Modification of the WLF model for characterization of the relaxation time-temperature relationship in trehalose-whey protein isolate systems.

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1. Abstract.

Understanding structural relaxations is an important task for materials engineering in biological, food, polymer and pharmaceutical areas. By now a number of theoretical models for describing structural relaxation were developed. Nevertheless, the complicity of real systems requires more detailed investigation and development of appropriate mathematical models. In the present study trehalose-WPI system was used as a carbohydrate-protein model. The ratio of components was varied over the range 0-100% with 20% intervals. The experimental part consisted of three main sections: (1) sample preparation and preliminary analysis (water content, water sorption, freeze drying), (2) thermal (DSC) and mechanical (DMA) analyses, (3) rheology of high water content systems. Water sorption isotherms were plotted, glass transition and α -relaxation temperature were obtained for each system. Based on the WLF relationship, the “strength” parameter: $S = \frac{dC_2}{C_1 - d}$ was defined, where d is the critical decrease of τ (from 100 to 0.01s) on the logarithmic scale, C_1 and C_2 – the “non-universal” constants in WLF equation. For all systems dependence of the “strength” parameter on the composition and water content was established.

Keywords: “strength” parameter, WLF, trehalose, whey protein isolate, relaxation.

2. Introduction

Thermodynamic properties and surrounding conditions define the physical state of materials. Pressure and temperature control the equilibrium of crystalline, liquid and gaseous states. As a result of comprehensive studies of physical properties (heat capacity, thermal conductivity, viscosity etc.), Parks (Parks and Huffman, 1927) defined the glassy state as the fourth state of matter, distinct from the liquid and crystalline states. Based on this definition, any material under certain conditions can be present as a nonequilibrium solid in the glassy state. However, the glassy state is metastable state of matter with time-dependent characteristics, which affect material behavior during processing and storage (Troy and Sharp, 1930; Levine and Slade, 1986).

The amorphous solid (e.g., glass) and supercooled liquid (e.g., rubber) are characterized by a lack of molecular order in structure and they exhibit a larger volume than the same material in the equilibrium crystalline state (Slade and Levine, 1995; Roos, 2008). The glassy state has

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extremely high viscosity (10^{12} Pa s) (Levine and Slade, 1986) and can be transformed to the more liquid-like “rubbery” state over the temperature region of the glass transition (T_g). The definition of glass transition is not clear and its properties are continuously discussed (Angell, 2002). Glass transition results in dramatic changes in molecular mobility and consequently in electric and mechanical properties (Williams et al., 1955; Slade et al., 1991; Roos, 1993). Below T_g amorphous materials demonstrate higher thermodynamic parameters (enthalpy, entropy, free volume) than their equilibrium state at the same conditions. The transition from the amorphous solid state to the rubbery state results in a rapid change in structural relaxation times (Angell et al., 2000; Priestley et al., 2005). Traditionally – Arrhenius (Peleg et al., 2002), Vogel-Tammar-Fulcher (VTF) (Rault, 2000) and Williams-Landel-Ferry (WLF) (Williams et al., 1955) relationships have been used to model temperature dependence of structural relaxation times. Structural relaxation time can be a characteristic of mechanical properties which control the stability and quality of food upon storage (the particle structure, viscosity, collapse, flow characteristics). The complicity of real multicomponent systems reduces the validity of such models, although a simple approach to describe material characteristics is often required.

Carbohydrates and proteins are important components of numerous food solids and particularly dairy powders and infant formulae. Such hydrophilic materials tend to exist as amorphous, noncrystalline structures at low water content (White and Cakebread, 1966; Slade et al., 1991; Roos, 1995). In the present study systems of trehalose-whey protein isolate (WPI) were used to investigate structural relaxation times in complex food solids. Trehalose is a naturally occurring disaccharide of glucose monomers with high T_g (Green and Angell, 1989) and significant ability to support cell viability in stress conditions (Clegg, 1965; Miller et al., 1999) and dormancy of multicellular organisms (Elbein et al., 2003). High molecular weight components, such as proteins WPI, may exhibit challenging glass transition behavior over a wide temperature range and slowly developing plasticization above the T_g (Roudaut et al., 2004; Roos, 2008). WPI due to its molecular composition (β -lactoglobulin $\sim 50\%$, α -lactalbumin $\sim 20\%$, bovine serum albumin $\sim 10\%$ of whey protein) and the complex tertiary structure does not have clear representative glass transition temperature (Roos and Potes, 2015).

The T_g of binary systems depends on the component properties and miscibility (Roos, 2008). Proteins with carbohydrates are reported to cause a slight increase of the T_g of the sugar, however, the hydrolysis of proteins can also dramatically decrease an overall T_g value (Aguilera et al., 1993; Netto et al., 1998). The T_g of carbohydrates and proteins significantly

depends on the water content and often become significantly plasticized by water (Slade and Levine, 1995; Roos, 2008). The T_g of dehydrated solids decreases as a result of water sorption and their properties may change from glassy solids to viscous liquids (e.g. sugar systems) or leathery materials (e.g. protein systems) in an isothermal process (Roos, 2008). The T_g of water is very low (-135°C) (Angell, 2002), and its experimental determination for system with high water content is often inhibited because of crystallization. For such systems T_g can be derived from the Gordon-Taylor relationship (Arvanitoyannis et al., 1993).

