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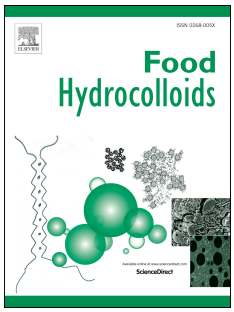

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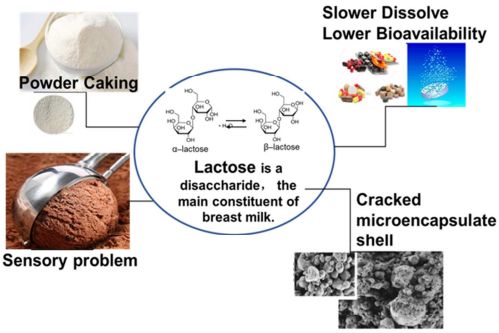
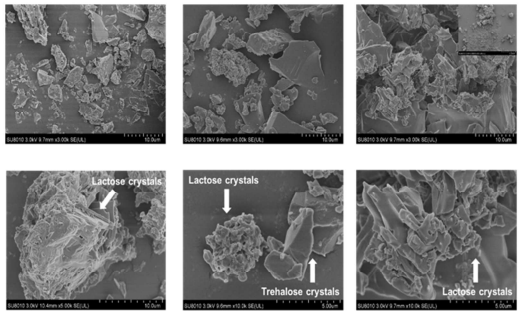
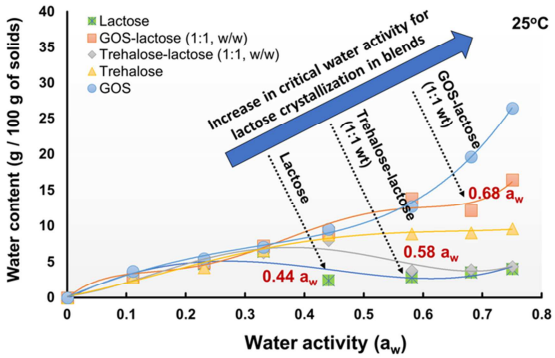




Galacto-oligosaccharide (GOS)

Prebiotics +
Crystallization inhibitor



Inhibition of lactose crystallization in the presence of galacto-oligosaccharide

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Abstract

The stabilization of lactose in the form of amorphous (i.e. non-crystalline form) is the basic requirement to maintain the quality of relevant food and pharmaceutical products. The physiochemical properties of amorphous lactose mixed with galacto-oligosaccharide (GOS) were investigated. Water sorption, glass transition temperature, and crystallization behaviour of lactose in the present of GOS (1:1 w/w) were measured at various water activity (0.11-0.75 a_w, 25 ^oC) and lactose mutarotation was also evaluated. All of them were compared with the physiochemical properties of trehalose-lactose (1:1 w/w). The addition of GOS to lactose increased the hygroscopicity of the mixture, as well as slightly increased the glass transition temperature compared to lactose alone. The critical water activity (at 0.68 a_w) of lactose crystallization was increased by the addition of GOS as compared to that of trehalose-lactose (1:1 w/w) (at 0.58 a_w) or lactose alone (at 0.44 a_w). The dramatical inhibition of lactose crystallization with a lower crystallization kinetic constant and the alternation of lactose crystal forms in the presence of GOS was observed as compared to the crystallization behaviour of trehalose-lactose (1:1 w/w) and pure lactose at 0.68 and 0.75 a_w, 25 ^oC. Without affecting its T_g, the significantly delayed crystallization of lactose in GOS-lactose mixture (1:1 w/w) was more likely due to the change of lactose mutarotation. As comparing to trehalose that is an effective inhibitor, GOS has a stronger ability to prevent lactose from crystallization in hydrous matrices.

Keywords: Galacto-oligosaccharide, lactose, crystallization, glass transition temperature, mutarotation

1. Introduction

Lactose is the main constituent of breast milk. It is commonly used as an ingredient in various food products and as an excipient and tableting agent in pharmaceutical products (*Carpin et al., 2016*). In many products, lactose amorphous state and crystallisation during storage cannot occur (*Hartel, 1991*). The tendency of amorphous lactose to form crystals is the leading cause of decreased powder flowability, caking of dairy powder (i.e. amorphous caking and humidity caking), and diminished rehydration properties (*Fitzpatrick et al., 2007*). The crystallinity of lactose, as an additive of drug tablets, is important for drug solubility and bioavailability. Dried solids containing amorphous compounds have a faster dissolution rate than solids containing crystals, as bonding between the amorphous molecules is weaker as compared to the crystalline regions (*Hancock & Parks, 2000*). Drug solubility is one of the key determinants of bioavailability. Additionally, lactose is an important type of microencapsulation wall materials. The amorphous form of lactose is essential for the entrapment and protection of active or non-compatible components in microencapsulation (*Miao & Roos, 2017*). In the research and development of food and drug products, it is important to use certain technologies and processes to ensure the amorphous structure of lactose and to postpone the undesirable crystallisation to improve product quality.

