

Water Activity–Temperature State Diagram of Amorphous Lactose

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Development in the glass transition temperature, T_g , to reach a stabilized value for amorphous lactose stored at 5, 25, and 38 °C at different water activities varying from $a_w = 0.21$ to 0.59 was followed by differential scanning calorimetry. Combinations of stabilized T_g and water activity were used for interpolation of the value of water activity, where T_g was equal to the storage temperature. These values of water activity were used to construct a state diagram in the (a_w, T) -plane for lactose in the amorphous state from which critical combinations of water activity and storage temperature may be obtained for optimization of storage conditions of lactose-based dry products.

KEYWORDS: Lactose; glass transition temperature; T_g ; water activity; DSC; milk powder

INTRODUCTION

The technical specifications of milk powder, such as particle size and flow properties, important for accuracy for dosage, depend strongly on the physical state of lactose as the most abundant component (1). Milk powder is produced industrially by spray-drying of pre-concentrated, heat-treated milk and consists of a continuous mass of mainly amorphous lactose, in which proteins and fat globules are embedded (1). Milk powder, based on whole milk or skim milk and with lactose in an amorphous glassy state, forms the basis for various products, such as infant formula, which is often enriched with vitamins and iron. Instant powders for cocoa- or coffee-flavored hot or cold beverages also contain milk powder as an important ingredient. The lipids and flavor components in such products with low water activity easily oxidize, resulting in off-flavors, and lactose may also react with lysine side chains in proteins, resulting in loss of nutrients and browning. Both lipid oxidation and Maillard reactions depend on the physical state of lactose, and amorphous lactose in the glassy state prevents, or at least slows down, such deteriorative reactions (2). Accordingly, the physical state of lactose is very important for the shelf life of milk powder and products based on milk powder. Amorphous lactose is also used in pharmaceutical formulations, and again, the physical state of lactose is important for shelf life and also for delivery of active compounds embedded in the lactose matrix.

Crystallization of amorphous lactose is a spontaneous process, which is very slow under conditions of low temperature and high viscosity, as in products of low water activity (1). However, water is a plasticizer, and above the glass transition temperature, T_g , the rate of crystallization increases, and crystallization

becomes important in the product (3, 4). To map the conditions for which lactose stays in the glassy state, we have designed experiments in which amorphous lactose was followed with respect to development in T_g during storage at constant temperatures of 5, 25, and 38 °C at varying water activities. The resulting values of stabilized T_g and corresponding water activities for the three storage temperatures allowed the construction of a (a_w, T) -state diagram for long-term storage stability of amorphous lactose. The purpose of such a diagram is two-fold. In the case of production of dry lactose-based products (milk powder, pharmaceutical products, etc.), the final water activity (after production) may be deduced to ensure that lactose remains in the glassy state at a given temperature. In this respect, the diagram can be used as a convenient alternative to the conventional water content/temperature state diagram. The other purpose relates to the storage stability of the product as the diagram can be used in the process of evaluating combinations of external conditions (humidity and temperature) and packaging barrier demands in order to ensure stability over a given period.

MATERIALS AND METHODS

Lactose. Amorphous lactose was prepared by freezing a 20% (w/v) α -lactose monohydrate solution overnight at -80 °C, followed by freeze-drying using a Edwards Modulyo (Edwards High Vacuum International, Crawley, United Kingdom) laboratory freeze dryer at a pressure of 0.15 mbar and a condenser temperature of -50 °C. Immediately after freeze drying, the lactose was transferred to a glass desiccator over P_2O_5 ($a_w = 0$) in order to keep the material as dry as possible until use.