The calorimetric T_g is often measured by differential scanning calorimeter (DSC). Glass transition of amorphous solids, including most mono- and disaccharides (Roos, 1993; Roos, 2008) occurs at about $100\text{-}150^{\circ}\text{C}$ below their equilibrium melting temperature. Despite the fact, that the value of T_g is an important material property it does not provide information on the kinetics of the transition. In general, amorphous structures are fairly stable in the glassy state (Sperling, 1991; Slade and Levine, 1995; Roos, 2008). However, at temperatures close to T_g , more rapid time-dependent flow appears (White and Cakebread, 1966; Roos, 1995; Roudaut et al., 2004). Changes in mechanical properties around the glass transition result in α -relaxation which can be investigated by dynamic mechanical analysis (DMA) and detected from changes in loss modulus (E'' , mechanical energy dissipation), storage modulus (E' , mechanical energy storage) and $\tan \delta$ ($\tan \delta = E''/E'$). DMA has been applied for the analysis of carbohydrates (Roos and Karel, 1991; Kalichevsky and Blanshard, 1993; Silalai and Roos, 2011; Potes and Roos, 2012) and proteins (Gearing et al., 2010; Kokini et al., 1994). DMA studies in multi-frequency mode demonstrated a significant decrease in relaxation times around the T_g (Roos, 2013). On the other hand, rheometry allows determination of time-temperature dependence of relaxations for systems of high water contents (OLLETT and Parker, 1990; Miller et al., 1999). Measurements of viscosity in the T_g region are extremely difficult (Ferry, 1980; Peleg et al., 2002). Knowledge of the relaxation times during temperature changes is fundamental for understanding of properties of food materials.

The WLF equation is one of the popular models, describing dependence of relaxation time and viscosity on the temperature. Originally, this relationship showed that inorganic and organic glassy materials possessed similar decreases in relaxation times and viscosity over the temperature range of T_g to $T_g+100\text{K}$. Williams et al. (Williams et al., 1955) offered “universal” values for WLF constants: $C_1 = 17.44$ and $C_2 = 51.6$ and suggested their use for a wide range of materials. However, many authors (Ferry, 1980; Peleg, 1992; Roos, 1995) advised against the use of these “universal” coefficients and suggested to determine the real constants for each system using experimental data (Eq. 6). C_1 and C_2 constants could describe

the state of the material, in relation to its free volume, since changes in free volume are reflective of changes at the molecular level (Sanford and McCullough, 1990). While C_1 gives the number of decades for decreasing of τ (the limiting τ in the solids), C_2 shows the temperature below T_g where τ become infinitive (Landel and Nielsen, 1993). Application of WLF equation is successful for temperatures well above the T_g . However, when the WLF kinetics is extrapolated to lower temperatures, a significant disagreement between the model and experimental data at higher temperatures appears (Peleg and Chinachoti, 1996). Several studies have confirmed that WLF model is suitable for amorphous materials to describe relaxation time and viscosity dependence on the temperature. Also this relation was used for relating viscosities of supercooled liquids to their stickiness (Downton et al., 1982; Boonyai et al., 2004).

The WLF relationship is often used to define mobility in terms of the non-Arrhenius temperature behavior of structural relaxation processes at temperatures above the T_g . WLF relationship uses structural relaxation times at a reference temperature and the corresponding relaxation time above the glass transition (T_s) (at a certain conditions: $T_s = T_g$ (Williams et al., 1955; Peleg, 1992; Roos and Roos, 1995). Numerous studies have reported a good fit of the WLF model with unfixed constants to experimental data at temperatures above the T_g (Peleg, 1992; Slade and Levine, 1995; Peleg and Chinachoti, 1996). However, the extrapolation of the WLF equation to the range around T_g , proposes non-realistic fit for systems (Peleg and Chinachoti, 1996). The use of calculated C_1 and C_2 constants gives WLF plot matching the experimental data, but does not allow predicting system behavior under changing conditions (composition, water content and i.e.). The objective of the present study was to determine structural relaxation times of sugar-protein systems and assess the use of the WLF equation in a simple and convenient form, that allows describing of the relaxation time-temperature dependence over a wide temperature range, including region around T_g . A new parameter for system characterization was introduced and the influence of protein and water contents on structural relaxation times in tertiary systems was investigated.

3. Materials and Methods

3.1 Materials

D-(+)-Trehalose crystalline dihydrate (Hayashibara Co., Ltd., Okayama, Japan) and whey protein isolate (Isolac[®], Carbery Food Ingredients, Ballineen, Cork, Ireland) were used without purification. De-ionized water (KB Scientific, Cork, Ireland) was used for all experimental work.

3.2 Determination of the initial water content.

The initial water content of trehalose-WPI mixtures was determined using WTB Binder vacuum oven (Mason Technology®, Tuttingen, Germany). Six samples with different contents of trehalose and WPI were prepared in glass vials (10 ml) by mixing of pure powders. The amounts of components were varied over the range 0-100% with 20% intervals. Samples with the final weight 0.5-1 g were dried at 70°C and absolute pressure $P_{abs} < 10$ mbar during 20 hours. The water content in samples was calculated as the weight loss and expressed in percentage.