The mechanism of α-monohydrate lactose crystallisation in solution and the main influencing factors have been studied in great detail. Kinetic models for each of the different steps of mutarotation, nucleation, and crystal growth have been developed. At a low concentration of lactose (≤0.6 g / g H_2O), the rate of growth of lactose crystals is predominantly governed by the lactose mutarotation, which is the reversible transition between two anomeric forms of lactose (α-form with low solubility and β-form with high solubility) and α-lactose supersaturation. A rapid conversion of β-lactose to α-lactose could decrease the solubility of lactose and increase α-lactose supersaturation, which could provide a strong driving force to increase the rate of the crystallisation process. At a high concentration of lactose (>0.6 g / g H_2O), the rate of lactose crystallisation is dependent on the degree of α-lactose supersaturation and the crystal surface area (*Mimouni, Schuck & Bouhallab, 2009*). Lactose crystallisation is also significantly influenced by pH and temperature. In solid matrices, lactose mutarotation (or the proportion of α- and β- lactose) and its molecular mobility are two major influences governing water sorption-induced lactose nucleation formation and crystal growth (*Zhou, Zhang, Law, Grant & Schmitt, 2008; Heljo, Nordberg, Tenho, Virtanen, Jouppila, Salonen, Maunu & Juppo, 2011; Champion, Le Meste & Simatos, 2000; Miu & Tag, 2010; Timmermann, Steckel & Trunk, 2006*).

Prevention or delay of lactose crystallisation can be achieved by the addition of impurities, such as mineral salts, proteins, milk fats and carbohydrates (*Ame & Roos, 2007; Fan & Roos, 2016b; Kelly, 2009; Miao & Roos, 2010; Potes, Kerry & Roos, 2012*). The effects of different additives on the inhibition of crystallisation of lactose vary. Many kinds of mineral salts, such as LiCl, $MgSO_4$ and K_2HPO_4, inhibit lactose crystallisation by altering lactose solubility and/or formation of the complex between lactose and the mineral salt (*Wong & Hartel, 2014; Haase & Nickerson, 1967*). Milk fat is an effective inhibitor as it acts as a hydrophobic barrier that limits the diffusion of hydrophilic lactose and the growth of lactose crystals (*Kelly, 2009*). Delayed crystallisation of lactose in the presence of whey protein has been described (*Fan & Roos, 2015*). Whey protein isolates may increase the solubility of lactose crystals by changing the proportion of the α- and β-lactose anomeric forms and limiting the molecular diffusion of lactose during crystal growth (*Mimouni et al., 2005; Fan & Roos, 2015*). Carbohydrates have diverse structure with pronounced differences in molecular weight between monosaccharides and polysaccharides. Carbohydrates with different molecular sizes and structures can influence lactose crystallisation accordingly. Their addition can inhibit or promote lactose crystallisation in dried solids (*Li, Roos & Miao, 2017; Potes, Kerry & Roos, 2012*). The effect of various carbohydrates on the behaviour of lactose crystallisation mainly depends on the molecular mobility of composite systems, which is influenced by the solubility of lactose, mutarotation associated with solid-solid transformation, molecular size-related steric hindrance, and effects of glass transition temperature (T_g) (*Biliaderis et al., 2002; Potes, Kerry & Roos, 2012; Li, Roos & Miao, 2017*). As carbohydrates differ markedly in their physicochemical properties, the effect of carbohydrates on the lactose crystallisation could vary from different type of carbohydrates.

Galacto-oligosaccharide (GOS) is a non-digestible food ingredient with health-promoting benefits for some species of gut bacteria. Thus, GOS can be considered to be prebiotics. Their health benefits and favourable physiochemical properties, including high solubility, colourless appearance, and mild sweetness, have spurred innovative efforts to utilise health-promoting compounds in commercial food and pharmaceutical products. GOS comprises a mixture of oligomers. Purified GOS (97% w/w) is predominantly composed of trisaccharides (47% w/w) and tetrasaccharides (42% w/w), with the minor presence of pentasaccharides (8% w/w) (*Torres, Bastos, Goncalves, Teixeira & Rodrigues, 2011*). The stabilising effect of GOS could be expected on the basis of their high T_g, ability to form amorphous matrices of high viscosity and low molecular mobility (Torres et al., 2011; Potes, Kerry & Roos, 2012). GOS might stabilise lactose, which could prevent lactose crystallisation in solid matrices.

This study examined lactose crystallisation in the presence of GOS in dehydrated mixtures. The two major influencing factors (i.e. lactose mutarotation and molecular mobility) were investigated by testing the physicochemical properties of GOS and lactose mixtures. Trehalose was used for comparison, as it is an effective inhibitor and is a popular component in many food and pharmaceutical formulations. The characterisation of physicochemical properties of GOS-lactose mixtures will allow for the prediction of stability and quality changes during storage of related products.

2. Materials and methods

2.1. Materials

α-Lactose monohydrate was obtained from Sinopharm Chemical Reagent Co., Ltd., (Shanghai, China), D-(+)-trehalose dihydrate (purity >99%) from Aladdin Industrial Corporation (Shanghai, China) and GOS (purity approximately 78%) from Yuanye Biotechnology Co., Ltd., (Shanghai, China). GOS was confirmed to be free of lactose by high-performance liquid chromatography analysis conducted in our laboratory. LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, NaBr, KI, NaCl and P_2O_5 were obtained from Sinopharm Chemical Reagent Co., Ltd.