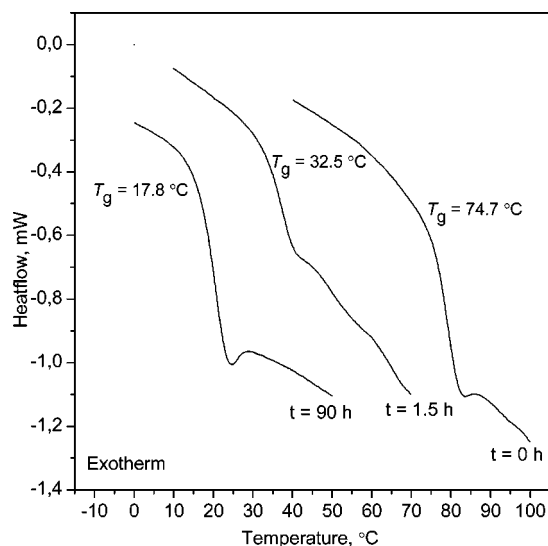
Differential Scanning Calorimetry (DSC) Measurements. The glass transition temperature was determined by conventional DSC using a DSC 820, Mettler Toledo (Schwerzenbach, Switzerland), which is based on the heat flux principle and cooled with liquid nitrogen. Calibration of heat flow and temperature was performed with indium

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Table 1. Different Combinations of Temperature and Water Activity Used in the Experimental Setup for Determination of T_g at Varying Conditions^a

saturated salt solution	a_w^b		
	5 °C	25 °C	38 °C
CH ₃ COOK		0.23	0.21 ^c
MgBr ₂		0.31	
MgCl ₂ ·6H ₂ O	0.34	0.33	0.32
K ₂ CO ₃	0.43	0.43	0.43 ^d
Mg(NO ₃) ₂ ·6H ₂ O	0.59		

^a The values of a_w depend on temperature and have been corrected according to ref 17. ^b Value obtained by extrapolation. ^c Value obtained by extrapolation. ^d Extrapolated value, since a_w of K₂CO₃ is temperature independent within statistical limit from 0 to 30 °C (17).

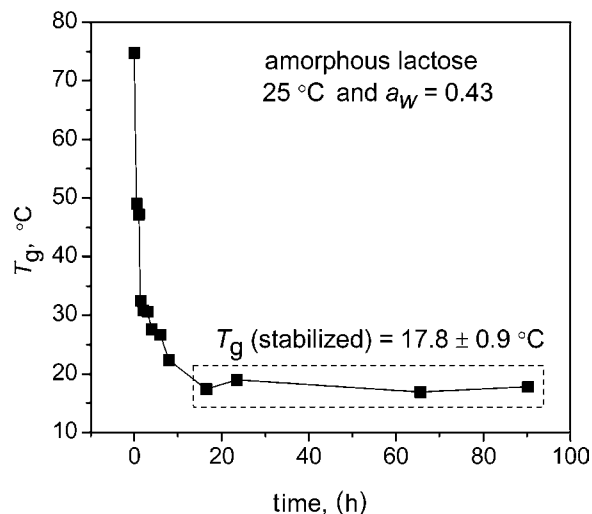
**Figure 1.** Thermograms of amorphous lactose stored at 25 °C and $a_w = 0.43$ at varying times. The glass transition temperature, T_g , is taken as the onset temperature.

as the standard (mp = 156.6 °C, Mettler Toledo calibration kit, ME 119442). The linearity of the calibration was verified with zinc (mp = 419.5 °C, Mettler Toledo calibration kit, ME 119442), decane ($T_m = -29.66$ °C), and cyclohexane ($T_m = 6.47$ °C). **Table 1** outlines the different combinations of temperature and water activity used for storage of amorphous lactose in the present experiments. For each experiment, a number of 40 μ L aluminum DSC crucibles were loaded with 10–20 mg of amorphous lactose and placed without a lid in glass desiccators at the desired combination of temperature and water activity (**Table 1**). At 25 and 38 °C, desiccators were thermostated in a water bath, whereas at 5 °C the desiccator was placed in a refrigerator. Temperature loggers placed inside the desiccators measured the temperature (5 ± 1 , 25 ± 0.9 , and 38 ± 0.9 °C). For each measurement, a crucible was taken from the desiccator, hermetically sealed immediately, and transferred to the DSC. Samples were scanned over an appropriate temperature range with the heating scan rate 5 °C/min (linear up-scan), using an empty crucible as reference.

