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# Structural strength analysis of partially crystalline trehalose.

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### 5 1. Abstract

6

7 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 8 present study, semi-crystalline trehalose powders with various (100:0; 80:20; 60:40; 40:60; 9 20:80) amorphous to crystalline ratios were analyzed. Amorphous components were prepared 10 from water solution by freeze-drying. Strength analysis, which included water sorption, 11 differential scanning calorimetry, dynamic mechanical analysis and microscopy, was applied. 12 The results indicated that water content significantly decreases glass transition (~100°C) and 13  $\alpha$ -relaxation temperatures (~90°C) as well as structural strength parameter (~10°C), while, 14 the effect of crystalline component is less pronounced. This study can be used in processing 15 and characterization of various partially crystalline food products including nutritional 16 formulations and infant formulas. 17

18

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

20

## 21 **2. Introduction**

22

The improvement of processing and storage techniques requires a deep and fundamental 23 24 knowledge of thermodynamics and kinetics. Food solids can exist in crystalline, amorphous 25 or partially (e. g. semi) crystalline powders. Crystalline and amorphous materials show 26 significantly different physicochemical properties (Bhandari, Bansal, Zhang & Schuck, 2013), due to differences in microstructure. Crystalline structures have long range molecular 27 order, while amorphous structures are more disordered (short range molecular alignment) 28 (Nurhadi & Roos, 2016). To preserve the taste, flavor and color of food the materials should 29 be maintained in the amorphous form (Roos & Drusch, 2015). Amorphous materials are 30 thermodynamically unstable compared to crystalline structures, however they are fairly stable 31 in the glassy state (e. g. glass) (Slade, Levine & Reid, 1991). At temperatures close to the 32 33 calorimetric glass transition temperature  $(T_g)$ , physical properties of solids, such as molecular mobility, viscosity, etc. significantly change and materials are converted to supercooled 34

35 liquids (e. g. rubber) showing time-dependent flow (Angell, Ngai, McKenna, McMillan & 36 Martin, 2000). Hence, obtaining the time-dependent characteristics of thermal, electric and 37 mechanical changes is practically important. Structural strength concept, proposed by Roos 38 and co-workers combine temperature differences (T-T<sub>g</sub>) and a practically important time 39 factor (critical change in structural relaxation time) (Roos et al., 2015).

Trehalose is a natural disaccharide of glucose with a high T<sub>g</sub> (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 50 quantification of the amorphous components in carbohydrate systems. For this, sample is 51 52 heated to above the T<sub>g</sub> to get dehydration (T<sub>h</sub>) (endotherm peak in DSC thermogram) and recrystallization (exotherm peak in DSC thermogram). The areas of exothermal and 53 54 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 55 56 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 57 (light) microscopy usually shows that the partially crystalline structure tends to increase in 58 transparency while approaching the transition temperature of dehydration without losing their 59 60 external morphology (Sussich, Urbani, Princivalle & Cesaro, 1998). A dynamic mechanical analysis (DMA) in multi-frequency mode is a useful tool to characterize mechanical 61 properties including  $\alpha$ ,  $\beta$ ,  $\gamma$ -relaxations, which happen due to variation in molecular mobility 62 below and around Tg (Moates, Noel, Parker & Ring, 2001). Knowing the frequency of 63 measurements allows obtaining the value of structural relaxation time of  $\alpha$ -relaxation process 64 (Noel, Parker & Ring, 2000), which may be related to particle structure, collapse and viscous 65 flow. As the determination of viscous flow characteristics at temperatures close to the Tg is 66 extremely difficult, strength concept provides an estimation of resistance to structural 67 changes for amorphous materials above the calorimetry onset temperature during heating. 68

69 Strength analysis, which included water sorption, DSC and DMA, was successfully applied for various carbohydrate-protein, carbohydrate-carbohydrate and miscible models such as 70 71 trehalose-whey protein isolate (WPI) (Fan & Roos, 2016a, b; Maidannyk & Roos, 2016), lactose-WPI (Fan & Roos, 2016a, b; Maidannyk & Roos, 2017), lactose-trehalose (Fan & 72 73 Roos, 2016a) and trehalose-maltodextrin (Maidannyk, Nurhadi & Roos 2017). These studies showed that structural strength linearly depends on concentration of components and 74 75 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 76 present study was to develop the strength model using partially crystalline systems of 77 trehalose. For this, effect of crystalline component on structural strength of amorphous 78 trehalose was under investigation and effects of water content on structural strength of the 79 80 partially crystalline systems were also studied.