2.2. Preparation of freeze-dried powders

Fifteen grams of α-lactose monohydrate (lactose), GOS and trehalose or the mixtures of GOS-lactose and trehalose-lactose at a mass ratio of 1:1 were completely dissolved in 85.0 g of distilled water to obtain 15% (w/w) solutions. A 30-min treatment in an ultrasonic water bath (Jielite Ultrasonic Cleaner Co., Ltd., Shenzhen, China) facilitated complete dissolution. The resulting solutions were individually frozen at –80 ^{o}C for 12 h and then freeze-dried for 48 h under a pressure *(p)* < 0.2 mbar using an Alpha 1-4 LD plus device (Marin Christ Gefriertrocknunsanlagen, Germany). Freeze-dried materials were quickly crushed into powder and kept over phosphorus pentoxide (P_2O_5) in a Vaseline-sealed desiccator to avoid future moisture uptake from the surrounding environment.

2.3. Methods

2.3.1. Initial water activity and water content of freeze-dried powders

The initial water activity (a_w) of freeze-dried powders was determined at 25 ^{o}C using an AQUA LAB 4TE apparatus, (Decagon Devices, Pullman, WA, USA). The water content (m) was measured using a model DE 401 rapid moisture analyser (Shenzheng, Yuanya Technology Co., Ltd.)

and was expressed as the difference of tested sample weight before and after heating at 160 ^{o}C for 40 s.

2.3.2 Water sorption

Freeze-dried powders were placed into vacuum desiccators containing saturated solutions (LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, NaBr, KI and NaCl) to maintain a relative humidity (RH) of 11%, 23%, 33%, 44%, 58%, 68% or 75%, respectively (*Greenspan, 1977*). The $a_w = p/p_o$ = RH/100, with the assumption that the ratio of vapor pressure (p) in food and that of pure water (p_o) equals a_w at a steady state (*Roos, 1995*). Approximately 1.0 g of freeze-dried powder was placed into a 30 mm-diameter pre-weighted petri dish. The total weight of each petri dish containing freeze-dried powder was determined at 0, 3, 6, 9, 12 and 24 h and every 24 h thereafter up to 288 h at 25 ^{o}C. The absorbed m value at each time point was obtained as the difference of the sample before and after incubation under various RHs (11-75%) at 25 ^{o}C. The total m value was the sum of the initial water content and the absorbed water content.

The Guggenheim-Anderson-de Boer (GAB) equation (1) was used to predict the m of non-crystalline material at any given water activity at a certain temperature (*Torres, Bastos, Goncalves, Teixeira & Rodrigues, 2011*).

$$\frac{m}{m_m} = \frac{CKa_w}{(1-Ka_w)(1-Ka_w+CKa_w)} \quad (1)$$

The GAB isotherm parameters were determined by plotting a_w/m against a_w (equation 2). The total m of freeze-dried powder and its a_w were used to calculate the constant values of α, β and γ. The GAB isotherm parameters C, K and m_m (g / 100 g of solids) were obtained from equations (3) to (5).

$$\frac{a_w}{m} = \alpha a_w + \beta a_w + \gamma \quad (2)$$

$$\mathrm{C} = \frac{1}{m_m \gamma K} \quad (3)$$

$$\mathrm{K} = \frac{\beta - (\frac{1}{m_m})}{-2\gamma} \quad (4)$$

$$m_m{}^2 = -\frac{1}{4\alpha\gamma - \beta^2} \quad (5)$$

2.3.3 Glass transition temperature

The glass transition temperature (T_g) of freeze-dried powder was analysed using differential scanning calorimetry (DSC) with a DSC 1(500^{o}C), STAR^{e} System device (Mettler Toledo,

Küsnacht Switzerland). Freeze-dried powder (10-15 mg) stored over P_2O_5 was transferred into a pre-weighted DSC aluminium pan (40 μL) and sealed with a punched cap. Samples were scanned from 5 to 100 ^oC at a rate of 10 ^oC / min and then cooled at the same speed to the initial temperature to remove the residual water from the freeze-dried powder (*Liu, Bhandari and Zhou, 2006*). Crystallisation does not occur in freeze-dried power at low a_w when heating to 100 ^oC. The anhydrous powder (0 a_w) was scanned for a second time from 5 to 140 ^oC at a rate of 10 ^oC / min. Onset T_g derived from the second scan was recorded. The onset T_g of freeze-dried powder was determined using the heating-cooling-heating operation program to remove the irreversible endothermic peak due to enthalpy relaxation of aged low molecular weight sugars (*Liu, Bhandari & Zhou, 2006*). The freeze-dried powder (10-15 mg) was immediately transferred to the pre-weighted standard DSC aluminium pan (40 μL), and hermetically sealed. Samples were scanned from 5 ^oC under the T_g to a temperature exceeding the T_g at a rate of 10 ^oC / min and then cooled at a rate of 10 ^oC / min to the initial temperature. A second scan was run at the same conditions of temperature increase. The onset T_g derived from the second scan was recorded (*Fan & Roos, 2015*).

The Gordon-Taylor equation (6) was used to predict T_g of a binary mixture (T_{gmix}) at any given water content:

$$T_{gmix} = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2} \quad (6)$$

where T_{gmix} is the predicted glass transition temperature of a binary system; w_1 and w_2 are the mass fractions of solids and of water; T_{g1} and T_{g2} are the T_g of anhydrous sample and water, respectively. In this study, the T_{g2} for water was –135 ^oC (*Madeka & Kokinii, 2002*). The *k* value was calculated from the average of *k* values obtained from experimental T_g and its corresponding water content (*Shrestha, Howes, Adhikari & Bhandari, 2007*).