3.2 Preparation of amorphous freeze-dried materials.

Freeze-dried amorphous solids of trehalose-WPI mixtures were obtained from the solution with 20% (w/w) of total solids. The ratios of components trehalose:WPI in solids were 100:0, 80:20, 60:40, 40:60, 20:80, 0:100. Aliquots of these trehalose-WPI solutions (5 ml, approximately 1g of freeze-dried material) were frozen in pre-weighted and semi-closed with septum 10 ml glass vials at -20°C for 24 h, then at -80°C for 3 h, followed by freeze-drying for 60 h at pressure $p < 0.1$ mbar (Lyovac GT2, Steris®, Hürth, Germany) to obtain amorphous materials. All vials were hermetically sealed under vacuum inside the freeze dryer at $p < 0.1$ mbar and stored over P_2O_5 in vacuum desiccators (Roos and Karel, 1990) at room temperature ($23 \pm 2^\circ\text{C}$) to protect samples from water uptake.

3.3 Water sorption analysis.

Samples with different ratios of trehalose-WPI (described above) were freeze-dried and stored in vacuum desiccators over P_2O_5 . Each sample was monitored during storage in vacuum desiccators at $23 \pm 2^\circ\text{C}$ for 120 h over the saturated solutions of CH_3COOK , MgCl_2 , K_2CO_3 , $\text{Mg}(\text{NO}_3)_2$, NaNO_2 , NaCl and KCl (Sigma Chemical Co., St. Louise, MO, U.S.A.), which provided 0.23, 0.33, 0.44, 0.545, 0.66, 0.76 and 0.85 a_w respectively. Samples were weighted at the time points 0, 2, 4, 6, 8, 10, 24, 48, 72, 96 and 120 hours upon storage. Possible crystallization of trehalose was detected from loss of sorbed water. AQUALAB 4 (TE) (Decagon Devices Inc., NE) water activity meter was used to check the water activity for each sample. The water content in the mixtures was plotted as a function of time, and the Guggenheim-Anderson-de Boer (GAB) equation was fit to the data (Eq. 1):

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

3.4 Differential Scanning Calorimetry (DSC)

The T_g was determined by DSC (Mettler Toledo Schwerzenbach, Switzerland) for anhydrous solids and solids at 0.23, 0.33, 0.44 and 0.545 a_w . Freeze-dried anhydrous materials were transferred to the pre-weighted DSC aluminium pans (40 μL , Mettler Toledo Schwerzenbach,

Switzerland) and hermetically sealed. The lids of DSC aluminium pans were punctured for anhydrous systems only to allow evaporation of residual water upon the measurement. The freeze-dried materials at different a_w were transferred to the pre-weighted DSC aluminium pans and stored in evacuated desiccators over saturated salt solutions at room temperature ($23 \pm 2^\circ\text{C}$) until constant weight. After equilibration the pans were hermitically sealed and reweighed. An empty pan with punctured lid was used as reference. The DSC was calibrated for temperature and heat flow. Samples were scanned from $\sim 30^\circ\text{C}$ below to over the T_g region at $5^\circ\text{C}/\text{min}$ heating and then cooled at $10^\circ\text{C}/\text{min}$ to the starting temperature. The second heating scan was performed to well above the T_g . STARE software version 8.10 (Mettler Toledo Schwerzenbach, Switzerland) was used to calculate the onset, midpoint and endset T_g values. For high water content systems (10, 20, 30, and 40% of total solids) the onset T_g values were calculated by Gordon-Taylor equation (Eq. 2):

$$T_g = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2} \quad (2)$$

3.5 Dynamical Mechanical Analyses (DMA)

Dynamic mechanical analyzer DMA (Tritec 2000 DMA, Triton Technology Ltd., UK) was used to measure dynamic-mechanical properties of the freeze-dried materials. Samples as described for the DSC experiments were analyzed and recorded using DMA control software version 1.43.00. A metal pocket-forming sheet (Triton Technology Ltd., UK) with approximately 60 mg of the powdered sample was fixed directly between the stationary and the driveshaft clamps (balanced to zero) inside the measuring head of the DMA. All three dimensions of the sample - length, width and thickness - were determined. Then sample was scanned over the temperature range -50°C - $+180^\circ\text{C}$ with cooling rate $5^\circ\text{C}/\text{min}$ and heating rate $1^\circ\text{C}/\text{min}$ at frequencies 0.1, 0.2, 0.5, 1, 2, 5, 10 Hz by using the single cantilever bending mode. To provide efficient cooling, the DMA device was connected to a liquid nitrogen tank (1 l; Cryogun, Brymill Cryogenic Systems, Labquip Ltd., Dublin, Ireland). The α -relaxation temperature (T_α) of freeze-dried anhydrous and humidified (0.23, 0.33, 0.44 a_w) trehalose-WPI was calculated from peak temperature of tangents δ , measured by DMA in multi-frequency mode. This calculation provided higher accuracy, than data from decreasing of loss modulus (Hardy et al., 2001).

The relaxation times (τ) of peak T_α , measured by DMA at various frequencies (f), were calculated using the Equation 3 (Noel et al., 2000; Potes et al., 2012):

$$\tau = \frac{1}{2\pi f} \quad (3)$$

3.6 Rheology.

Trehalose and WPI were dissolved separately in water (considering the initial water contents in the powders) and mixed together at ratios 100:0, 80:20, 60:40, 40:60, 20:80 after 2 hours. The total solids contents were 10, 20, 30 and 40%. Apparent viscosities of solutions were measured using a rheometer ARES-G2 (TA Instruments[®], USA) with aluminum “plane-plane” (40 mm) geometry. The shear rate 100s was constant for all systems. The temperature range was 0 - 50°C. To convert the raw viscosity (η) data into shear relaxation times (τ_s), the Maxwell relation (Eq. 4) was used (Angell, 2002):

$$\eta = G_{\infty} \tau_s \quad (4)$$

where, G_{∞} is the infinite frequency shear modulus.