81

### 82 **3. Materials and Methods**

83

### 84 3.1 Materials

85

B6 D-(+)-Trehalose crystalline dihydrate (Hayashibara Co., Ltd., Okayama, Japan) and deionized water (KB scientific, Cork, Ireland) were used without purification.

- 88
- 89 *3.1.1 Amorphous structure*

90

Amorphous structure was obtained by freeze-drying (Lyovac GT2, Steris<sup>®</sup>, Hürth, Germany). 91 For this, trehalose solution (total solid of 20%) in water was prepared. After that,  $5 \times 10^{-3}$  L 92 aliquots of solution were frozen in pre-weighted and semi-closed with septum in  $1 \times 10^{-2}$  L 93 glass vials (Schott, Mulheim, Germany) at -20°C for 24 h, then at -80°C for 3 h, followed by 94 freeze-drying for 60 h at pressure p < 10 Pa. All vials were hermetically sealed under the 95 vacuum conditions inside the freeze dryer at p < 10 Pa and stored over  $P_2O_5$  in vacuum 96 desiccators (Roos & Karel, 1990) at room temperature  $(25 \pm 1^{\circ}C)$  to protect samples from 97 water uptake. 98

99

100 3.1.2 Crystalline structure

102 Crystalline trehalose powder was prepared by grinding commercial trehalose powder and 103 stored in a desiccator over  $P_2O_5$  at  $25\pm1^{\circ}C$  for 3 days. A small amount of amorphous 104 structure can be detected after grinding procedure (Willart, Dujardin, Dudognon, Danede & 105 Descamps, 2010; Nurhadi & Roos, 2016).

106

# 107 3.1.3 Partially crystalline structures

108

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).

113

# 114 3.2 Determination of the initial water content

115

116 Samples of trehalose powder with final weight 0.5-1.0 g were dried at 70°C with absolute 117 pressure  $P_{abs} < 10^3$  Pa for 24 hours in a WTB Binder vacuum oven (Mason Technology<sup>®</sup>, 118 Tuttingen, Germany) to measure the initial water content of the material. The difference in 119 mass of samples before and after drying was defined as initial water content.

120

### 121 3.3 Water sorption analysis

122

123 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 124 saturated solutions of P<sub>2</sub>O<sub>5</sub>, LiCl, CH<sub>3</sub>COOK, MgCl<sub>2</sub>, K<sub>2</sub>CO<sub>3</sub>, Mg(NO<sub>3</sub>)<sub>2</sub>, NaNO<sub>2</sub>, NaCl and 125 KCl (Sigma Chemical Co., St. Louis, MO. U.S.A.), which at equilibrium provided 0, 0.11, 126 0.23, 0.33, 0.44, 0.545, 0.66, 0.76 and 0.85 a<sub>w</sub>, respectively. AQUALAB 4 (TE) (Decagon 127 Devices Inc., Pullman, WA., U.S.A.) water activity meter was used to measure water activity 128 for each material after storage. Samples were weighted at intervals of 0, 2, 4, 6, 8, 10, 24, 48, 129 72, 96 and 120 hours upon storage. Possible crystallization of amorphous trehalose was 130 assessed from the loss of sorbed water. The water content in each mixture was plotted as a 131 function of time, and the Guggenheim-Anderson-deBoer (GAB) relationship was fitted to 132 data to know water activity-water content dependence of amorphous trehalose systems (Eq. 133 1): 134

136 
$$\frac{m}{m_0} = \frac{Cka_w}{(1 - ka_w)(1 - ka_w + Cka_w)}$$
(1)

137

138 where, m is water content,  $m_0$  is the monolayer value and C and k were respectively 139 calculated from  $m_0$ .