2.3.4 Lactose mutarotation in GOS-lactose and trehalose-lactose solutions

Lactose mutarotation in the presence or absence of GOS (or trehalose) in solutions was investigated polarimetrically (*Patel & Nickerson, 1970*). The light source was a sodium-vapor lamp with a wavelength 589 nm. Fifty-millimetre polarimetric tubes were used to obtain accurate readings. Timing began as soon as the first drop of water touched the freeze-dried powder and the first reading was taken 2 min after the reaction had started. A series of readings was taken 2, 10, 30, 60, 90 and 120 min at 25 ^oC. The equilibrium reading was obtained at 48 h at 25 ^oC. The kinetics of mutarotation was evaluated by analysis of the mutarotation constant K, which was calculated using equation (7):

$$K = \frac{1}{t} \times log \frac{(V_o - V_\infty)}{(V_t - V_\infty)} \quad (7)$$

where V_∞ is the equilibrium rotation and V_t is the rotation at t hours after initial observation V_o (*Patel & Nickerson, 1970).*

2.3.5 Time-dependent crystallisation

2.3.5.1 Kinetics of crystallisation

The crystallisation of amorphous lactose results in dehydration at the time of formation of anhydrous crystals (*Schebor, Mazzobre & Buera, 2010*). The kinetics of crystallisation of lactose, GOS-lactose (1:1 w/w) and trehalose-lactose (1:1 w/w) were investigated by evaluating the dynamic loss of absorbed water from the maximum to the minimum amounts during the storage period. The rate constant (K_1) of dynamic loss of absorbed water was representative of the rate of crystallisation in each mixture (*Potes, Kerry & Roos, 2012*).

2.3.5.2. X-ray diffraction (XRD) analysis

The extent of crystallinity and the crystalline forms in samples were confirmed by XRD. The crystallised samples were frozen at –80 ^{o}C for at least 3 h followed by 24 h freeze-drying (*Fan & Roos, 2016*). The freeze-dried samples were immediately crushed and stored in sealed plastic vials to prevent exposure to air and water uptake. XRD patterns were determined by using a model XRD-7000 X-ray diffractometer (Shimadzu, Kyoto, Japan) operating with an anode current of 40 mA and an accelerating voltage of 40 kV. Samples were slightly pressed on a steel sample tray and exposed to CuKα radiation at diffraction angles (2θ) from 5^{o} to 30^{o} (step size 0.2^{o}; time per step 5 s). The peak search program was Jade 6.0 software. Intensity is expressed as CuKα net peak height in counts at CuKα position in degrees. The type of lactose crystals was identified based on the peaks at specific diffractive angles (2θ) in the XRD spectra, and the crystallinity of lactose in varied samples was compared by the intensities of the characteristic peaks (*Fan & Roos, 2016*).

2.3.5.3. Scanning electron microscopy (SEM)

Freeze-died powder was humidified at a RH of 75% at 25 ^{o}C for 288 h. The microstructure of freeze-dried powder and crystals were determined by SEM using a model JSM-7800F device (JEOL Ltd. Japan) at 0, 48 and 288 h. Small amounts of samples were mounted on SEM specimen stubs and coated with gold in a sputter coater under high vacuum. The coated samples were viewed by SEM at 20 kV in the secondary electron mode.

2.4 Statistical analysis

Water sorption experiment was carried out by duplicates at least. Glass transition temperature, mutarotation experiment and kinetics of crystallization measurements were carried out by duplicates. XRD analysis was carried out by duplicates as well. SEM was measured by using two prepared samples. One-way analysis of variance (ANOVA) was used to evaluate the significant differences between the mean values. The significance level of $p<0.05$ was used throughout the study.

3. Results and discussion

The water-sorption induced crystallization of lactose mixed with GOS have been investigated, meanwhile, trehalose is used as the comparative inhibitor for lactose crystallization as it has a desired effect on the inhibition of lactose crystallization (*Mazzobre, Soto, Aguilera & Buera, 2001; Gharsallaoui, Rogé & Mathlouthi, 2008*).

3.1. Initial a_w and m value of freeze-dried powders

The a_w of freeze-dried powders varied from 0.08 to 0.10 at 25 ^oC when stored over P_2O_5 overnight. Only minor changes were observed after 7 days of storage under the same conditions. The m values of freeze-dried lactose, trehalose, GOS, trehalose-lactose (1:1 w/w) and GOS-lactose (1:1 w/w) were 2.35%, 2.30%, 2.70%, 2.32% and 2.22% (w/w), respectively (p >0.05).

3.2. Water sorption

The experimental and GAB predicted m values of lactose, GOS, trehalose, GOS-lactose (1:1 w/w) and trehalose-lactose (1:1 w/w) are presented in Table 1, and the values of the lactose, trehalose-lactose and GOS-lactose comparators are plotted in Figure 1. The experimental m of lactose increased with increasing a_w up to 0.33 and then decreased to the minimum value at 0.44 a_w. At high, a_w (i.e. 0.58, 0.68 and 0.75 a_w), the m of lactose was slightly increased after the release of to the minimum value. The trend in the m values of lactose over the range of a_w indicated that lactose crystals began to form when the m value of lactose exceeded 6.2 g / 100 g solids ($\geq$ 0.44 a_w). After the release of water during crystallisation, the marginally increased m value of lactose indicated that the anhydrous lactose crystals reabsorbed a small amount of water for recrystallisation to form monohydrated crystals when a_w exceeded 0.58. This water sorption isotherm of lactose was consistent with a previous report (*Haque & Roos, 2005*). When mixing GOS with lactose at a mass ratio of 1:1, a change in water sorption behaviour over the whole range of a_w was detected. As shown in Figure 1, the water content of GOS-lactose (1:1 w/w) increased

