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Structural strength analysis of partially crystalline trehalose.

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1. Abstract

Strength concept, which is based on the Williams-Landel-Ferry (WLF) model, was developed using mixed structured powders containing amorphous and crystalline components. At the present study, semi-crystalline trehalose powders with various (100:0; 80:20; 60:40; 40:60; 20:80) amorphous to crystalline ratios were analyzed. Amorphous components were prepared from water solution by freeze-drying. Strength analysis, which included water sorption, differential scanning calorimetry, dynamic mechanical analysis and microscopy, was applied. The results indicated that water content significantly decreases glass transition (~100°C) and α-relaxation temperatures (~90°C) as well as structural strength parameter (~10°C), while, the effect of crystalline component is less pronounced. This study can be used in processing and characterization of various partially crystalline food products including nutritional formulations and infant formulas.

Keywords: Structural relaxation, WLF, glass transition, semi-crystalline structure.

2. Introduction

The improvement of processing and storage techniques requires a deep and fundamental knowledge of thermodynamics and kinetics. Food solids can exist in crystalline, amorphous or partially (e. g. semi) crystalline powders. Crystalline and amorphous materials show significantly different physicochemical properties (Bhandari, Bansal, Zhang & Schuck, 2013), due to differences in microstructure. Crystalline structures have long range molecular order, while amorphous structures are more disordered (short range molecular alignment) (Nurhadi & Roos, 2016). To preserve the taste, flavor and color of food the materials should be maintained in the amorphous form (Roos & Drusch, 2015). Amorphous materials are thermodynamically unstable compared to crystalline structures, however they are fairly stable in the glassy state (e. g. glass) (Slade, Levine & Reid, 1991). At temperatures close to the calorimetric glass transition temperature (T_g), physical properties of solids, such as molecular mobility, viscosity, etc. significantly change and materials are converted to supercooled

liquids (e. g. rubber) showing time-dependent flow (Angell, Ngai, McKenna, McMillan & Martin, 2000). Hence, obtaining the time-dependent characteristics of thermal, electric and mechanical changes is practically important. Structural strength concept, proposed by Roos and co-workers combine temperature differences ($T-T_g$) and a practically important time factor (critical change in structural relaxation time) (Roos et al., 2015).

Trehalose is a natural disaccharide of glucose with a high T_g (Green & Angell, 1989). Trehalose is widely used in food and biotechnology areas due to specific physicochemical properties such as prevention of biomolecules degradation (Crowe, Crowe, Rudolph, Womersley & Appel, 1985; Uritani, Takai & Yoshinaga, 1995); preservation of vaccines and medical proteins capabilities (Xie & Timasheff, 1997; Miller & de Pablo, 2000).

Amorphicity of materials can be detected gravimetrically from changing mass during water sorption (Buckton & Darcy, 1995; Mackin et al., 2002; Lehto et al., 2006; Nurhadi & Roos, 2016). Amorphous materials have larger porosity and more hydroscopic properties, hence amorphous structures show a higher sorption capacity than crystalline forms (Bhandari, Bansal, Zhang & Schuck, 2013; Nurhadi & Roos, 2016).

Differential scanning calorimetry (DSC) has been used as a method for detection and quantification of the amorphous components in carbohydrate systems. For this, sample is heated to above the T_g to get dehydration (T_h) (endotherm peak in DSC thermogram) and recrystallization (exotherm peak in DSC thermogram). The areas of exothermal and endothermal peaks are proportional to the amorphous content in a partially crystalline sample. Another approach to detect amorphous component in material by DSC is to determine the change of specific heat capacity (C_p) over the glass transition (Saleki-Gerhardt, Ahlneck & Zografi, 1994; Sebhatu, Angberg & Ahlneck, 1994; Lehto et al., 2006). Optical (light) microscopy usually shows that the partially crystalline structure tends to increase in transparency while approaching the transition temperature of dehydration without losing their external morphology (Sussich, Urbani, Princivalle & Cesaro, 1998). A dynamic mechanical analysis (DMA) in multi-frequency mode is a useful tool to characterize mechanical properties including α, β, γ-relaxations, which happen due to variation in molecular mobility below and around T_g (Moates, Noel, Parker & Ring, 2001). Knowing the frequency of measurements allows obtaining the value of structural relaxation time of α-relaxation process (Noel, Parker & Ring, 2000), which may be related to particle structure, collapse and viscous flow. As the determination of viscous flow characteristics at temperatures close to the T_g is extremely difficult, strength concept provides an estimation of resistance to structural changes for amorphous materials above the calorimetry onset temperature during heating.

Strength analysis, which included water sorption, DSC and DMA, was successfully applied for various carbohydrate-protein, carbohydrate-carbohydrate and miscible models such as trehalose-whey protein isolate (WPI) (Fan & Roos, 2016a, b; Maidannyk & Roos, 2016), lactose-WPI (Fan & Roos, 2016a, b; Maidannyk & Roos, 2017), lactose-trehalose (Fan & Roos, 2016a) and trehalose-maltodextrin (Maidannyk, Nurhadi & Roos 2017). These studies showed that structural strength linearly depends on concentration of components and significantly decreases with increasing water content in a system. However, structural strength analysis of partially crystalline systems has not been addressed. The main aim of the present study was to develop the strength model using partially crystalline systems of trehalose. For this, effect of crystalline component on structural strength of amorphous trehalose was under investigation and effects of water content on structural strength of the partially crystalline systems were also studied.

3. Materials and Methods

3.1 Materials

D-(+)-Trehalose crystalline dihydrate (Hayashibara Co., Ltd., Okayama, Japan) and de-ionized water (KB scientific, Cork, Ireland) were used without purification.

3.1.1 Amorphous structure

Amorphous structure was obtained by freeze-drying (Lyovac GT2, Steris®, Hürth, Germany). For this, trehalose solution (total solid of 20%) in water was prepared. After that, $5x10^{-3}$ L aliquots of solution were frozen in pre-weighted and semi-closed with septum in $1x10^{-2}$ L glass vials (Schott, Mulheim, Germany) at -20°C for 24 h, then at -80°C for 3 h, followed by freeze-drying for 60 h at pressure $p < 10$ Pa. All vials were hermetically sealed under the vacuum conditions inside the freeze dryer at $p < 10$ Pa and stored over P_2O_5 in vacuum desiccators (Roos & Karel, 1990) at room temperature (25 ± 1°C) to protect samples from water uptake.

3.1.2 Crystalline structure

Crystalline trehalose powder was prepared by grinding commercial trehalose powder and stored in a desiccator over P_2O_5 at 25±1°C for 3 days. A small amount of amorphous structure can be detected after grinding procedure (Willart, Dujardin, Dudognon, Danede & Descamps, 2010; Nurhadi & Roos, 2016).

3.1.3 Partially crystalline structures

Partially crystalline trehalose systems were prepared by blending accurately weighted amount of 100% crystalline and 100% amorphous structures at 100:0; 80:20; 60:40; 40:60; 20:80 and 0:100 amorphous:crystalline ratios. Mixing was done by spatula and shaking the closed vials (Nurhadi & Roos, 2016).

3.2 Determination of the initial water content

Samples of trehalose powder with final weight 0.5-1.0 g were dried at 70°C with absolute pressure $P_{abs}<10^3$ Pa for 24 hours in a WTB Binder vacuum oven (Mason Technology®, Tuttingen, Germany) to measure the initial water content of the material. The difference in mass of samples before and after drying was defined as initial water content.