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

(2)

142

143 
$$W_{mixture} = n_1 W_{crystalline} + n_2 W_{amorphous}$$

144

145 where,  $W_{mixture}$  is the total equilibrium water content in the mixture;  $n_1$  and  $n_2$  are mass 146 fractions of crystalline and amorphous components in the system  $(n_1+n_2 = 1)$ ;  $W_{crystalline}$  and 147  $W_{amorphous}$  are water contents in crystalline and amorphous component.

148

# 149 3.4 Differential Scanning Calorimetry (DSC)

150

Differential scanning calorimeter (DSC) (Mettler Toledo Schwerzenbach, Switzerland) was 151 used to measure the glass transition temperature, dehydration and spontaneous 152 recrystallization of partially crystalline trehalose mixtures with 0, 0.11, 0.23, 0.33 and 0.44 153 a<sub>w</sub>. Samples of all mixtures were transferred to pre-weighted standard DSC aluminium pans 154 (40 µL, Mettler Toledo, Schwerzenbach, Switzerland) and hermetically sealed. An empty 155 156 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 157 measurement. All samples were scanned with 5°C/min heating rate. The onset of T<sub>g</sub>, heat 158 capacity of endotherm and exotherm were determined by the STAR<sup>e</sup> software version 8.10 159 (Mettler Toledo, Schwerzenbach, Switzerland). 160

161

# 162 3.5 Dynamical Mechanical Analyses (DMA)

163

164 Dynamic mechanical analyzer (DMA) (Tritec 2000 DMA, Triton Technology Ltd., 165 Grantham, Lincolnshire, UK) was used to measure mechanical properties (E'' – loss 166 modulus, E' – storage modulus and  $\tan \delta = E''/E'$ ) of anhydrous and humidified partially 167 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 168 starting. Approximately 60 g of ground samples were spread on a metal pocket-forming sheet 169 (Triton Technology Ltd., UK). This sheet was fixed between the stationary and drive shaft 170 clamps inside the measuring head of the DMA. Length, width and thickness were measured 171 for each sample. All results were obtained using 1.43.00 DMA software version. To control 172 temperature the DMA was connected to a liquid nitrogen tank (1L; Cryogun, Brymill 173 Cryogenic Systems, Labquip Ltd., Dublin, Ireland). Samples were scanned from ~ 50°C 174 below to over the α-relaxation region with cooling rate of 5°C/min and heating rate of 175 2°C/min using the single cantilever bending mode (Fan & Roos, 2016a, b; Maidannyk & 176 Roos, 2016). The  $\alpha$ -relaxation temperatures (T<sub> $\alpha$ </sub>) were determined from peaks of tan $\delta$  above 177 178 the glass transition.

179 Equation 3 was used to calculate the relaxation times ( $\tau$ ) of peak T<sub>a</sub>, measured by DMA at 180 various frequencies (*f*) (Noel, Parker & Ring, 2000):

182 
$$\tau = \frac{1}{2\pi f} \tag{3}$$

183

181

# 184 3.6 Optical light microscopy

185

Microscope observation (OLYMPUS BX51 (Olympus Corporation, Tokyo, Japan), with magnification x20) was done on humidified (0, 0.11, 0.23 and 0.44 a<sub>w</sub>) 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.

191

# 192 3.7 Calculation of WLF model constants and Structural Strength

193

194 The WLF equation in the form of (Eq. 4) was used to fit DMA and DSC data (Williams,195 Landel & Ferry, 1955):

196

197 
$$log_{10}\frac{\tau}{\tau_s} = log_{10}\frac{\eta}{\eta_s} = \frac{-C_1(T - T_g)}{C_2 + (T - T_g)}$$
 (4)

- 199 where,  $\tau$  is relaxation time,  $\tau_s$  is reference relaxation time,  $\eta$  is viscosity,  $\eta_s$  is reference 200 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:
- 203

204 
$$\frac{1}{lg\frac{\tau}{\tau_s}} = \frac{1}{-C_1} - \frac{C_2}{C_1(T-T_g)}$$
 (5)

205

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

208 Mathematically, structural strength parameter (S) is based on WLF relationship and can be209 calculated by Equation (6):

210

211 
$$S = \frac{dC_2}{C_1 - d}$$
 (6)

212

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:

218

219 
$$S = \frac{w_1 S_1 + k w_2 S_2}{w_1 + k w_2}$$
(7)

220

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).