with increasing a_w up to 0.58 and slightly decreased at a GOS-lactose a_w>0.68. The finding indicated that the crystallisation of lactose in the GOS-lactose mixture occurred at a higher a_w than that in pure lactose. The mild reduction of m values indicated the slow rate of crystal growth when lactose was mixed with GOS. When GOS-lactose a_w increased to 0.75, the equilibrium m value was higher than that at an a_w of 0.68. This could be due to the reformation of monohydrated lactose crystals at the end of the storage period. In trehalose-lactose mixtures (1:1 w/w), m was initially elevated as a_w increased from 0.11 to 0.44, and then sharply declined to the minimum amount at an a_w of 0.58. The water sorption isotherm of trehalose-lactose mixtures agreed with the findings from a previous study (*Fan & Roos, 2016*). This water sorption curve indicated that trehalose-lactose began to form crystals when the m value exceeded 8.0 g / g of solids (a_w >0.58).

The calculated GAB m values for non-crystalline (or amorphous powder) are presented in Table 1. In pure carbohydrate systems, the water sorption capacity for non-crystalline lactose was the lowest, followed by trehalose and GOS. This could reflect the high-water solubility of GOS and trehalose compared to that of lactose (*Fan & Roos, 2016; Lans & Vodovotz, 2018*). In the lactose-carbohydrate mixtures, the hygroscopicity was obviously changed by mixing GOS and trehalose with lactose. The addition of GOS to lactose significantly increased the hygroscopicity of the blend as compared to that of pure lactose. On the contrary, comparison of the GAB water sorption isotherm revealed that the hygroscopicity of non-crystalline trehalose-lactose (1:1 w/w) was close to that of pure lactose. In addition, the total water content of the mixture may be not equal to the sum of the water content of the single composite in this study (Figure 1). For example, the total water content of GOS-lactose (1:1 w/w) was higher than the sum of the water content of the signal composite, and the water content of trehalose-lactose (1:1 w/w) was similar to the total absorbed water of signal trehalose and lactose. This observation was inconsistent with previous statements that the water sorption of the mixture could be affected proportionally by the additives when using the GAB equation to investigate the water sorption isotherm in lactose-maltodextrin systems (*Potes, Kerry & Roos, 2012*). The non-proportionality obtained in this study could be because the solubility of lactose in the mixture was enhanced by the altered transformation of α-lactose to β-lactose, which consequently increased the moisture absorption of the mixture. The cause of this phenomenon could also be related to the effect of aging on the alternation of hygroscopicity of amorphous sugars (Surana, Pyne & Suranarayanan, 2004). Nevertheless, an error in the GAB calculation cannot be ruled out, although the goodness-of-fit was <5% in the majority samples (*Potes, Kerry & Roos, 2012*).

3.3. Glass transition of freeze–dried powders

The glass transition temperature of a material is a reference temperature for the evolution of molecular mobility in a matrix. A high glass transition temperature indicates low molecular mobility in the matrix. The glass transition temperature (onset T_g) of freeze-dried powders (i.e. lactose, trehalose, GOS, trehalose-lactose (1:1 w/w) and GOS-lactose (1:1 w/w) at a_w between 0 and 0.44 were determined using DSC. For anhydrous powders (0 a_w), the T_g of anhydrous GOS was 115 ^oC. This value was lower than the previously reported T_g of 135 ^oC for anhydrous GOS (purity >98%) (*Torres et al., 2011*). This may be related to the different purities of GOS containing varied proportions of oligosaccharides and monosaccharides. A recent study reported that the glass transition temperature of the GOS was proportional to its purity (*Lans & Vodovotz, 2018*). Presently, the T_g of anhydrous lactose and trehalose were 108 ^oC and 113 ^oC, both of which were consistent with previously published values (*Li, Roos & Miao, 2017; Maidannyk, Nurhadi & Roos, 2017*).

By mixing GOS with lactose at a mass ratio of 1:1, the T_g of the mixture was slightly higher than that of pure lactose, but lower than the T_g of GOS (Figure 2A). The effect of mixing trehalose with lactose at this mass ratio on the T_g of their mixture was not obvious, as the T_g of anhydrous trehalose was close to that of lactose (Figure 2B). It is conceivable that the addition of highly purified GOS (purity >80% and T_g >115 ^oC for anhydrous GOS) to lactose was more likely to enhance the T_g of the mixture as the T_g of a material can be increased by adding another material that has a high T_g. The T_g of the mixture of anhydrous pullulan (T_g approximately 160 ^oC) and lactose was higher than that of lactose, with further increases with the increasing proportion of pullulan in the mixture (*Biliaderis, Lazaridou & Arvanitoyannis, 1999; Biliaderis, Lazaridou, Mavropoulos & Barbayyiannis, 2002*). However, the change in T_g upon the addition of various proportions of maltodextrin (T_g approximately 160 ^oC for anhydrous maltodextrin) to lactose was small as compared to the T_g of lactose alone (*Potes, Kerry & Roos, 2012*). The effect of the addition of highly purified GOS (purity >80%) on the enhancement of the T_g of GOS-lactose mixture may require further investigation, as the glass transition temperature of a material is a complex physicochemical property.

