of the SNF in our milk powders) crystallized as the anhydrous $\beta$-lactose at RH $\leq$66.2%, most of the water remaining was probably sorbed by protein and minor components (261, 26, and 24 g of H$_2$O/100 g of protein at 44.4, 53.8, and 66.2% RH after 400 h, respectively). At 76.4% RH, lactose crystallized probably as $\alpha$-lactose monohydrate with 5% (wt/wt) water, and the remainder of the water was sorbed by other SNF (26 g of H$_2$O/100 g of protein), as reported by Jouppila and Roos (9). Vuataz (29) reported that lactose crystallized as anhydrous $\beta$-lactose in skim milk powder stored at RH from 39 to 49% and as $\alpha$-lactose monohydrate in skim milk powder stored at 57% RH. However, we assume that different crystal forms may form, depending on the method used for powder production.

CONCLUSIONS

Stability of milk powders is governed by the physical state of component compounds. The physical state is related to the $T_g$ of the component carbohydrates, and most deteriorative changes occur at temperatures above the $T_g$. Crystallization of amorphous lactose occurs above $T_g$ but is delayed by other components in the milk solids. Milk powders with hydrolyzed lactose have a lower $T_g$. Stability of milk powders in storage can be described in terms of $T_g$ and the corresponding critical values for water content and $a_w$. Data on water sorption and $T_g$ are important in establishing state diagrams, which can be used for evaluation of proper dehydration, agglomeration, and storage conditions for milk powders and other dairy foods.

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REFERENCES

Lactose crystallization in dry products of milk. II. The effects of moisture and alcohol. J. Dairy Sci. 34:850.

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