3.3 Water sorption analysis

Partially crystalline systems with various ratios of amorphous and crystalline structures (described above) were stored at evacuated desiccators (25±1°C) for 10 days over the saturated solutions of P_2O_5, LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $NaNO_2$, NaCl and KCl (Sigma Chemical Co., St. Louis, MO. U.S.A.), which at equilibrium provided 0, 0.11, 0.23, 0.33, 0.44, 0.545, 0.66, 0.76 and 0.85 a_w, respectively. AQUALAB 4 (TE) (Decagon Devices Inc., Pullman, WA., U.S.A.) water activity meter was used to measure water activity for each material after storage. Samples were weighted at intervals of 0, 2, 4, 6, 8, 10, 24, 48, 72, 96 and 120 hours upon storage. Possible crystallization of amorphous trehalose was assessed from the loss of sorbed water. The water content in each mixture was plotted as a function of time, and the Guggenheim-Anderson-deBoer (GAB) relationship was fitted to data to know water activity-water content dependence of amorphous trehalose systems (Eq. 1):

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

where, m is water content, m_0 is the monolayer value and C and k were respectively calculated from m_0.

Equation 2 was used to determine the combined effects of amorphous and crystalline components (Bronlund & Paterson, 2004):

$$W_{mixture} = n_1 W_{crystalline} + n_2 W_{amorphous} \quad (2)$$

where, $W_{mixture}$ is the total equilibrium water content in the mixture; n_1 and n_2 are mass fractions of crystalline and amorphous components in the system ($n_1+n_2 = 1$); $W_{crystalline}$ and $W_{amorphous}$ are water contents in crystalline and amorphous component.

3.4 Differential Scanning Calorimetry (DSC)

Differential scanning calorimeter (DSC) (Mettler Toledo Schwerzenbach, Switzerland) was used to measure the glass transition temperature, dehydration and spontaneous recrystallization of partially crystalline trehalose mixtures with 0, 0.11, 0.23, 0.33 and 0.44 a_w. Samples of all mixtures were transferred to pre-weighted standard DSC aluminium pans (40 µL, Mettler Toledo, Schwerzenbach, Switzerland) and hermetically sealed. An empty punctured pan was used as a reference. For anhydrous systems only, the lids of DSC aluminium pans were punctured to allow evaporation of residual water upon the measurement. All samples were scanned with 5°C/min heating rate. The onset of T_g, heat capacity of endotherm and exotherm were determined by the STAR^e software version 8.10 (Mettler Toledo, Schwerzenbach, Switzerland).

3.5 Dynamical Mechanical Analyses (DMA)

Dynamic mechanical analyzer (DMA) (Tritec 2000 DMA, Triton Technology Ltd., Grantham, Lincolnshire, UK) was used to measure mechanical properties (E'' – loss modulus, E' – storage modulus and tanδ = E''/E') of anhydrous and humidified partially crystalline trehalose systems (described above for the DSC experiments). The DMA

instrument was balanced or set at zero to determine the zero displacement position before starting. Approximately 60 g of ground samples were spread on a metal pocket-forming sheet (Triton Technology Ltd., UK). This sheet was fixed between the stationary and drive shaft clamps inside the measuring head of the DMA. Length, width and thickness were measured for each sample. All results were obtained using 1.43.00 DMA software version. To control temperature the DMA was connected to a liquid nitrogen tank (1L; Cryogun, Brymill Cryogenic Systems, Labquip Ltd., Dublin, Ireland). Samples were scanned from ~ 50°C below to over the α-relaxation region with cooling rate of 5°C/min and heating rate of 2°C/min using the single cantilever bending mode (Fan & Roos, 2016a, b; Maidannyk & Roos, 2016). The α-relaxation temperatures (T_α) were determined from peaks of tanδ above the glass transition.

Equation 3 was used to calculate the relaxation times (τ) of peak T_α, measured by DMA at various frequencies (f) (Noel, Parker & Ring, 2000):

$$\tau = \frac{1}{2\pi f} \tag{3}$$

3.6 Optical light microscopy

Microscope observation (OLYMPUS BX51 (Olympus Corporation, Tokyo, Japan), with magnification x20) was done on humidified (0, 0.11, 0.23 and 0.44 a_w) partially crystalline trehalose powders (described above for the DSC experiments) placed between cover glass and mounted Linkam 120 (TP 94) temperature controller stage. The samples were scanned from 5 to 150°C, with 2°C/min heating rate.

3.7 Calculation of WLF model constants and Structural Strength

The WLF equation in the form of (Eq. 4) was used to fit DMA and DSC data (Williams, Landel & Ferry, 1955):

$$log_{10}\frac{\tau}{\tau_s} = log_{10}\frac{\eta}{\eta_s} = \frac{-C_1(T-T_g)}{C_2+(T-T_g)} \tag{4}$$

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where, τ is relaxation time, τ_s is reference relaxation time, η is viscosity, η_s is reference viscosity, T is temperature, T_g is glass transition temperature, C_1 and C_2 are constants.

The WLF equation in the form of (Eq. 5) suggested that the plot of $1/lg(\tau/\tau_s)$ versus $1/(T-T_g)$ gives a linear correlation:

$$\frac{1}{lg\frac{\tau}{\tau_s}} = \frac{1}{-C_1} - \frac{C_2}{C_1(T-T_g)} \quad (5)$$

The WLF constants C_1 and C_2 were derived from the slope and intercept of the straight line (Roos & Drusch, 2015).

Mathematically, structural strength parameter (S) is based on WLF relationship and can be calculated by Equation (6):

$$S = \frac{dC_2}{C_1 - d} \quad (6)$$

where d is a parameter, showing the critical decrease in the number of logarithmic decades for the flow (e.g., 100 s to 0.01 s corresponds to $d = 4$; can be chosen for each system as an integer depending on the critical time for the process (Fig. 4)), C_1 and C_2 are "non-universal" constants in the WLF equation.

Equation 7 was used to predict structural strength at different water contents:

$$S = \frac{w_1 S_1 + k w_2 S_2}{w_1 + k w_2} \quad (7)$$

where w_1 – weight fraction of dry solid; w_2 – weight fraction of water; k – coefficient; S_1 – structural strength for anhydrous system; S_2 – structural strength of pure water (S_2 = 6.0) (Maidannyk & Roos, 2017).

3.8 Data analysis

All experiments were performed in triplicate. Mean data of the water sorption analyses, DSC, DMA were calculated from 3 replicates with standard deviations expressed in error bars.

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4. Results and discussion

4.1 Water sorption analysis

Table 1 and Figure 1 shows experimental water content of partially crystalline trehalose systems, over the whole range of water activities at 25±1°C.

Crystalline structures humidified at low water activities (0-0.76 a_w) adsorb very little amount of water while at high water activities (0.85-0.99 a_w) the amount of sorbed water increases exponentially (Table 1) due to capillary condensation and dissolution (Bronlund & Paterson, 2004).