We determined the apparent viscosity for high water content (10, 20, 30, 40 % of total solids) trehalose-WPI systems with components ratios 100:0, 80:20, 60:40, 40:60 and 20:80.

The WLF relation in a form (Eq. 5) was used to fit DMA and rheometry data (Williams et al., 1955; Roos, 2013).

$$\log_{10} \frac{\tau}{\tau_s} = \log_{10} \frac{\eta}{\eta_s} = - \frac{C_1(T-T_g)}{C_2+(T-T_g)} \quad (5)$$

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 Eq. 6 suggests that the plot of $1/\lg(\tau/\tau_s)$ versus $1/(T-T_g)$ gives a straight line:

$$\frac{1}{\lg \frac{\tau}{\tau_s}} = \frac{1}{-C_1} - \frac{C_2}{C_1(T-T_g)}, \quad (6)$$

The WLF constants C_1 and C_2 derive from the slope and intercept of the straight line (Roos, 1995).

3.7 Data analysis

Mean values of the water content, glass transition and α -relaxation temperatures were calculated from 3 replicates with standard deviations expressed in error bars. To ensure consistency, all the experiments were performed in triplicates.

4. Results and discussion.

4.1 Water sorption analysis.

Water contents in the freeze-dried trehalose-WPI systems, equilibrated in desiccators with different water activities (a_w), are given in Table 1.

Table 1.

Water content of freeze-dried Trehalose-WPI systems stored at different water activities for 120 hours at $23\pm 2^\circ\text{C}$.

Water content (g/100 g of solids)						
a_w	100:0	80:20	60:40	40:60	20:80	0:100
0.23	4.13 $\pm$ 0.44	4.98 $\pm$ 0.03	5.12 $\pm$ 0.19	5.97 $\pm$ 0.23	6.50 $\pm$ 0.09	7.33 $\pm$ 0.09
0.33	5.92 $\pm$ 0.39	6.61 $\pm$ 0.02	7.02 $\pm$ 0.21	7.73 $\pm$ 0.16	8.27 $\pm$ 0.13	9.24 $\pm$ 0.12
0.44	9.00 $\pm$ 0.21	8.75 $\pm$ 0.06	8.66 $\pm$ 0.13	9.24 $\pm$ 0.34	9.98 $\pm$ 0.05	11.24 $\pm$ 0.21
0.55	9.49 $\pm$ 0.11	12.25 $\pm$ 0.17	11.14 $\pm$ 0.22	11.05 $\pm$ 0.08	11.04 $\pm$ 0.22	13.04 $\pm$ 0.24
0.65	9.45 $\pm$ 0.27	12.00 $\pm$ 0.16	15.95 $\pm$ 0.15	14.62 $\pm$ 0.14	13.38 $\pm$ 0.17	16.20 $\pm$ 0.19
0.76	9.28 $\pm$ 0.40	12.52 $\pm$ 0.07	14.46 $\pm$ 0.25	21.23 $\pm$ 0.24	18.88 $\pm$ 0.18	19.82 $\pm$ 0.15
0.85	9.46 $\pm$ 0.41	13.65 $\pm$ 0.21	17.66 $\pm$ 0.29	22.21 $\pm$ 0.22	26.19 $\pm$ 0.19	24.96 $\pm$ 0.24

The sorption kinetic profiles of trehalose-WPI mixtures 100:0 (Fig.1 a), 80:20 (Fig.1 b), 60:40 (Fig.1 c), 40:60 (Fig.1 d), 20:80 (Fig.1 e) and 0:100 (Fig.1 f) at different relative humidities demonstrate the time required for the system equilibration.

Figure 1 show that samples reached the equilibrium water content in about 96 hours, except for the “40:60” system, which required about 120 hours. All systems demonstrated an overall increase of the water content for the higher water activity and with an increase in the WPI concentration for the certain water activity. We observed a spike in the water content for “100:0” trehalose-WPI system at the water activity 0.55-0.85 a_w , for “80:20” system at 0.65-0.85 a_w , for “60:40” system at 0.76-0.85 a_w and for “40:60” system at 0.85 a_w . The further decrease of the water content for these systems during several hours of monitoring may indicated trehalose *crystallization* above 0.55 a_w ; above 0.65 a_w for “80:20” system; above 0.76 a_w for “60:40” system and above 0.85 a_w for “40:60” system. For all further experiments in this work, only the amorphous noncrystallizing systems were used. To study the influence of water content on the system, all samples were equilibrated in desiccators with 0, 0.23, 0.33 and 0.44 a_w .

The GAB sorption isotherms (lines), experimental data (solid symbols) and predicted water contents (clear symbols) for non-crystalline trehalose-WPI system are shown at Fig. 2. Previous studies suggested that the GAB equation could be used to fit to the experimental data of amorphous trehalose below 0.55 a_w at 20°C (Bell and Labuza, 2000). However, the extrapolated sorption data above 0.44 a_w provided false results of sorbed water contents. In the present study the water sorption data for non-crystalline trehalose-WPI systems were obtained from the sum of water contents of amorphous component at different a_w (0.11-

0.85 a_w), i.e. the sorbed water contents of trehalose-WPI systems were matched and compared with the sum of water contents of individual amorphous components at each a_w (Potes et al., 2012) according to Equation 7 (Potes, 2014):

$$W_t = n_1 W_1 + n_2 W_2 \quad (7)$$

where, W_t is the total equilibrium water content in the system; n_1 and n_2 are multipliers of each component in the system; W_1 and W_2 are water contents in each non-crystallized composition.