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225 3.8 Data analysis
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226

227 All experiments were performed in triplicate. Mean data of the water sorption analyses, DSC,

228 DMA were calculated from 3 replicates with standard deviations expressed in error bars.

- 4. Results and discussion 230
- 231

#### 4.1 Water sorption analysis 232

233

234 Table 1 and Figure 1 shows experimental water content of partially crystalline trehalose systems, over the whole range of water activities at  $25\pm1^{\circ}$ C. 235

236 Crystalline structures humidified at low water activities (0-0.76 a<sub>w</sub>) adsorb very little amount of water while at high water activities (0.85-0.99 a<sub>w</sub>) the amount of sorbed water increases 237 238 exponentially (Table 1) due to capillary condensation and dissolution (Bronlund & Paterson, 2004). 239

Steady-state water contents of each mixture at 120 hours were used in GAB model. The GAB 240 model was fitted to experimental data of 100:0 amorphous:crystalline systems below 0.55 a<sub>w</sub> 241 (Labuza, 1984; Maidannyk & Roos, 2016). However, at higher water activities (≥0.55 a<sub>w</sub>), the 242 crystallization of trehalose occurred and extrapolated sorption data from GAB relationship 243 give misleading results for sorbed water contents (Potes, 2014). Fractional isotherm approach 244 (Eq. 2) allowed calculating water contents at high water activities. Using previously 245 published data for pure amorphous trehalose (Maidannyk & Roos, 2016; Maidannyk, 246 Nurhadi & Roos, 2017) and experimental data for pure crystalline trehalose, the GAB model 247 248 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 249 presence of a small amount of amorphous component in crystalline structure results in 250 significant difference to the water sorption isotherm of material. Water sorption isotherm and 251 Eq. 2 allows predicting water content for partially crystalline trehalose at all water activities 252 and all amorphous:crystalline ratios. 253

254

#### 4.2 Differential Scanning Calorimetry and Microscopy 255

256

The onset of calorimetric glass transition temperature (T<sub>g</sub>) was obtained by DSC for each 257 anhydrous and humidified (0-0.44a<sub>w</sub>) partially crystalline trehalose system (Table 2). 258

Detected values of T<sub>g</sub> belong to amorphous component in partially crystalline systems. The 259 obtained data for pure anhydrous trehalose is in agreement with previous study (Green & 260 Angell, 1989; Roos 1993; Miller & de Pablo, 2000; Maidannyk & Roos, 2016; 2017 a). As 261 expected crystalline fraction has no significant effect on the  $T_g$  of the amorphous fraction in 262

the partially crystalline systems (Table 2). Glass transition is that of the amorphous fraction 263 which is shown by the proportional decrease of heat capacity  $(C_p)$  over the  $T_g$  region. A slight 264 decrease of Tg value with increase of crystalline component content in the systems is in 265 agreement with water sorption data (Table 1). Water as a good and strong plasticizer 266 significantly increases mobility of molecules of the amorphous component, which results in 267 the decrease of  $T_g$  values with increasing water content (Maidannyk & Roos, 2016). For 268 example, in the present study T<sub>g</sub> of 100:0 amorphous:crystalline system decreased from 269 112°C (at 0 a<sub>w</sub>) to 15°C (at 0.44 a<sub>w</sub>) due to water plasticization. 270

Microscope observation, carried out on the same samples placed between cover glasses, 271 showed that crystalline and amorphous components of trehalose became more transparent 272 during heating to above T<sub>g</sub>. However, at the same time the external morphology of the 273 structure was not changed until the temperature about 135°C, that the "solid" sample appears 274 to change morphological shape ("edge roundness") with an increasing mobility of the phase, 275 although still firm (sticky) (Sussich et al, 1998) (Figure 2). These observations were the same 276 for anhydrous and humidified (0.11, 0.23, 0.33 and 0.44a<sub>w</sub>) partially crystalline trehalose 277 systems. 278