Steady-state water contents of each mixture at 120 hours were used in GAB model. The GAB model was fitted to experimental data of 100:0 amorphous:crystalline systems below 0.55 a_w (Labuza, 1984; Maidannyk & Roos, 2016). However, at higher water activities (≥0.55 a_w), the crystallization of trehalose occurred and extrapolated sorption data from GAB relationship give misleading results for sorbed water contents (Potes, 2014). Fractional isotherm approach (Eq. 2) allowed calculating water contents at high water activities. Using previously published data for pure amorphous trehalose (Maidannyk & Roos, 2016; Maidannyk, Nurhadi & Roos, 2017) and experimental data for pure crystalline trehalose, the GAB model was fitted to obtain sorption isotherms at high water activities (Fig. 1). Amorphous structure adsorbs around 100 times more water than crystalline structure (Table 1). That's why the presence of a small amount of amorphous component in crystalline structure results in significant difference to the water sorption isotherm of material. Water sorption isotherm and Eq. 2 allows predicting water content for partially crystalline trehalose at all water activities and all amorphous:crystalline ratios.

4.2 Differential Scanning Calorimetry and Microscopy

The onset of calorimetric glass transition temperature (T_g) was obtained by DSC for each anhydrous and humidified (0-0.44a_w) partially crystalline trehalose system (Table 2).

Detected values of T_g belong to amorphous component in partially crystalline systems. The obtained data for pure anhydrous trehalose is in agreement with previous study (Green & Angell, 1989; Roos 1993; Miller & de Pablo, 2000; Maidannyk & Roos, 2016; 2017 a). As expected crystalline fraction has no significant effect on the T_g of the amorphous fraction in

the partially crystalline systems (Table 2). Glass transition is that of the amorphous fraction which is shown by the proportional decrease of heat capacity (C_p) over the T_g region. A slight decrease of T_g value with increase of crystalline component content in the systems is in agreement with water sorption data (Table 1). Water as a good and strong plasticizer significantly increases mobility of molecules of the amorphous component, which results in the decrease of T_g values with increasing water content (Maidannyk & Roos, 2016). For example, in the present study T_g of 100:0 amorphous:crystalline system decreased from 112°C (at 0 a_w) to 15°C (at 0.44 a_w) due to water plasticization.

Microscope observation, carried out on the same samples placed between cover glasses, showed that crystalline and amorphous components of trehalose became more transparent during heating to above T_g. However, at the same time the external morphology of the structure was not changed until the temperature about 135°C, that the "solid" sample appears to change morphological shape ("edge roundness") with an increasing mobility of the phase, although still firm (sticky) (Sussich et al, 1998) (Figure 2). These observations were the same for anhydrous and humidified (0.11, 0.23, 0.33 and 0.44a_w) partially crystalline trehalose systems.

4.3 Dynamical Mechanical Analyses (DMA)

Partially crystalline trehalose systems with different ratios of amorphous and crystalline components (described above for DSC experiments) were scanned by DMA in multi-frequency mode.

The rapid changes in mechanical properties, which related to the amount of energy converted to heat during relaxation can be detected by DMA, while DSC directly detects the difference in the amount of heat (Gonnet, Guillet, Sirakov, Fulchiron & Seytre, 2002; Roos & Drusch, 2015). Figure 3 shows typical frequently-dependent thermogram obtained by DMA. The α-relaxation temperature values were determined from the temperature peak of tanδ, at ~ 20-30ºC above the onset of the T_g. Systems with high amount of crystalline component showed slightly lowered α-relaxation temperature peak with less intensity, due to significant difference between crystalline and amorphous particle sizes (crystalline particles needs less mechanical energy than amorphous and α-relaxations occurs at lower temperature). Also crystalline phase may slightly decreased α-relaxation temperature due to sliding effect inside the partially crystalline system (Cano-Chauca, Stringheta, Ramos & Cal-Vidal, 2005). The big differences (~6-10ºC) were observed for anhydrous partially crystalline trehalose

systems, while for humidified systems (0.44 a_w) these differences were not significant (~2-5ºC) and α-relaxation occurred at similar temperatures at all amorphous and crystalline ratios (Table 3).

Water as a good plasticizer, increases free volume in the systems as well as molecular mobility of amorphous components (Slade et al., 1991; Royall et al., 2005; Meinders and van Vliet, 2009) that result in significant decrease (~85ºC for 100:0 amorphous:crystalline) of the T_α for every partially crystalline system with increasing water content. These results are in agreement with previous studies (Fan & Roos, 2016 a, b; Maidannyk & Roos, 2016; 2017; Maidannyk, Nurhadi & Roos, 2017).

4.4 Applications of WLF equation and Strength

Williams-Landel-Ferry or WLF model (Eq. 4) is a popular equation, which can describe dependence of relaxation time and viscosity on the temperature close to onset T_g. Original relationship (Williams et al., 1955) showed that glass formers possessed similar decreases in relaxation times over the temperature range of T_g to T_g + 100 K. Because of that authors offered "universal" values for WLF constants: C_1 =17.44 and C_2 = 51.6 for many different materials (inorganic and organic). However, using WLF equation with "non-universal" constants C_1 and C_2 provides more realistic modelling with good fit to experimental data (Ferry, 1980; Peleg, 1992; Slade & Levine, 1995; Peleg & Chinachoti, 1996; Roos & Drusch, 2015;). In the present study, WLF constants C_1 and C_2 were calculated by Eq. 5 and summarized in table 4. C_1 and C_2 constants were determined with 0.9651, 0.9210, 0.9116, 0.9491 and 0.7622 R^2 for 100:0; 80:20; 60:40; 40:60 and 20:80 amorphous:crystalline ratios at RH 0%; 0.9707, 0.8933, 0.9281, 0.9681 and 0.9013 at RH 11%; 0.8079, 0.8694, 0.7596, 0.9664 and 0.7279 at RH 23%; 0.9987, 0.9491, 0.9506, 0.9627 and 0.7639 at RH 33%; 0.7440, 0.9742, 0.9715, 0.9807 and 0.9698 at RH 44% respectively.

In calculations, the viscosity and relaxation time of supercooled liquid approached 10^{12} Pa s and 100 s respectively. Upon heating, values of viscosity and relaxation time were decreasing down to 10^5 Pa s and 10^{-14} s respectively (Angell, 1991; Angell, Ngai, McKenna, McMillan & Martin, 2000; Roos & Drusch, 2015; Maidannyk & Roos, 2016). Using 10^{12} Pa s and 100 s as reference values of viscosity and structural relaxation time respectively, Roos and co-workers (Roos et al., 2015; Fan & Roos, 2016 a ,b; Maidannyk & Roos, 2016; 2017; Maidannyk, Nurhadi & Roos, 2017) adapted WLF equation in a simple and convenient form, named "Strength" concept. Structural strength measures solid flow characteristics of amorphous

materials above T_g and links the material state with a practically important time factor. As large and critical changes in structural relaxation time upon heating happened between 100 s and 0.01 s (between 2 and -2 in logarithmic scale) (Fig. 4), the corresponding temperature difference, $T-T_g$, was defined as strength, S (Eq. 6) (Roos et al., 2015; Fan & Roos, 2016 a ,b; Maidannyk & Roos, 2016; 2017; Maidannyk, Nurhadi & Roos, 2017).

S was obtained (at d = 4) for partially crystalline trehalose systems at all ratios (Table 5). Strength curves, which show structural strength dependence on water content, were calculated by Eq. 7, which allows predicting S value at all water contents (from 0 to 100 g/100g of dry Solids) (Fig. 5). The k value was 15.2, 26.0, 17.1, 21.0 and 26.0 for 100:0, 80:20, 60:40, 40:60 and 20:80 amorphous:crystalline trehalose systems respectively.