RESULTS

For each of the different combinations of water activity and storage temperature shown in **Table 1**, a series of DSC measurements was performed from beginning ($t = 0$) to the observed glass transition temperature, T_g , had reached a stable level at the given conditions. Typical thermograms are shown in **Figure 1** for amorphous lactose at a water activity of 0.43 prior to storage and following storage for 1.5 and 90 h.

The time required to reach a stable level for T_g lasted from 2 h to 15 days depending on the storage temperature. The

**Figure 2.** Changes in glass transition temperature, T_g , for amorphous lactose stored at 25 °C and $a_w = 0.43$. The frame marks the part of the curve where T_g is stabilized, and the average value of T_g is shown above the frame.**Table 2.** Stabilized T_g Values Determined for Amorphous Lactose at Varying Storage Temperatures and Water Activities

saturated salt solution ^a	stabilized T_g (°C)		
	5 °C	25 °C	38 °C
CH ₃ COOK		48.4 ± 1.5	60.4 ± 1.1
MgBr ₂		43.8 ± 0.6	
MgCl ₂ ·6H ₂ O	18.4 ± 0.8	38.9 ± 0.3	33.5 ± 1.4
K ₂ CO ₃	4.8 ± 1.5	17.8 ± 0.9	16.4 ± 0.8
Mg(NO ₃) ₂ ·6H ₂ O	-13.2 ± 2.6		

^a Refer to **Table 1** for corresponding water activity.

duration of stabilizing T_g was shortest at the higher water activities at 5 and 38 °C, and when the water activity was lowered, the duration of the adjustment process of T_g was longer. At 25 °C, there was no marked difference in the duration of T_g stabilization. To illustrate the principle of the experiment, the results of T_g stabilization for samples stored at 25 °C and $a_w = 0.43$ are shown in **Figure 2**. Similar curves were obtained for 5 and 38 °C for $a_w = 0.43$ and for each of the three temperatures at the 3–4 different values of a_w (see **Table 1**).

Figure 2 shows that at these storage conditions ($T = 25$ °C, $a_w = 0.43$), T_g decreased within 8 h from 75 to 22 °C, after which the observed glass transition temperature, T_g , leveled at 17.8 ± 0.9 °C and remained stable for at least 90 h. The decrease in T_g reflects that water, absorbed by lactose during the adjustment to the higher water activity, acted as a plasticizer, thereby lowering T_g . The values of the stabilized T_g at all given 10 combinations of storage temperature and water activity are shown in **Table 2**.

From **Table 2**, it is clear that the observed T_g for lactose is strongly dependent upon both storage temperature and water activity. The differences in observed stabilized T_g are a consequence of differences in water content depending on both temperature and water activity, since water is an efficient plasticizer. Upon increasing temperature, absorption characteristics of lactose are changed toward absorption of less water, and the water activity of the saturated salt solution used as a water activity buffer is slightly decreased. The combination ($a_w = 0.43$, $T = 4.8 \pm 1.5$ °C) is to be regarded as a border point, since it appeared to be the combination of water activity and storage temperature where the stabilized glass transition

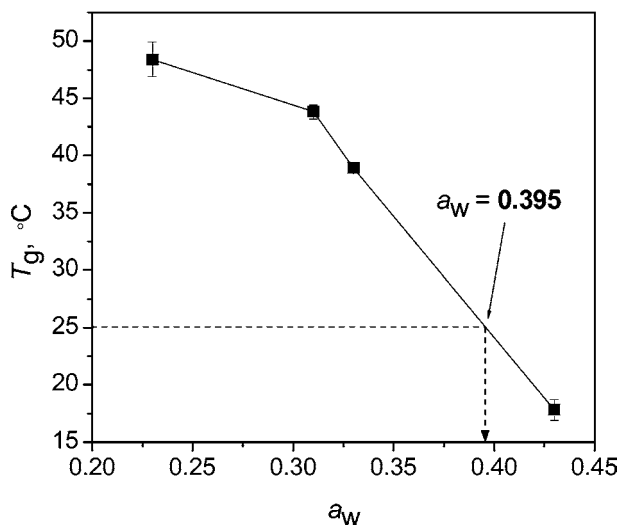


Figure 3. Average values of stabilized T_g of amorphous lactose stored at 25 °C and varying a_w . The broken line arrow indicates the value of a_w when the storage temperature equals the glass transition temperature, T_g .