Calculated water contents were derived from experimental water sorption data of trehalose-WPI “20:80” and WPI systems (steady-state sorbed water contents of non-crystalline components). The experimental data of water contents are in agreement with calculated water contents at 0.11-0.44 a_w for pure trehalose; at 0.11-0.55 a_w for “80:20” trehalose-WPI system; at 0.11-0.65 a_w for “60:40” system and at 0.11-0.76 a_w for “40:60” system. This result allows obtaining the GAB sorption isotherms for non-crystalline trehalose-WPI systems with different ratios of components.

4.2 Glass transition temperature

The onset of calorimetric glass transition T_g for anhydrous and humidified (0-0.44 a_w) freeze-dried trehalose-WPI systems are shown in the Table 2. As expected, the T_g decreased with increasing of the water content (Figure 3). For anhydrous trehalose we obtained the same T_g value, which reported by numerous authors such as Green and others (Green and Angell, 1989), Miller and others (Miller et al., 1999). The T_g of trehalose-WPI systems were slightly higher than those of pure trehalose in the anhydrous state. The increasing of T_g in the presence of WPI may be explained by effect of the high molecular weight molecules, which can interact with trehalose to some extent. On the other hand, trehalose was unable to form strong hydrogen bonds between molecules in the anhydrous amorphous state, which explained strong interactions with protein compare to the other saccharides (López-Díez and Bone, 2004). These changes in T_g are in agreement with previous study (Haque and Roos, 2004), where an increase of lactose T_g after addition of WPI was reported.

For the pure WPI glass transition temperature was not observed. It is known, that T_g of globular proteins is difficult to detect due to their complex secondary and tertiary structures, which results in gradual increase of the heat capacity during glass transition (Zhou and Labuza, 2007). For humidified samples (0.23-0.44 a_w) the differences in T_g for pure trehalose and trehalose-WPI systems are not significant. Haque and others (Haque and Roos, 2004) explained similar changes in T_g for lactose-WPI systems with various water sorption

properties. Indeed, during storage at $a_w \leq 0.33$, trehalose sorbed less water than trehalose-WPI systems. However, the amounts of sorbed water at 0.44 a_w for pure trehalose and trehalose-WPI systems are almost equal (table 1), what is in agreement with detected changes of T_g .

Table 2.

Measured by DSC the onset of calorimetric glass transition temperatures (T_g) of freeze-dried trehalose-WPI systems stored for 48 hours at $23 \pm 2^\circ\text{C}$ and various water activities.

Glass transition temperatures (T_g) of trehalose-WPI (w/w) systems, $^\circ\text{C}$					
a_w	100:0	80:20	60:40	40:60	20:80
0	112.0 $\pm$ 1.4	116.1 $\pm$ 1.2	123.7 $\pm$ 1.8	128.0 $\pm$ 0.2	133.0 $\pm$ 0.1
0.23	43.2 $\pm$ 2.1	40.0 $\pm$ 1.4	40.2 $\pm$ 0.2	41.0 $\pm$ 1.2	41.5 $\pm$ 1.1
0.33	31.6 $\pm$ 0.9	30.9 $\pm$ 1.1	31.8 $\pm$ 1.0	38.4 $\pm$ 1.0	32.8 $\pm$ 1.1
0.44	14.5 $\pm$ 0.4	16.5 $\pm$ 0.7	18.4 $\pm$ 1.1	18.8 $\pm$ 0.9	19.1 $\pm$ 1.3

Table 3 gives predicted by Gordon-Taylor equation (Eq. 2) values of T_g for high water content trehalose-WPI systems (10, 20, 30 and 40% of total solids). We used experimental T_{gl} (Table 2) for anhydrous trehalose-WPI systems, and $T_{g2} = -135^\circ\text{C}$ (Angell, 2002) for water. This equation was successfully used to fit the experimental data of amorphous trehalose-WPI systems (Fig. 3). We obtained the following constants k for trehalose-WPI systems: 8.1 $\pm$ 1.0 at ratio 100:0; 7.9 $\pm$ 0.4 at 80:20; 8.3 $\pm$ 0.7 at 60:40; 7.6 $\pm$ 0.2 at 40:60 and 7.4 $\pm$ 0.6 at 20:80.

Table 3.

Glass transition temperatures (T_g) predicted by Gordon-Taylor equation for trehalose-WPI systems with high water contents.

Water content,	Glass transition temperatures (T_g) of trehalose-WPI (w/w) systems, $^\circ\text{C}$				
%	100:0	80:20	60:40	40:60	20:80
90	-132.0	-131.9	-131.9	-131.6	-131.4
80	-129.0	-128.8	-128.9	-128.3	-127.9
70	-126.2	-125.8	-125.9	-125.0	-124.6
60	-123.4	-122.9	-123.1	-121.9	-121.3

4.3 Dynamic-mechanical properties.

The α -relaxation temperature (T_α) calculated from peak temperature of tangents δ , measured by DMA in multi-frequency mode is presented on Figure 4.