Table 5 and Figure 5 shows that for anhydrous systems (RH 0%) the S value significantly decreased (ΔS = 9.1ºC) with increasing crystalline component in a system, while for humidified systems (RH 44%) these differences were not so pronounced (ΔS = 1.7 ºC). This result is similar to our previous findings and can be explained by molecular mobility of amorphous component and by strong plasticization properties of water (Roos et al., 2015; Fan & Roos, 2016 a, b; Maidannyk & Roos, 2016; 2017; Maidannyk, Nurhadi & Roos, 2017).

Even small variation in material structure significantly changes the structural strength in a system (Fig. 6). Amorphous systems show more “strong” behavior compare to “weak” highly crystalline trehalose systems. However, this difference is significant only for anhydrous systems, while humidified systems shows very similar values of strength for all partially crystalline system. Hence, the presence of water is a very important factor, which significantly influences a structural strength and structure of food material. Despite the fact that for humidified systems, the values of S are similar, they are still decreasing linearly (with R^2 0.9879, 0.9310, 0.9045, 0.8860, 0.8816, 0.8789, 0.8767, 0.8750, 0.8743 and 0.8737 at 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% of water respectively) with increasing crystalline content in partially crystalline systems (Fig. 7).

Knowing structural strength of pure amorphous component and reference partially crystalline systems allows calculation of S value for systems at any crystalline content at any water content, as well as prediction of crystalline content in unknown partially crystalline system. Therefore, the present study showed that strength concept is a suitable model for determining crystalline content in partially crystalline system and allows controlling stability and quality in various food systems.

5. Conclusions

This study was focused on applying and further development of the structural strength concept. For this, strength analysis was applied in partially crystalline trehalose system. Water sorption analysis allows controlling water contents in different partially crystalline mixtures at low and high water activities. The glass transition and α-relaxation temperatures were detected by DSC and DMA respectively. The values of T_g and T_α as well as structural strength are significantly decreasing with increasing water content in a system. At very low content of amorphous component in a system, the determination of strength is practically complex. However, strength analysis showed good consistency in predicting the crystalline content in unknown systems at different water contents. The structural strength shows linear decrease with increase of crystalline content in a system and significant decrease with increase of the water content in a system. Hence structural strength concept can be used in adaptation of processing and characterization of various food materials as well as in quality and stability control during production and storage.

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474 *Table 1. Water content of partially crystalline trehalose systems (ratios of components 100:0,*
475 *80:20, 60:40, 40:60, 20:80, 0:100) stored at different water activities (0.11, 0.23, 0.33, 0.44,*
476 *0.55, 0.65, 0.76, 0.85, 0.99) for 120 hours at 25±1°C.*

a_w	Experimental water content for partially crystalline (amorphous:crystalline) trehalose systems (g/100 g of solids)					
	100:0	**80:20**	**60:40**	**40:60**	**20:80**	**0:100**
0.11	2.2±0.1	1.7±0.1	1.3±0.1	0.9±0.1	0.4±0.1	0.010±0.01
0.23	4.1±0.4	3.3±0.1	2.5±0.3	1.7±0.2	0.8±0.2	0.014±0.03
0.33	5.9±0.4	4.7±0.2	3.7±0.1	2.4±0.2	1.2±0.2	0.019±0.03
0.44	9.0±0.2	6.9±0.2	5.3±0.1	3.4±0.3	1.8±0.2	0.023 ±0.05
0.55	9.5±0.1	7.6±0.1	5.6±0.3	3.8±0.3	1.9±0.3	0.029±0.04
0.65	9.4±0.3	7.6±0.2	5.7±0.1	3.8±0.1	1.9±0.3	0.037±0.02
0.76	8.8±0.4	7.2±0.3	5.5±0.2	3.6±0.2	1.8±0.2	0.049±0.05
0.85	9.5±0.4	7.6±0.1	5.5±0.2	3.7±0.2	1.9±0.3	0.066±0.02
0.99	9.7±0.3	7.8±0.3	5.7±0.3	3.8±0.3	2.3±0.2	0.598±0.09

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479 *Table 2. The onset of calorimetric glass transition temperatures (T_g) of anhydrous (0 a_w) and*
480 *humidified (0.11-0.44 a_w) partially crystalline trehalose systems (amorphous:crystalline)*
481 *stored for 120 hours at 25±1°C.*

	Glass transition temperature (T_g) of partially crystalline (amorphous:crystalline) trehalose systems, ^{O}C				
a_w	**100:0**	**80:20**	**60:40**	**40:60**	**20:80**
0	112±3	112±2	111±3	111±2	111±2
0.11	62±1	61±3	60±2	59±2	59±2
0.23	43±2	43±1	42±3	42±1	41±1
0.33	32±2	32±1	32±2	31±3	31±2
0.44	15±2	15±3	15±2	15±1	14±3

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484 *Table 3. α-Relaxation temperature (T_α) detected by DMA for partially crystalline trehalose*
485 *systems with different amorphous:crystalline ratios, equilibrated at different relative*
486 *humidities (RH).*

RH 0%		**Amorphous:Crystalline trehalose**				
		100:0	**80:20**	**60:40**	**40:60**	**20:80**
f, Hz	**logτ, s**	**T_α, °C**	**T_α, °C**	**T_α, °C**	**T_α, °C**	**T_α, °C**
0.1	**0.20**	123±2	122±2	118±2	117±2	116±1
0.5	**-0.49**	125±1	123±1	119±1	118±1	116±2
1.0	**-0.80**	127±2	125±2	120±2	119±2	117±2
2.0	**-1.10**	128±2	126±3	121±1	120±2	118±1
5.0	**-1.50**	128±3	127±1	123±3	121±2	119±3
10.0	**-1.80**	130±1	128±2	124±2	122±1	120±2
RH 11%		**Amorphous:Crystalline trehalose**				
		100:0	**80:20**	**60:40**	**40:60**	**20:80**
f, Hz	**logτ, s**	**T_α, °C**	**T_α, °C**	**T_α, °C**	**T_α, °C**	**T_α, °C**
0.1	**0.20**	71±2	68±2	66±2	64±2	64±2
0.5	**-0.49**	73±2	69±2	67±2	65±2	65±2
1.0	**-0.80**	75±1	70±3	68±2	66±2	65±3
2.0	**-1.10**	77±2	71±2	69±1	67±2	66±1
5.0	**-1.50**	78±2	73±1	70±2	68±3	67±2
10.0	**-1.80**	79±3	75±2	72±2	69±3	68±2
RH 23%		**Amorphous:Crystalline trehalose**				
		100:0	**80:20**	**60:40**	**40:60**	**20:80**
f, Hz	**logτ, s**	**T_α, °C**	**T_α, °C**	**T_α, °C**	**T_α, °C**	**T_α, °C**
0.1	**0.20**	52±1	51±2	50±3	49±1	47±2
0.5	**-0.49**	53±1	52±3	50±2	50±2	47±1
1.0	**-0.80**	53±2	52±2	51±2	51±3	48±2
2.0	**-1.10**	54±2	53±2	52±3	51±1	48±2
5.0	**-1.50**	55±1	54±3	53±1	52±1	49±2
10.0	**-1.80**	57±2	55±3	53±2	52±2	50±2
RH 33%		**Amorphous:Crystalline trehalose**				
		100:0	**80:20**	**60:40**	**40:60**	**20:80**
f, Hz	**logτ, s**	**T_α, °C**	**T_α, °C**	**T_α, °C**	**T_α, °C**	**T_α, °C**
0.1	**0.20**	38±2	38±1	37±1	36±1	36±1
0.5	**-0.49**	40±2	39±2	39±1	37±2	36±1
1.0	**-0.80**	41±2	40±1	39±2	38±2	37±1
2.0	**-1.10**	42±3	41±2	41±1	39±1	38±2
5.0	**-1.50**	43±3	42±2	41±2	39±2	38±2
10.0	**-1.80**	44±2	43±1	41±2	40±2	39±2
RH 44%		**Amorphous:Crystalline trhalose**				
		100:0	**80:20**	**60:40**	**40:60**	**20:80**
f, Hz	**logτ, s**	**T_α, °C**	**T_α, °C**	**T_α, °C**	**T_α, °C**	**T_α, °C**
0.1	**0.20**	19±1	18±2	18±1	18±1	17±1