temperature is equal to the storage temperature. Below the border point temperature, lactose remained in the glassy state, whereas above this temperature, but at the same water activity ($a_w = 0.43$), lactose was found to be amorphous but nonglassy. At 25 and 38 °C, border points could not be observed directly, and thus, the glass transition temperature became equal to the storage temperature at a water activity in the region between 0.33 and 0.43 and 0.21–0.32, respectively. To obtain the border points at 25 and 38 °C, interpolation of the experimental values in **Table 2** was necessary, and **Figure 3** shows a plot of the stabilized T_g against a_w for amorphous lactose stored at 25 °C to illustrate the principle.

The glass transition temperature, T_g , was found to be equal to the storage temperature 25 °C at water activity 0.395, which is a combination that thus represents a border point between glassy and nonglassy states. In the same manner, the border point at storage temperature 38 °C was found by interpolation to be ($a_w = 0.30$, $T = 38$ °C). The border points obtained through this procedure are plotted in the water activity/temperature plane in **Figure 4**. The line is drawn between the points in order to guide the eyes; however, for measurements of the glass transition temperature, it is important to consider that the glass transition is represented by a temperature interval in the thermogram, of which T_g can be determined in various manners (5, 6). In the present study, T_g was taken as the onset of the second-order transition; see **Figure 1**. Furthermore, the value of T_g is influenced, although to a lesser degree for instance of the scan rate of the DSC. Therefore, the border between glassy and nonglassy lactose in **Figure 4** must, rather than a thin line, be considered to be a region around the line, in which glass transition occurs as illustrated in **Figure 4**.

Figure 4 depicts the physical state of lactose for in principle any combinations of water activity and temperature. For temperatures below the T_g borderline, lactose remains in the amorphous glassy state, which can be considered as meta-stable, due to very high viscosity and limited molecular mobility (2). Above the line, lactose enters the more unstable amorphous rubbery state, in which viscosity is lowered, and thus, molecular mobility is increased (2). Thus, **Figure 4** constitutes a water activity/temperature state diagram, which is an alternative to the traditional water content/temperature state diagram. With the help of this state diagram, it is thus possible to predict the

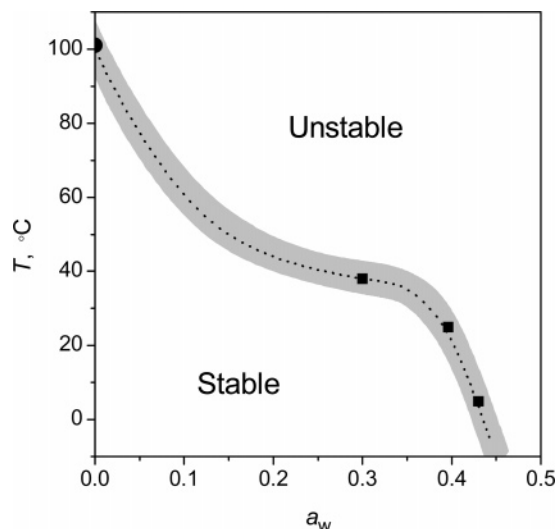


Figure 4. Water activity–temperature state diagram of amorphous lactose. The gray area indicates a transition zone separating stable amorphous lactose from unstable nonglassy lactose. The dotted line marks the borderline, where the storage temperature equals the glass transition temperature, T_g . ■ = experimental value; ● from ref 6. The line and transition zone are drawn to guide the eyes and are not based on actual measurements.