279

# 280 4.3 Dynamical Mechanical Analyses (DMA)

281

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

The rapid changes in mechanical properties, which related to the amount of energy converted 285 286 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, 287 288 2015). Figure 3 shows typical frequently-dependent thermogram obtained by DMA. The  $\alpha$ relaxation temperature values were determined from the temperature peak of tan $\delta$ , at ~ 20-289 30°C above the onset of the T<sub>g</sub>. Systems with high amount of crystalline component showed 290 slightly lowered  $\alpha$ -relaxation temperature peak with less intensity, due to significant 291 difference between crystalline and amorphous particle sizes (crystalline particles needs less 292 mechanical energy than amorphous and  $\alpha$ -relaxations occurs at lower temperature). Also 293 crystalline phase may slightly decreased  $\alpha$ -relaxation temperature due to sliding effect inside 294 the partially crystalline system (Cano-Chauca, Stringheta, Ramos & Cal-Vidal, 2005). The 295 big differences (~6-10°C) were observed for anhydrous partially crystalline trehalose 296

systems, while for humidified systems (0.44  $a_w$ ) these differences were not significant (~2-5°C) and  $\alpha$ -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 (~85°C for 100:0 amorphous:crystalline) of the T<sub> $\alpha$ </sub> 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).

306

# 307 4.4 Applications of WLF equation and Strength

308

Williams-Landel-Ferry or WLF model (Eq. 4) is a popular equation, which can describe 309 dependence of relaxation time and viscosity on the temperature close to onset T<sub>g</sub>. Original 310 relationship (Williams et al., 1955) showed that glass formers possessed similar decreases in 311 relaxation times over the temperature range of  $T_g$  to  $T_g + 100$  K. Because of that authors 312 offered "universal" values for WLF constants:  $C_1 = 17.44$  and  $C_2 = 51.6$  for many different 313 314 materials (inorganic and organic). However, using WLF equation with "non-universal" constants C<sub>1</sub> and C<sub>2</sub> provides more realistic modelling with good fit to experimental data 315 (Ferry, 1980; Peleg, 1992; Slade & Levine, 1995; Peleg & Chinachoti, 1996; Roos & Drusch, 316 2015;). In the present study, WLF constants C1 and C2 were calculated by Eq. 5 and 317 summarized in table 4. C<sub>1</sub> and C<sub>2</sub> constants were determined with 0.9651, 0.9210, 0.9116, 318 0.9491 and 0.7622 R<sup>2</sup> for 100:0; 80:20; 60:40; 40:60 and 20:80 amorphous:crystalline ratios 319 at RH 0%; 0.9707, 0.8933, 0.9281, 0.9681 and 0.9013 at RH 11%; 0.8079, 0.8694, 0.7596, 320 0.9664 and 0.7279 at RH 23%; 0.9987, 0.9491, 0.9506, 0.9627 and 0.7639 at RH 33%; 321 0.7440, 0.9742, 0.9715, 0.9807 and 0.9698 at RH 44% respectively. 322

In calculations, the viscosity and relaxation time of supercooled liquid approached  $10^{12}$  Pa s 323 and 100 s respectively. Upon heating, values of viscosity and relaxation time were decreasing 324 down to 10<sup>5</sup> Pa s and 10<sup>-14</sup> s respectively (Angell, 1991; Angell, Ngai, McKenna, McMillan & 325 Martin, 2000; Roos & Drusch, 2015; Maidannyk & Roos, 2016). Using 10<sup>12</sup> Pa s and 100 s as 326 reference values of viscosity and structural relaxation time respectively, Roos and co-workers 327 (Roos et al., 2015; Fan & Roos, 2016 a ,b; Maidannyk & Roos, 2016; 2017; Maidannyk, 328 Nurhadi & Roos, 2017) adapted WLF equation in a simple and convenient form, named 329 "Strength" concept. Structural strength measures solid flow characteristics of amorphous 330