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0.5	**-0.49**	19±1	19±2	19±1	19±1	18±2
1.0	**-0.80**	20±2	20±1	20±2	20±2	19±1
2.0	**-1.10**	21±1	21±1	21±2	21±2	20±1
5.0	**-1.50**	23±1	22±1	23±1	22±1	20±1
10.0	**-1.80**	25±1	24±2	23±2	23±1	22±2

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489 *Table 4. Calculated WLF (τ_s = 100s; η_s = 10^{12} Pa s) constants C_1 and C_2 for partially*
490 *crystalline trehalose systems, stored at various relative humidities (RH).*

Amorphous: Crystalline Trehalose	***RH 0%***		***RH 11%***		***RH 23%***		***RH 33%***		***RH 44%***	
	-C_1, s	***-C_2, °C***	***-C_1, s***	***-C_2, °C***	***-C_1, s***	***-C_2, °C***	***-C_1, s***	***-C_2, °C***	***-C_1, s***	***-C_2, °C***
100:0	5.0±0.3	40.7±0.5	23.7±0.5	123.8±2.4	3.8±0.9	25.6±1.7	31.8±2.6	111.4±2.3	-10.0±0.7	-14.6±1.1
80:20	5.5±0.3	38.7±0.6	27.4±1.5	104.0±3.1	3.2±1.1	21.0±1.5	11.9±1.3	43.6±1.2	-9.6±0.5	-12.5±1.0
60:40	15.1±0.3	61.0±0.5	20.9±0.7	70.8±1.3	3.8±1.4	22.1±0.7	24.0±2.1	71.5±1.6	-9.5±0.9	-12.3±0.7
40:60	11.9±0.2	43.6±1.2	36.4±1.1	101.1±1.7	2.8±0.6	17.4±1.7	10.7±0.9	33.9±2.9	-11.1±1.3	-15.0±0.9
20:80	44.6±0.2	109.6±2.7	9.1±0.6	28.4±2.1	4.6±1.3	18.8±1.0	8.9±1.1	25.9±1.4	-13.4±1.6	-18.8±2.0

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494 *Table 5. "Strength" S (calculated at d = 4) for partially crystalline trehalose systems at all*
495 *ratios (amorphous:crystalline) stored at different relative humidities (RH).*

Amorphous: Crystalline Trehalose	RH 0%	RH11%	RH 23%	RH 33%	RH 44%
100:0	18.1±2.9	17.9±2.1	13.2±1.8	12.5±1.2	9.7±1.1
80:20	16.2±2.5	13.2±1.9	11.6±1.3	10.9±1.1	8.9±1.4
60:40	12.8±2.3	11.4±1.1	11.4±1.1	10.2±2.1	9.0±0.4
40:60	10.9±1.7	10.0±1.2	10.3±0.9	9.2±1.3	8.4±0.5
20:80	9.0±0.9	8.7±0.4	8.7±0.5	8.1±0.7	8.0±0.7

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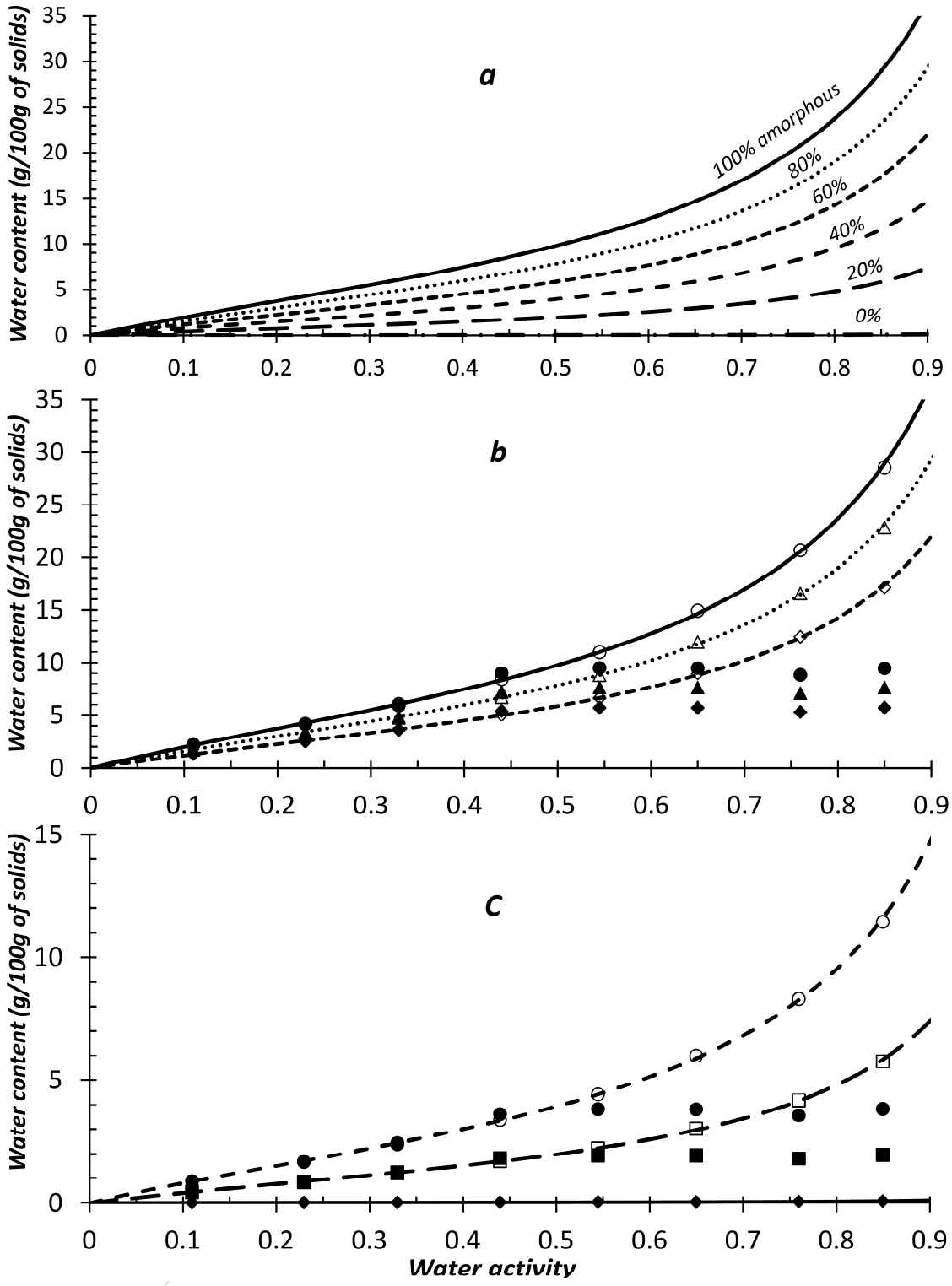

Figure 1. (a) The GAB sorption isotherms at 25±1°C, predicted with Eq. 1 for 0:100 (– · – ·), 20:80 (— —), 40:60 (— –), 60:40 (- - -), 80:20 (·····) and 100:0 (——) partially crystalline trehalose; comparison of modeled (lines), experimental data (solid symbols) and predicted by Eq. 1 (empty symbols): (b) for 100:0 (●,○), 80:20 (▲,△) , 60:40 (♦,◇) partially crystalline systems; (c) for 40:60 (●,○), 20:80 (▪,▫), 0:100 (♦) partially crystalline systems.