The value of T_α for amorphous trehalose and trehalose-WPI systems was highly dependent on the water content (Table 4), what is in agreement with previous works For example, the value

of T_α at 10 Hz for pure trehalose is varied from 128.5°C for anhydrous system to 33.6°C for humidified system at 0.44 a_w . These changes in systems and increase of diffusivity could be explained by the free volume theory (Royall et al., 2005; Meinders and van Vliet, 2009). Probably, water increases the free volume in the system and molecular mobility of amorphous material consequently.

For anhydrous and humidified (0.23, 0.33, 0.44 a_w) systems the T_α of amorphous trehalose and trehalose-WPI systems occurred at ~20-30°C above the onset T_g . Table 4 gives T_α from peak temperature of tangents δ measured by DMA. It is well known, that T_α from the mechanical analysis are highly dependent on the frequencies of the measurements (Talja and Roos, 2001) (Fig. 4 a).

The presence of WPI in the system significantly increased the T_α values (Table 4). The amorphous trehalose showed lowest T_α , while 20:80 trehalose-WPI system - the highest T_α for all humidities. This dependence demonstrated that the WPI could affect the molecular mobility of amorphous trehalose. The WPI is macromolecular component and could reduce diffusion process in the system. The intensity of $\tan \delta$ showed independence on the water content (Fig. 4 b, c, d), however, it inversely correlates with T_α values. During this study, we show that presence of water or proteins is important due to the changes of molecular mobility, which could be detected by DMA.

Table 4.

α -relaxation temperature (T_α) detected by DMA for trehalose-WPI mixtures, equilibrated at different relative humidities (RH), measured at different frequencies (f) and relaxation times ($\log_{10}\tau$, s).

RH 0%		Trehalose-WPI systems				
f, Hz	$\lg\tau$, s	100:0 T_α , °C	80:20 T_α , °C	60:40 T_α , °C	40:60 T_α , °C	20:80 T_α , °C
0.1	0.20	123.0	130.1	143.3	161.2	179.8
0.2	-0.09	124.2	131.4	144.1	163.1	182.3
0.5	-0.49	125.2	131.9	147.1	166.2	184.5
1.0	-0.80	127.3	133.9	147.6	166.5	184.4
2.0	-1.09	127.4	134.8	149.0	166.7	187.5
5.0	-1.50	128.4	135.8	150.8	168.8	189.5
10.0	-1.80	128.5	136.5	151.1	169.6	189.6
RH 23%		Trehalose-WPI systems				
f, Hz	$\lg\tau$, s	100:0 T_α , °C	80:20 T_α , °C	60:40 T_α , °C	40:60 T_α , °C	20:80 T_α , °C
0.1	0.20	54.8	54.5	57.3	64.8	69.1
0.2	-0.09	56.0	55.5	59.1	66.5	71.2

0.5	-0.49	57.9	57.0	61.4	67.9	71.6
1.0	-0.80	58.3	58.0	61.5	68.7	72.8
2.0	-1.09	58.5	58.8	62.1	69.4	73.2
5.0	-1.50	60.2	60.3	63.6	70.2	73.8
10.0	-1.80	60.9	61.0	64.2	70.9	74.2
RH 33%		Trehalose-WPI systems				
		100:0	80:20	60:40	40:60	20:80
f, Hz	lgτ, s	T_a, °C	T_a, °C	T_a, °C	T_a, °C	T_a, °C
0.1	0.20	43.9	45.4	49.7	58.2	62.0
0.2	-0.09	45.1	46.5	49.8	59.6	62.4
0.5	-0.49	46.5	47.5	52.5	60.9	63.4
1.0	-0.80	46.7	48.9	53.4	61.6	63.7
2.0	-1.09	48.3	50.3	53.8	62.2	64.7
5.0	-1.50	48.9	50.8	55.5	62.8	65.1
10.0	-1.80	50.0	51.4	55.6	63.3	66.2
RH 44%		Trehalose-WPI systems				
		100:0	80:20	60:40	40:60	20:80
f, Hz	lgτ, s	T_a, °C	T_a, °C	T_a, °C	T_a, °C	T_a, °C
0.1	0.20	28.1	30.9	37.7	45.0	48.2
0.2	-0.09	28.4	32.3	38.8	46.1	49.8
0.5	-0.49	30.1	33.8	41.0	47.1	50.1
1.0	-0.80	31.4	34.7	41.2	47.8	50.6
2.0	-1.09	31.8	35.6	41.3	48.2	50.9
5.0	-1.50	32.7	36.5	42.8	49.2	51.6
10.0	-1.80	33.6	36.8	43.5	49.4	51.9

4.4 Rheology.

Figure 5 is a graphical presentation of apparent viscosity of aqueous trehalose and trehalose-WPI systems.

The apparent viscosity values for trehalose solution increased with increasing content of solids in a system that correlates with the previous study (Miller et al., 1999). The addition of WPI significantly increased apparent viscosity, especially for systems with 30 and 40% of total solids. Such behavior of systems is typical for addition of WPI macromolecules to carbohydrate aqueous solution (Adhikari et al., 2007). As expected, the values of apparent viscosity are decreasing upon heating (Parks and Gilkey, 1929; Miller et al., 1999).