- 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<sub>g</sub>, 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 ( $\Delta S = 9.1^{\circ}C$ ) with increasing crystalline component in a system, while for humidified systems (RH 44%) these differences were not so pronounced ( $\Delta S = 1.7 \,^{\circ}C$ ). 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 347 system (Fig. 6). Amorphous systems show more "strong" behavior compare to "weak" highly 348 crystalline trehalose systems. However, this difference is significant only for anhydrous 349 350 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 351 significantly influences a structural strength and structure of food material. Despite the fact 352 that for humidified systems, the values of S are similar, they are still decreasing linearly (with 353  $R^2$  0.9879, 0.9310, 0.9045, 0.8860, 0.8816, 0.8789, 0.8767, 0.8750, 0.8743 and 0.8737 at 0%, 354 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% of water respectively) with increasing 355 crystalline content in partially crystalline systems (Fig. 7). 356
- 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.
- 363

### 364 5. Conclusions

This study was focused on applying and further development of the structural strength 366 concept. For this, strength analysis was applied in partially crystalline trehalose system. 367 Water sorption analysis allows controlling water contents in different partially crystalline 368 mixtures at low and high water activities. The glass transition and  $\alpha$ -relaxation temperatures 369 were detected by DSC and DMA respectively. The values of  $T_g$  and  $T_\alpha$  as well as structural 370 371 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 372 complex. However, strength analysis showed good consistency in predicting the crystalline 373 content in unknown systems at different water contents. The structural strength shows linear 374 decrease with increase of crystalline content in a system and significant decrease with 375 increase of the water content in a system. Hence structural strength concept can be used in 376 adaptation of processing and characterization of various food materials as well as in quality 377 and stability control during production and storage. 378

379

# 380 6. Acknowledgments

381

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Table 1. Water content of partially crystalline trehalose systems (ratios of components 100:0, 474

- 80:20, 60:40, 40:60, 20:80, 0:100) stored at different water activities (0.11, 0.23, 0.33, 0.44, 475
- 0.55, 0.65, 0.76, 0.85, 0.99) for 120 hours at 25±1°C. 476

		Experimen	tal water cont	tent for partia	lly crystalline	(amorphous	crystalline):							
	$\mathbf{a}_{\mathbf{w}}$	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	$0.023 \pm 0.05$							
	0.55	9.5±0.1	7.6±0.1	5.6±0.3	3.8±0.3	1.9±0.3	$0.029 \pm 0.04$							
	0.65	9.4±0.3	7.6±0.2	5.7±0.1	3.8±0.1	1.9±0.3	$0.037 \pm 0.02$							
	0.76	$8.8 \pm 0.4$	7.2±0.3	5.5±0.2	3.6±0.2	1.8±0.2	$0.049 \pm 0.05$							
	0.85	9.5±0.4	7.6±0.1	5.5±0.2	3.7±0.2	1.9±0.3	$0.066 \pm 0.02$							
	0.99	9.7±0.3	7.8±0.3	5.7±0.3	3.8±0.3	2.3±0.2	$0.598 \pm 0.09$							

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  $25\pm1^{\circ}C$ .

	Glass transition temperature $(T_g)$ of partially									
	crystalline (amorphous:crystalline) trehalose systems, <sup>o</sup> C									
$\mathbf{a}_{\mathbf{w}}$	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					

482

484 Table 3.  $\alpha$ -Relaxation temperature ( $T_{\alpha}$ ) 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 <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °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%		A	morphous:C	Crystalline tre	halose							
		100:0	80:20	60:40	40:60	20:80						
f, Hz	logτ, s	T <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °C						
0.1	0.20	71±2	$68 \pm 2$	66±2	64±2	64±2						
0.5	-0.49	73±2	69±2	67±2	$65 \pm 2$	$65 \pm 2$						
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 <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °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	$47 \pm 1$						
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	$48\pm2$						
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%		A	morphous:C	Crystalline tre	halose							
		100:0	80:20	60:40	40:60	20:80						
f, Hz	logτ, s	T <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °C						
0.1	0.20	38±2	38±1	37±1	36±1	36±1						
0.5	-0.49	$40 \pm 2$	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%		A	morphous:C	Crystalline trh	alose							
		100:0	80:20	60:40	40:60	20:80						
f, Hz	logτ, s	T <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °C	T <sub>α</sub> , °C						
0.1	0.20	19±1	18±2	18±1	18±1	17±1						