In this study, the T_g of lactose was not greatly affected by the addition of GOS or trehalose at a mass ratio of 1:1, while the T_g of all samples decreased with increasing m values because of the water plasticization effect (Figure 2). The decrease in the measured T_g of trehalose-lactose mixture was similar to that of GOS-lactose mixture at low water activity (0 – 0.22 a_w). When water activity continues to increase (0.33 – 0.44 a_w), the measured T_g of trehalose-lactose decreased more than that of GOS-lactose (Figure 2). The different drop range of T_g indicated that GOS has anti-plasticization effect of water at relative high-water activity ranges by comparing to trehalose. It may be due to the

restricted water molecular motion (diffusion) in the presence of GOS in the system. The anti-plasticization effect of water could be taken account into polymer-water interactions, which could affect the state of water in the system. Many hypothesises have been explained on the basis of the formation of supplementary hydrogen bonds between the polymeric matrix or the surface of the polymeric matrix (Pittia & Sacchetti, 2008).

3.4. Lactose mutarotation in GOS-lactose and trehalose-lactose solutions

Lactose mutarotation refers to a simultaneously reversible reaction between two anomeric forms (i.e. an α-form with a specific rotation of +88º and a β-form with a specific rotation of 34º) in solution and in solids in amorphous or crystalline states (*Hartel & Shastry, 1991; Lefort, Caron, Willart & Descamps, 2006*). The optical rotation values of GOS (7.5% w/v) and trehalose (7.5% w/v) were 147.8º and 36.8º, respectively (Table 2), which indicated the absence of mutarotation. These values were confirmed by determinations using dilute GOS and trehalose solutions at concentrations of 2.5% (w/v) and 0.5% (w/v) (data not shown). The optical rotation value of lactose was concentration-dependent with values of 88.6º in 0.5% (w/v) lactose, 82.9º in 2.5% (w/v) lactose and 83.4º in 7.5% (w/v) lactose at an initial time of 2 min. The corresponding equilibrium values of lactose after 48 h were 60.1º, 48.3º and 44.8º, in the same respective order. The lactose mutarotation velocity (K) was 0.575, 0.485 and 0.435 in 0.5% (w/v), 2.5% (w/v) and 7.5% (w/v) lactose, respectively (Table 2). These results strongly agreed with previously published data (*Haase & Nickerson, 1967; Patel & Nickerson, 1970*). The addition of different proportions of GOS or trehalose to 7.5% (w/v) lactose solution reduced the optical rotation values of GOS-lactose observed at 48 h, with the decrease being more pronounced with the increase in the concentration of added GOS from 0.5% to 7.5 (w/v). The K values of GOS-lactose solutions were also raised from 0.429 to 0.469 by increasing the concentration of the mixture. Compared to the K of pure lactose (7.5% w/v), the addition of GOS accelerated lactose mutarotation. This could contribute to the formation of more β-lactose. The results highlighted that the solubility (or hygroscopicity) of lactose could increase in the presence of GOS, while the K value of trehalose-lactose decreased with an increase in the amount of added trehalose in 7.5% (w/v) lactose solution with corresponding values of 0.495, 0.455 and 0.248 for 0.5%, 2.5% and 7.5% (w/v) added trehalose, respectively. These results indicated that trehalose affected lactose mutarotation. Thus, the similar reduction of optical rotation values and a smaller K value of trehalose-lactose (7.5%:7.5% w/v) than that of pure lactose solution (7.5% w/v) suggested that trehalose significantly impedes the transformation between α-lactose and β-lactose in the mixed solution but may not change the ratio of α-lactose and

β-lactose after reaching equilibrium. The effect of carbohydrates on lactose mutarotation (i.e. a hydration reaction) may be related to their different chemical structures. Various molecular associations take place in aqueous carbohydrate solutions (e.g. disaccharides). There are at least three types of associations (water-water, water-sugar and sugar-sugar) occur together with collisions between the different associates (Gharsallaous, Rogé & Mathlouthi, 2006). The higher concentration of hydroxy groups in GOS-lactose solutions than that in trehalose-lactose may guide intermolecular interactions (e.g. hydrogen bonding and hydration) differently in their solutions (< 40% w/w), consequently leading to an intervention effect on lactose-lactose and lactose-water interactions (Vilén & Sandström, 2013). These impurities change the mutarotation of molecules that could lead to lower effective supersaturation of the system during crystal growth (*Mimouni, Schuck, Bouhallab, 2009*). The change in lactose mutarotation by the addition of GOS or trehalose could play a crucial role in the modification of lactose crystallisation.