4.5 Application and modification of the WLF equation.

In the present study we determined the constants C_1 and C_2 for both low and high water content systems. In our calculations we considered that relaxation time was typically 100s and assumed to approach 10^{-14} s at high temperatures, what corresponds to decrease in viscosity from 10^{12} Pa s to 10^5 Pa s (Angell, 1991; Angell, 2002; Roos, 2013).

Depending on the positive or negative values of C_1 and C_2 we obtained different types (I-IV) of hyperbole for WLF curves (Fig.6).

In the real situation $T - T_g > 0$, what is shown with magnification on the Figure 6b. At the low temperatures: $T - T_g < C_2$, the behavior of WLF curves is different from those of “universal” WLF curves. The type I of hyperbole corresponds to the liquid phase state, while type III is typical for the amorphous state (Fig. 7)

For both amorphous and “liquid” systems, the WLF equation was used to fit the experimental data. The calculated C_1 and C_2 constants are given in tables 5 and 6.

Table 5.

Calculated WLF constants C_1 , C_2 for trehalose-WPI systems humidified at different relative humidities (RH).

Trehalose-WPI	RH 0%		RH 23%		RH 33%		RH 44%	
	$-C_1$	$-C_2$	$-C_1$	$-C_2$	$-C_1$	$-C_2$	$-C_1$	$-C_2$
100:0	3.85	34.5	3.30	32.9	2.88	31.9	2.47	31.3
80:20	2.83	35.5	2.60	35.0	2.48	34.0	2.34	33.3
60:40	2.47	45.6	2.14	37.8	2.09	37.4	1.40	34.2
40:60	1.12	53.9	1.09	35.5	0.81	37.6	0.68	36.1
20:80	0.94	70.7	0.74	39.3	0.57	37.8	0.46	36.8

Table 6.

Calculated WLF constants C_1 and C_2 for aqueous trehalose systems with high water contents.

Trehalose-WPI	10% of total solids		20% of total solids		30% of total solids		40% of total solids	
	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
100:0	12.00	0.07	12.00	0.12	12.02	0.22	12.06	1.00
80:20	12.00	0.09	12.00	0.15	12.02	0.33	12.07	1.17
60:40	12.00	0.10	12.02	0.24	12.03	0.57	12.15	2.22
40:60	12.00	0.13	12.02	0.24	12.06	1.00	12.30	4.50
20:80	12.00	0.16	12.02	0.41	12.12	1.83	12.57	8.53

Angell (1991) classified glass materials using fragility parameter. According to this concept, “fragile” materials have significant change in the molecular mobility at temperatures close to T_g , while “strong” materials are following the Arrhenius relationship. However, this approach is not suitable for biological and food systems (Roos, 2013). Characterization of the glass-forming properties of those systems is an important question. Several studies have confirmed that WLF model is suitable for amorphous food and biological materials for describing of relaxation time and viscosity dependence on temperature. Also this equation was used for relating of supercooled liquids viscosities to their stickiness (Downton et al., 1982; Boonyai et al., 2004).

During heating above the onset of calorimetric T_g , amorphous materials rapidly loss their solid characteristics, expressed in decreasing of viscosity and increasing of the systems flowability (Levine and Slade, 1988; Lillie and Gosline, 1990). Equation 6 allows calculating of C_1 and C_2 constants from the WLF relation for each system. Figure 8 shows that upon

heating, the structural relaxation time dropped rapidly from 100s to 0.01s (from 2 to -2 in logarithmic scale). This decrease of structural relaxation is critical for material and can be defined as a parameter d . We defined the temperature difference $T-T_g$ at which this critical change happens as “strength” (S) parameter (Eq. 8):

$$S = \frac{dC_2}{C_1-d} \quad (8)$$

where, d is a parameter showing the critical decrease of τ (from 100 to 0.01s) in the logarithmic scale (can be chosen for each system as an integer depending on the required accuracy), C_1 and C_2 – the “non-universal” constants in the WLF equation that define S .

However, at temperatures well above the glass transition (rheometry data on Figure 7) the structural relaxation time is almost unchanged. This shows the physical limit of change of the structural relaxation time.

The “strength” parameter combines the characterization of material state of the system and practically important time factor. This parameter is simple in calculation and allows describing the strength of glass forming material. This allows using this concept for characterization of flow and mechanical properties (viscosity, stickiness, collapse, etc.) of food, pharmaceutical and biological materials such as food solids, proteins, lipids and carbohydrates. To summarize, S parameter shows the critical temperature difference, at which a sharp change in properties of the system occurs.

To prove the concept the “strength” parameter was calculated for each system at $d = 4$ (Fig. 9) and summarized in the Table 7. Importantly, the new parameter S correlates with the system resistance to changes: higher value of S refers to the stronger system.

Table 7.

The “strength” parameter S (at $d = 4$) for trehalose-WPI systems humidified at different relative humidities (RH).

Trehalose-WPI	RH 0%	RH 23%	RH 33%	RH 44%
100:0	17.6	18.0	18.5	19.3
80:20	20.8	21.2	21.0	21.0
60:40	28.2	24.6	24.6	25.4
40:60	42.1	30.2	31.2	30.8
20:80	57.3	33.2	33.1	33.0

Knowing the C_1 and C_2 parameter values for the pure substances allows predicting S values for any mixtures of these substances. The “strength” parameter is linearly depends on the protein content of the systems (Fig. 9, Table 7).