ACCEPTED MANUSCRIPT										
0.5 1.0 2.0	-0.49 -0.80 -1.10	19±1 20±2 21+1	19±2 20±1 21+1	19±1 20±2 21+2	19±1 20±2 21+2	18±2 19±1 20+1				
5.0 10.0	-1.10 -1.50 -1.80	23±1 25±1	21±1 22±1 24±2	23±1 23±2	21±2 22±1 23±1	$20\pm1$ $20\pm1$ $22\pm2$				
10.0	-1.80	25±1	24±2	23±2	23±1	22±2				
						, ,				
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	Y'									

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

Amorphous:	RH	0%	RH	11%	RH	23%	RH	33%	RH 4	44%
Crystalline Trehalose	-C <sub>1</sub> , s	- <i>C</i> <sub>2</sub> , • <i>C</i>	-C <sub>1</sub> , s	-C <sub>2</sub> , •C	- <i>C</i> <sub>1</sub> , s	-C <sub>2</sub> , •C	- <i>C</i> <sub>1</sub> , s	- <i>C</i> <sub>2</sub> , • <i>C</i>	- <i>C</i> <sub>1</sub> , s	- <i>C</i> <sub>2</sub> , • <i>C</i>
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

49	93
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494	Table 5.	"Strength"	S	(calculated	at	d =	4) fa	or	partially	crystalline	e trehalo	se sy	stems	at	all

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	$16.2 \pm 2.5$	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	$10.9 \pm 1.7$	$10.0{\pm}1.2$	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
				5	

*ratios (amorphous:crystalline) stored at different relative humidities (RH).* 



498

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 (•, 4), 60:40 (•, ◊) partially crystalline
systems; (c) for 40:60 (•,•), 20:80 (•,•), 0:100 (•) partially crystalline systems.



504

505 Figure 2. Microscope observation of 60:40 amorphous:crystalline trehalose, stored at 0.11

506  $a_w$ , scanned at 2 K min<sup>-1</sup> at the temperature of (a) 20°C; (b) 100°C; (c) 135°C and (d) 150°C.



Figure 3. Temperature dependence of tangents δ for humidified (0.23 a<sub>w</sub>) 80:20
amorphous:crystalline trehalose system determined by DMA in multi-frequency mode 0.1 Hz
(---), 0.5 Hz (----), 1 Hz (----), 2 Hz (----), 5 Hz (-----), 10 Hz (----).



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



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

521 crystalline trehalose systems (100:0 (---, □), 80:20 (----, 𝔅), 60:40 (---, △), 40:60 (---, ◊),

522 and 20:80 ( $-\cdot - \cdot$ ,  $\circ$ ) amorphous: crystalline ratios) at different water contents (g/100g of dry

- *solids*).
- 524





Figure 6. Modified WLF curves (lines) and experimental data (symbols) for partially crystalline trehalose (100:0 (---,  $\circ$ ), 80:20 (----,  $\checkmark$ ), 60:40 (---,  $\checkmark$ ), 40:60 (---,  $\diamond$ ) and 20:80 (----,  $\circ$ ) amorphous:crystalline ratios) stored 120 h (at 25±1°C) at different relative humidities (a (RH 0%), b (RH 11%), c (RH 23%), d (RH 33%) and e (RH 44%).



530

531 Figure 7. Structural strength versus crystalline component content of partially crystalline

trehalose (%) at different water contents (the data predicted by Eq. 7): 0% (•), 10% (•), 20%

533 (▲), 40% (♦), 50% (𝔅), 60% (𝔅), 70% (◊), 80% (◦) and 90% (△).

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.