3.5. Time-dependent crystallisation of freeze-dried powders

3.5.1 Crystallisation kinetics

The kinetics of water release (K) represent the rate of lactose crystallisation accompanied by dehydration (*Potes, Kerry & Roos, 2012*). Figure 3 shows the rate of water released from lactose, GOS-lactose (1:1 w/w) and trehalose-lactose (1:1 w/w) at a_w ranging between 0.44 and 0.75 at 25 ^oC. The loss of absorbed water from amorphous powders at a_w<0.44 was not observed during the storage period (Table 1). The rapid loss of absorbed water (or lactose crystallisation) appeared in lactose at $a_w \geq 0.44$. The rate of lactose crystallisation (K) was highest for pure lactose. The rate increased with increased a_w of 0.44, 0.58, 0.68 and 0.75, with the corresponding values of -0.20, -1.77, -1.61 and -2.04, respectively. The reduction in m values caused by the crystallisation of lactose was observed in trehalose-lactose (1:1 w/w) at $a_w \geq$ 0.58 and for GOS-lactose (1:1 w/w) at $a_w \geq$ 0.68. The rates of lactose crystallisation (K) in GOS-lactose (1:1 w/w) were lower than that in trehalose-lactose (1:1 w/w), with the corresponding values of 0.00, 0.00, -0.03 and -0.03 for aw of 0.44, 0.57, 0.68 and 0.75, respectively, for GOS-lactose and 0.00, -0.14, -0.41 and -0.21 in the same respective order of a_w for trehalose-lactose. The results indicated the excellent inhibitory effect of GOS and trehalose on lactose crystallisation, with the inhibitory effect of GOS being stronger than that of trehalose. The macrostructure of crystallised lactose, mixed trehalose-lactose and mixed GOS-lactose was assessed by SEM (Figure 4). The macrostructure of lactose crystals differed

somewhat with pure crystallised lactose, trehalose-lactose (1:1 w/w) and GOS-lactose (1:1 w/w) after storage at an RH of 75% and 25 ^{o}C for 288 h. The enhanced solubility of lactose in the presence of GOS caused by modified lactose mutarotation greatly reduced the driving force for the nucleic formation and crystal growth. As compared to trehalose, GOS avidly impeded.

3.5.2. Crystallinity and type of lactose crystal forms

The crystallinity and type of lactose crystal forms after storage were confirmed by XRD analysis. It is assumed that the quantity of each lactose crystalline form could be indicated by the intensity values at their characteristic diffraction angles (2θ). Several types of lactose crystals were evident, although the quantification was not precise (*Haque & Roos, 2005*). The amorphous lactose may crystallise into four crystal forms: α-lactose monohydrate, anhydrous β-lactose (stable and unstable forms), anhydrous crystals with α-/β-lactose in a molar ratio of 5:3 and α-/β-lactose in a molar ratio of 4:1 (*Miao & Roos, 2005*). We previously described that the degree of lactose crystallisation during storage at an RH of 68% and 75% for 288 h reached equilibrium (*Li, Roos & Miao, 2017*). Presently, α-lactose monohydrate (12.5º, 16.5º and 20.1º), α-/β-lactose in molar ratio of 5:3 (19.1º), α-/β-lactose in molar ratio of 4:1(19.7º) and anhydrous β-lactose (21.0º) were determined in crystallised lactose when stored at an RH of 68% and 75% at 25 ^{o}C for 288 h (Figure 5). Comparison of the intensities of the characteristic 2θ peaks revealed that the α-lactose monohydrate was the dominant crystalline form, followed by α-/β-lactose in a molar ratio of 5:3, α-/β-lactose in a molar ratio of 4:1 and anhydrous β-lactose. The peak intensity of anhydrous β-lactose was the lowest, which could be due to the participation of anhydrous lactose crystals participated in recrystallisation to forming α-lactose monohydrate crystals, α-/β-lactose in a molar ratio of 5:3 and α-/β-lactose in a molar ratio of 4:1 (*Fan & Roos, 2016*).

The addition of GOS to lactose at a mass ratio of 1:1 significantly reduced the lactose crystallinity, with the lowest intensities of characteristic 2θ peaks for lactose and altered the types of crystal forms after 288 h of storage at an RH of 65% and 75% at 25 ^{o}C (Figure 5). The types of lactose crystals in the crystallised GOS-lactose mixture (1:1 w/w) were α-lactose monohydrate, anhydrous β-lactose and α-/β-lactose in a molar ratio of 5:3. The absence of α-/β-lactose in a molar ratio of 4:1 in crystallised GOS-lactose (1:1 w/w) was observed. The α-lactose monohydrate can convert to α- or β-lactose at a molar ratio of 5:3 and α- or β-lactose at a molar ratio of 4:1 at the end of the crystallisation period. However, this solid-solid transformation might depend on the water content (m) and temperature (*Simpson, Parrish & Nelson, 2010; Fan & Roos, 2015*). The high m

value in GOS-lactose could limit the solid-solid transformation during lactose crystallisation. For crystallised trehalose-lactose (1:1 w/w), the intensities of the characteristic 2θ peaks for lactose were lower than the intensities for pure lactose, but higher than the intensities for GOS-lactose (1:1 w/w), suggesting that the presence of trehalose in lactose inhibits lactose crystallisation. The type of crystals in crystallised trehalose-lactose (1:1 w/w) were the same as the type of pure lactose, including α-lactose monohydrate, α-/β-lactose in a molar ratio of 5:3, α-/β-lactose in a molar ratio of 4:1 and anhydrous β-lactose (Figure 5). This result is consistent with the previously published results by (*Miao & Roos, 2005*). The ratio of α-lactose monohydrate to anhydrous β-lactose in the trehalose-lactose mixture (1:1 w/w) was remarkably lower than that in pure lactose. When mixed with lactose, trehalose can disturb the movement of lactose molecules and the recrystallisation from anhydrous lactose to monohydrates could also be depressed (*Fan & Roos, 2016*). Presently, the depressed solid-solid transformation of lactose crystals in crystallised trehalose-lactose mixture (1:1 w/w) could be related to a slow rate of α-/β-lactose mutarotation when trehalose was mixed with lactose (Table 2).