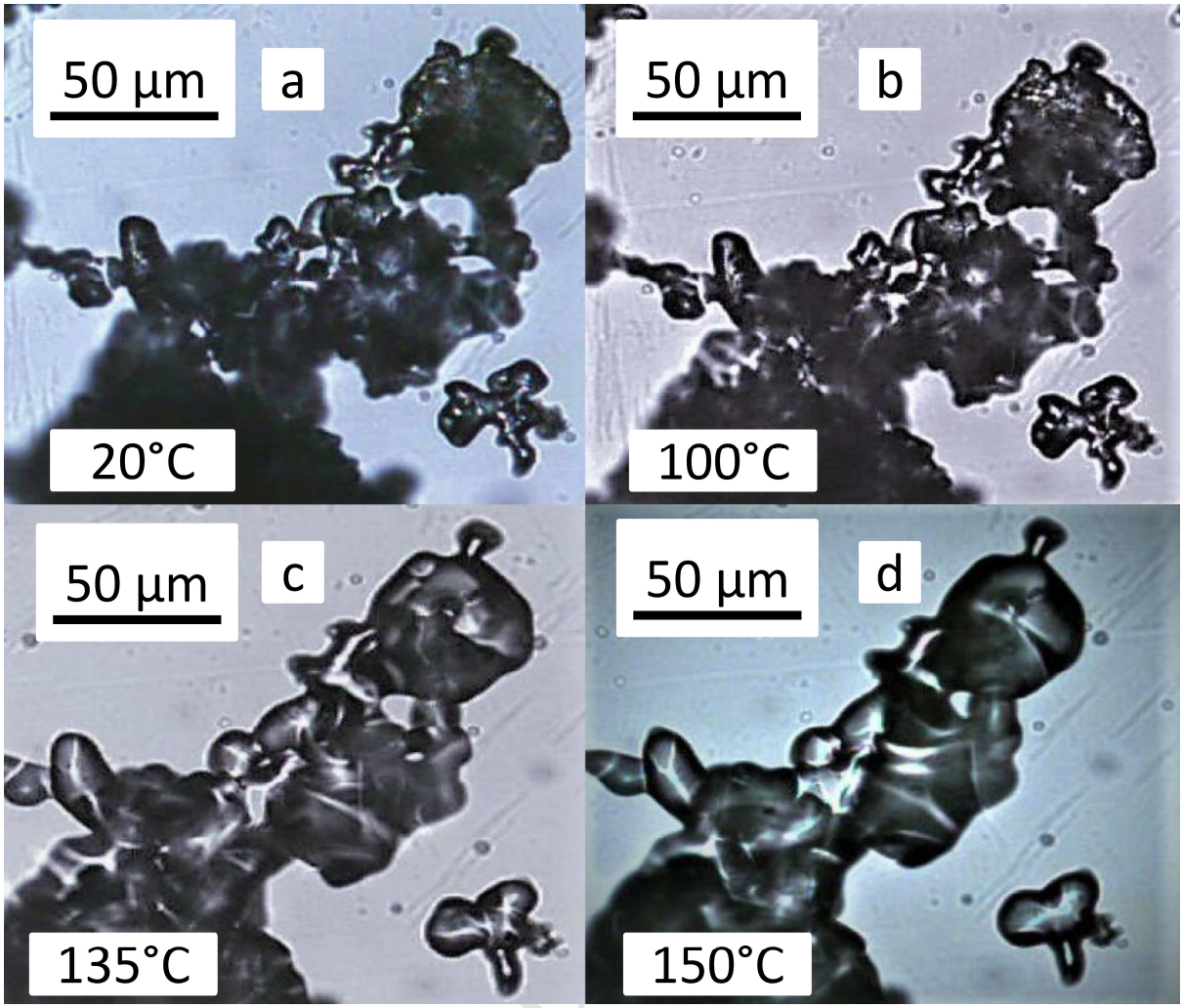

Figure 2. Microscope observation of 60:40 amorphous:crystalline trehalose, stored at 0.11 a_w, scanned at 2 K min^{-1} at the temperature of (a) 20°C; (b) 100°C; (c) 135°C and (d) 150°C.

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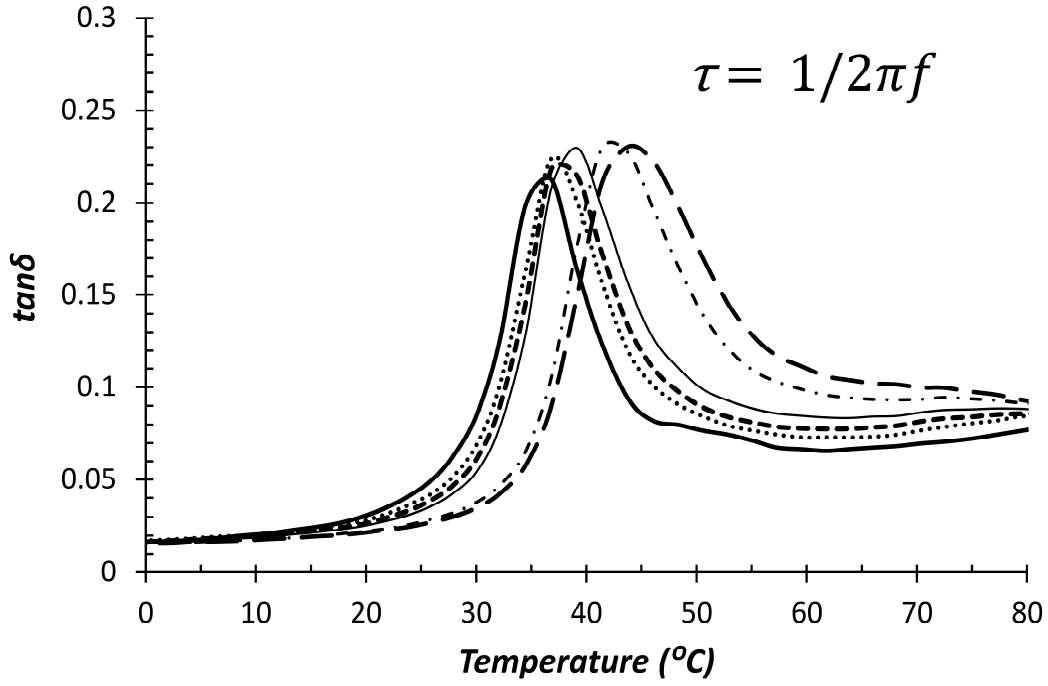

508

509 *Figure 3. Temperature dependence of tangents δ for humidified (0.23 a_w) 80:20*
510 *amorphous:crystalline trehalose system determined by DMA in multi-frequency mode 0.1 Hz*
511 *(—), 0.5 Hz (·····), 1 Hz (- - -), 2 Hz (—), 5 Hz (– · – ·), 10 Hz (––).*