Anhydrous trehalose, with $S = 17.6$, have a minimal value of S , while anhydrous 20:80 trehalose-WPI system have a highest value with $S = 57.3$. Consequently, the “Strength”

parameter is increasing upon addition of the protein to the system. The presence of water in the system has less impact than the protein amount. However, for the systems with high protein levels (60:40, 40:60, 20:80) the addition of water (RH 23%) significantly decreases the “strength” parameter. Further water addition (RH 33%, 44%) to the system does not have a strong influence on parameter.

5. Conclusions.

In the present study we have presented the new approach, which describes the temperature dependence of structural relaxation time, using trehalose-WPI system as a carbohydrate-protein model. Therefore preliminary, calorimetric and mechanical analyses were carried out. Water sorption isotherms show crystallization during water sorption above 0.65, 0.76, 0.85 a_w in samples with 20%, 40% and 60% of protein respectively. The glass transition temperature, measured by DSC, was strongly decreased by the addition of water and in a lesser extent by the addition of the carbohydrate. The α -relaxation temperature, measured by DMA had a similar behavior with glass transition temperature. We introduced the “strength” parameter, which shows the linear dependence on the concentration of components. This parameter can be used for stability and quality control during food processing and storage. Knowing values of parameter for pure substances allows predicting of S values for any mixtures of these components. The “strength” parameter combines the material state of the system and a practically important time factor. The parameter is increasing upon addition of protein to the system. The anhydrous systems with high level of protein (40%-80%) had significant decreasing S parameter with addition of water. Further water addition for humidified systems did not have a strong influence on parameter.

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Fig. 1. Sorption kinetics for freeze-dried trehalose-WPI mixtures 100:0 (a), 80:20 (b), 60:40 (c), 40:60 (d), 20:80 (e) and 0:100 (f), at different relative humidities 23, 33, 44, 55, 65, 76, 85%, monitored during 120 hours at $23\pm 2^\circ\text{C}$.

Fig.2. The GAB sorption isotherms at $23\pm 2^\circ\text{C}$ (lines), experimental data (solid symbols) and calculated water contents (clear symbols) for amorphous trehalose-WPI systems with components ratios 100:0 ($\blacksquare, \square$), 80:20 ($\bullet, \circ$), 60:40 ($\blacklozenge, \lozenge$), 40:60 ($\blacktriangle, \triangle$), compared to experimental data for trehalose-WPI 20:80($\times$) and 0:100($\mathbb{X}$) systems.

Fig. 3. Dependence of the glass transition temperatures (T_g) on the water content and water activity (a_w) of freeze-dried trehalose-WPI system with ratios 100:0 ($\square$), 80:20 ($\circ$), 60:40 ($\diamond$), 40:60 ($\triangle$), 20:80 ($\times$). Anhydrous samples were stored for 48 hours at $23 \pm 2^\circ\text{C}$ and various water activities (0, 0.23, 0.33, 0.44 a_w). Lines correspond to the T_g predicted by the Gordon-Taylor equation.

Fig. 4. (a) Temperature dependence of dynamic tangents δ for humidified (0.44 a_w) trehalose-WPI "80:20" system in multi-frequency mode (0.1, 0.2, 0.5, 1, 2, 5, 10 Hz). (b,c,d) Temperature dependence of dynamic tangents δ for anhydrous and humidified (0.23, 0.33 a_w) trehalose-WPI mixtures (100:0, 80:20, 60:40, 40:60, 20:80) at 1 Hz.

Fig. 5. Temperature dependence of the viscosity of aqueous trehalose-WPI (100:0; 80:20; 60:40; 40:60; 20:80) systems at 10, 20, 30 and 40% of total solids.

Fig. 6. Calculated WLF curves with positive and negative values of C_1 and C_2 constants.

Fig. 7. "Universal" and calculated WLF curves (lines) and experimental data (symbols) for anhydrous (DMA data) and dissolved (rheometry data) 40% of total solids trehalose-WPI mixtures (100:0, 80:20, 60:40, 40:60, 20:80).

Fig. 8. "Universal" and calculated WLF curves (lines) and experimental data (symbols) for anhydrous trehalose-WPI mixtures (100:0, 80:20, 60:40, 40:60, 20:80).

Fig. 9. Calculated WLF curves (lines) and experimental data (symbols) for trehalose-WPI mixtures (100:0, 80:20, 60:40, 40:60, 20:80) stored for 48 hours at various water activity (0, 0.23, 0.33, 0.44 a_w) at $23 \pm 2^\circ\text{C}$.

Fig. 10. “Strength” parameter versus WPI concentration (%) for anhydrous and humidified (0.23, 0.33, 0.44 a_w) trehalose-WPI systems.

Fig. 1. Sorption kinetics for freeze-dried trehalose-WPI mixtures 100:0 (a), 80:20 (b), 60:40 (c), 40:60 (d), 20:80 (e) and 0:100 (f), at different relative humidities 23, 33, 44, 55, 65, 76, 85%, monitored during 120 hours at $23\pm2^\circ\text{C}$.

Fig.2. The GAB sorption isotherms at $23\pm2^\circ\text{C}$ (lines), experimental data (solid symbols) and calculated water contents (clear symbols) for amorphous trehalose-WPI systems with components ratios 100:0 ($\square, \blacksquare$), 80:20 ($\bullet, \circ$), 60:40 ($\blacklozenge, \lozenge$), 40:60 ($\blacktriangle, \triangle$), compared to experimental data for trehalose-WPI 20:80($\times$) and 0:100($\times$) systems.

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