physical state of lactose at any given combination of water activity and storage temperature, to determine the long-term stability of pure lactose and lactose-rich products. The experimental effort of obtaining the border points is concentrated in a temperature interval relevant for storage of dry lactose-based formulation and milk powder (5–38 °C). Conventional water content/temperature state diagrams are constructed for a wide temperature interval extending from the glass transition of pure water to that of pure lactose. To conceptually show the interrelation with the conventional type state diagrams, we included information of the glass transitions temperature of dry lactose, $T_g = 101$ °C, corresponding to a water activity of 0 (6). It should be noted that in principle it is possible to extend the diagram by including the combination of water activity and temperature of supercooled pure water (1, -137 ± 1 °C) (7, 8). To guide the eyes and to qualitatively illustrate the interval nature of the glass transition, a soft bending glass transition region has been added to the state diagram to complement the actual experimentally based points.

DISCUSSION

A stability map comparable to **Figure 4** has previously been published for sucrose (9). In preparing that diagram, a more conventional experimental approach was used. Briefly, a set of moisture sorption isotherms corresponding to a set of storage temperatures was combined with a conventional state diagram in order to obtain the needed information. However, the details in this procedure were never published (9). In this context, it is worth noticing that the procedure used in this study automatically includes the temperature effect on moisture sorption without the need for moisture sorption isotherms to be worked out at every temperature under consideration.

When comparing the concept of water activity/temperature state diagrams with the more conventional water content/temperature state diagram, it is worth noticing some fundamental differences in measuring and controlling water content and water activity in heterogeneous materials. Most foods can be considered to be complex and nonuniform, and in the specific case of

products rich in amorphous lactose, other domains or phases (such as phases of crystalline lactose or fat) might be present. Thermodynamics ensures that water activity is uniform throughout a heterogeneous material when equilibrium is attained. Thus, the water activity reflects the local condition in a phase of amorphous lactose. This is not true in the case of water content as this quantity only reflects the overall composition of the product, and local concentrations of water in amorphous regions might deviate substantially due to the presence of other phases, such as crystalline lactose or fat phases. It can consequently be argued that the presented type of state diagram is more useful and of more general value in the case of heterogeneous foods materials.

Lactose is one of the main components in milk-based powders, and because glass transition temperatures of pure lactose and milk powders are very similar, it is likely that the physical state of lactose governs the state of the milk powders (10, 11). Consequently, it is reasonable to suppose that the stability of milk powders, with regards to crystallization of lactose, molecular mobility, and accordingly possibility for chemical reactions, can be predicted by Figure 4. To establish the potential use of Figure 4 in the design of drying processes for powder to be stored at different temperatures, experimental results from existing literature on milk powder stability, stored at constant water activity, have been collected. For whole milk powder (approximately 38% lactose), the critical water activity, defined as the water activity at which T_g is lower than the storage temperature, has been found to be 0.37 at 24 °C (10, 12), which is within the border region in Figure 4. Furthermore, crystallization was observed within 24 h of storage at 24 °C for whole milk powder, when the water activities were ≥ 0.44 (10, 13), which is in the "unstable" region of Figure 4, and thus, crystallization is predicted to occur within a short time scale. For infant formula, crystallization was observed within 17 days during storage at a water activity of 0.31 and 40 °C (14), which is almost at the borderline of Figure 4, and again, crystallization must be expected to occur within a short time scale. Infant formula stored at a water activity of 0.43 at 27 °C crystallized within 3–4 days of storage (15), which again is in accordance with Figure 4, as these conditions are above the border region of Figure 4. For pure amorphous lactose, crystallization was observed at water activities above 0.4 at 20 °C, above 0.32 at 30 °C, and above 0.31 at 38 °C (time scale not described) (16); all combinations of temperature and water activity are situated within the border region in Figure 4 and accordingly predicted to lead to crystallization within a short time scale. These examples confirm that the plot in Figure 4 may be a valuable tool in predicting the stability of lactose and lactose-based dry products.

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