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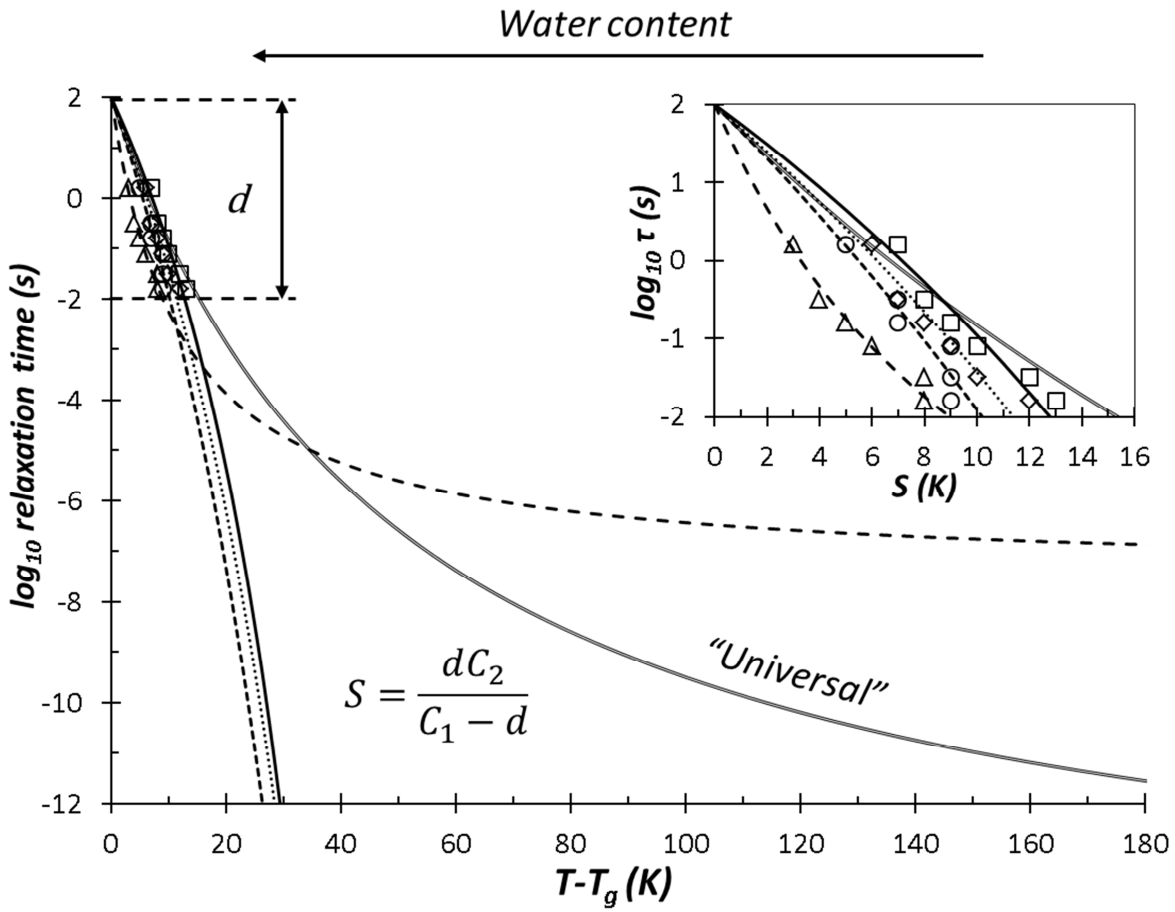

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514 *Figure 4. Modified WLF curves (lines), experimental data (symbols) and "strength" plot*
515 *(inset) for 60:40 amorphous:crystalline trehalose system humidified at different relative*
516 *humidities (0 (—, ▫), 11 (·····, ◇), 33 (- - -, ○), 44% RH (- - -, △)) with different water content*
517 *(0, 1.3, 3.7, 5.3 (g/100g of dry solids), respectively).*

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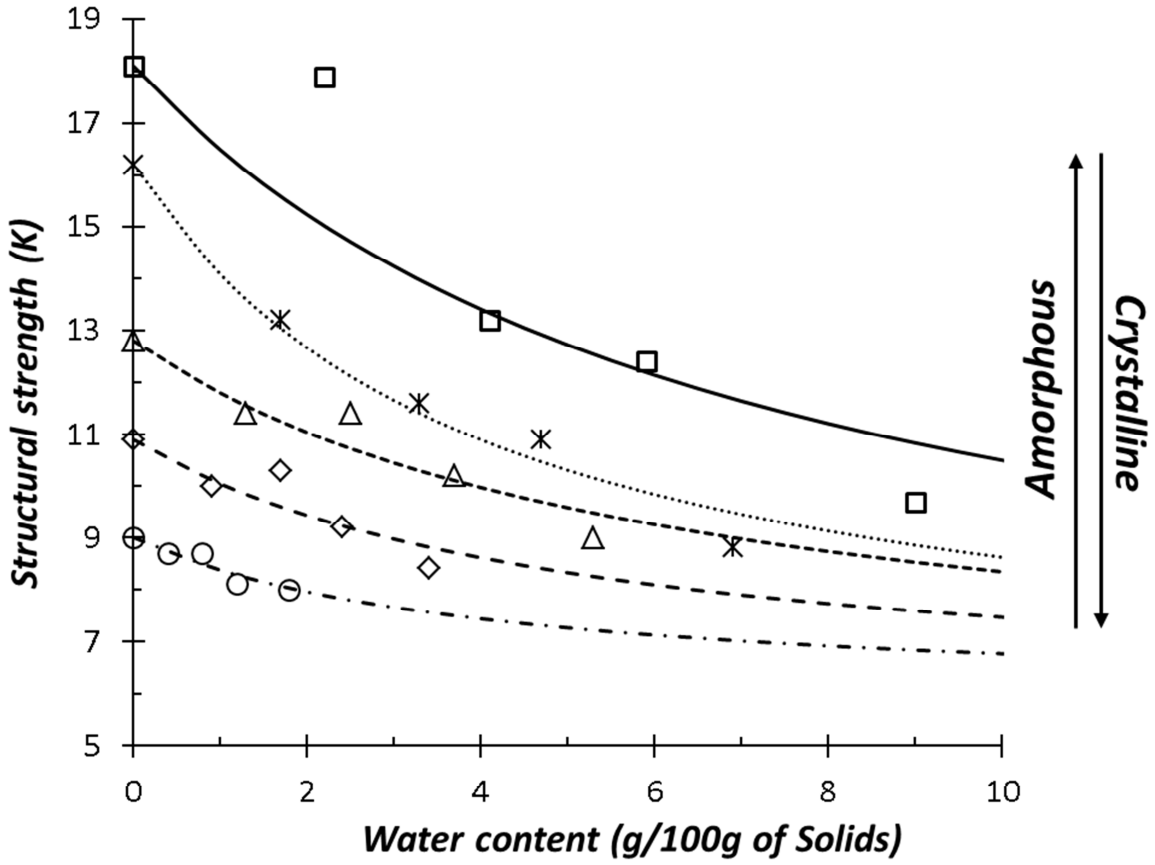

519

520 *Figure 5. Strength curves, modeled by Eq. 7 (lines) and experimental (symbols) S of partially*

521 *crystalline trehalose systems (100:0 (—, ▫), 80:20 (·····,Ж), 60:40 (- - -, △), 40:60 (- - -, ◇),*

522 *and 20:80 (– · – ·, ○) amorphous:crystalline ratios) at different water contents (g/100g of dry*

523 *solids).*

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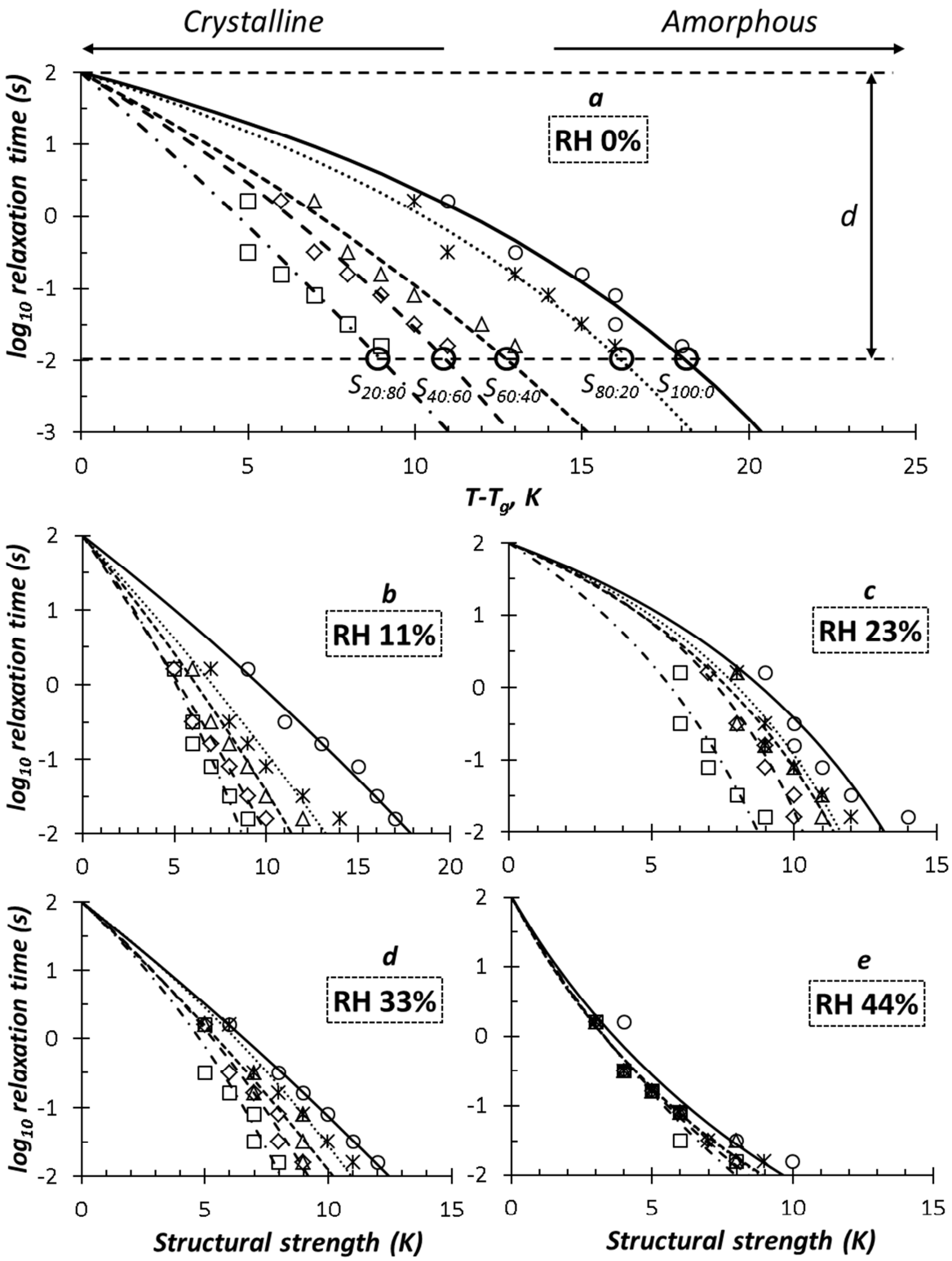
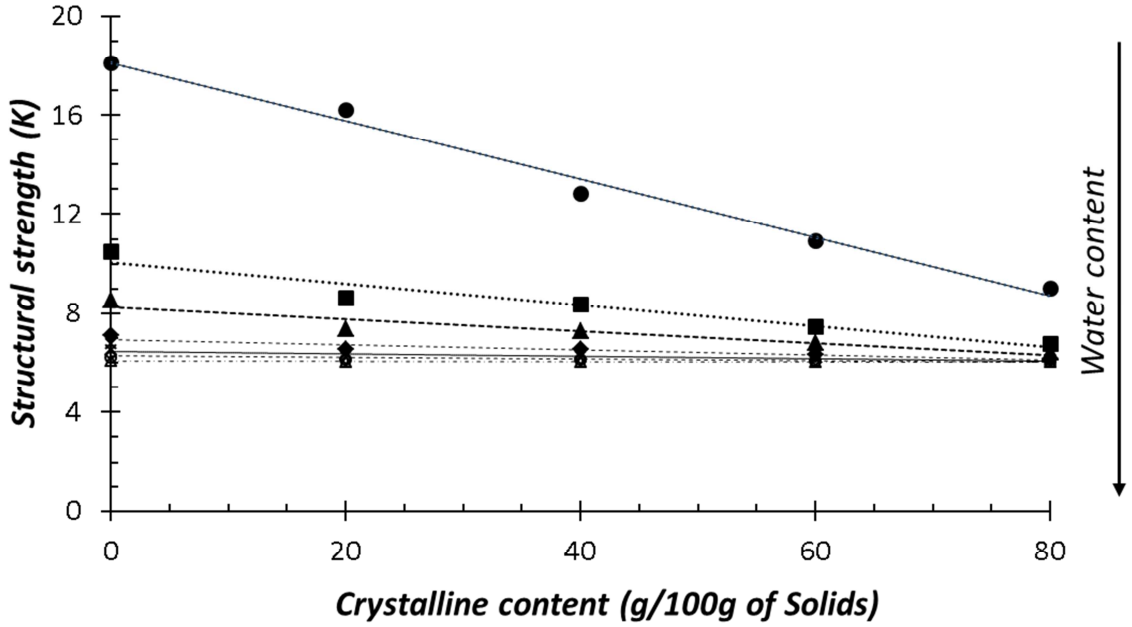


Figure 6. Modified WLF curves (lines) and experimental data (symbols) for partially crystalline trehalose (100:0 (——, ○), 80:20 (·····,Ж), 60:40 (- - -, △), 40:60 (- - -, ◇) and 20:80 (— · — ·,▫) amorphous:crystalline ratios) stored 120 h (at 25±1ºC) at different relative humidities (a (RH 0%), b (RH 11%), c (RH 23%), d (RH 33%) and e (RH 44%).



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531 *Figure 7. Structural strength versus crystalline component content of partially crystalline*
532 *trehalose (%) at different water contents (the data predicted by Eq. 7): 0% (●), 10% (▪), 20%*
533 *(▲), 40% (♦), 50% (Ж), 60% (X), 70% (◇), 80% (○) and 90% (△).*

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The concept of strength was developed using partially crystalline trehalose systems.

Strength shows linear decreasing with increasing of crystalline content in a system.

Fractional water sorption analysis was used in partially crystalline systems.

DSC shows crystalline phase-independent glass transition temperature.

The water content dependence on strength was shown.

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