The collective results indicate that the addition of GOS to lactose (1:1 w/w) significantly depressed the lactose crystallisation by reducing the initial driving force for the formation of lactose nuclei and the growth of lactose crystals. The addition of GOS also changed the proportion of lactose crystalline forms in comparison to pure lactose and trehalose-lactose (1:1 w/w).

5. Conclusion

This study investigated water sorption behaviours, glass transition temperature (onset T_g), and crystallisation behaviours of freeze-dried GOS-lactose (1:1 w/w) at a_w values of 0.11 to 0.75. These physicochemical properties of GOS-lactose were also compared with the same ratio of trehalose-lactose. The addition of GOS to lactose changed the water sorption isotherm and enhanced the hygroscopicity as compared to pure lactose and the trehalose-lactose mixture. The T_g of the mixtures were not greatly affected by the addition of GOS or trehalose to lactose at the mass ratio of 1:1. Without affecting the T_g of the mixtures, GOS displayed a greater ability to inhibit lactose crystallisation than trehalose and altered the type of lactose crystals that formed. The inhibition of lactose crystallisation by GOS was more likely due to an increased solubility of lactose, as GOS modified the lactose mutarotation in the aqueous phase. These results suggest that GOS can be used to inhibit crystallisation to stabilise and improve the quality of relevant products during processing and storage.

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Table 1. Total water content (experimental values) and Guggenheim-Anderson-de Boer (GAB) predicted values of lactose, GOS, GOS–lactose

	Water content (g H_2O / 100 g of solids) [b]

(1:1 w/w), trehalose, and trehalose-lactose (1:1 w/w) at 0.11-0.75 a_w, 25 ^oC. The GAB predicted values were for non-crystalline.

Water activity at 25 °C	Lactose		GOS		GOS–lactose (1:1 w/w)		Trehalose		Trehalose–lactose (1:1 w/w)	
	Experimental values	GAB predicted values [a]	Experimental values	GAB predicted values [a]	Experimental values	GAB predicted values [a]	Experimental values	GAB predicted values [a]	Experimental values	GAB predicted values [a]
0.11	2.89 ± 0.11	2.83	3.75 ± 0.78	3.70	3.01 ± 0.44	3.01	2.89 ± 0.15	3.05	3.16 ± 0.05	2.99
0.23	4.96 ± 0.34	4.82	5.51 ± 0.37	5.62	5.12 ± 0.47	5.16	4.97 ± 0.34	5.09	4.84 ± 0.49	4.92
0.33	6.20 ± 0.23	5.79	7.12 ± 0.20	7.13	7.09 ± 1.46	6.17	6.68 ± 0.17	6.67	6.37 ± 0.44	6.68
0.44	2.50 ± 0.48	7.94	9.61 ± 1.55	9.20	9.13 ± 0.45	8.18	8.84 ± 0.09	9.11	8.02 ± 0.77	8.65
0.58	2.93 ± 0.08	11.05	12.8 ± 0.38	13.4	12.59 ± 0.16	12.7	8.96 ± 0.12	12.9	3.13 ± 0.08	12.1
0.68	3.61 ± 0.15	14.6	19.7 ± 0.18	19.0	19.0 ± 0.08	19.0	9.12 ± 0.11	17.2	4.01 ± 0.13	15.8
0.75	4.08 ± 0.03	18.6	26.5 ± 0.18	26.4	29.2 ± 0.01	29.2	9.66 ± 0.07	21.9	4.45 ± 0.06	19.9

[a] Experimental data of lactose at water activity (a_w) of 0.11-0.33 a_w for GAB calculation; experimental data of GOS at a_w of 0.11-0.75 for GAB calculation; experimental data of GOS-lactose (1:1 w/w) at a_w of 0.11-0.58 for GAB calculation; experimental data of trehalose at a_w of 0.11-0.44 for GAB calculation; experimental data of trehalose-lactose (1:1 w/w) at a_w of 0.11-0.44 for GAB calculation; [b] The fitness (r^2) of polynomial regression of the experimental values for lactose, GOS, GOS-lactose (1:1 w/w), trehalose and trehalose-lactose (1:1 w/w) were 1, 0.973, 0.981,0.999, 0.999 and 0.995, respectively. The water sorption experiments for lactose and trehalose-lactose (1:1 w/w) were carried out in replicates with duplicate experiments. The water sorption experiments for GOS, GOS-lactose (1:1 w/w) and trehalose were carried out in duplicate experiments.

Table 2. Change in mutarotation velocity (K) of lactose with GOS or trehalose in distilled water at 25 °C.

Samples (g / 100 mL solution)	Initial optical	Final optical	Reduced	K	Increase (+) /

GOS	Trehalose	Lactose	value (2 min)	value (48 h)	values		decrease (-) [a]
□	7.5	□	146.5º	147.8º	N/A	N/A	N/A
7.5	□	□	33.56º	36.75º	N/A	N/A	N/A
□	□	0.5	88.55º	60.11º	28.44º	0.575	(+) 0.140
□	□	2.5	82.91º	48.32º	34.59º	0.485	(+) 0.050
□	□	7.5	83.44º	44.78º	38.66º	0.435	0.000
0.5	□	7.5	79.78º	46.49º	33.29º	0.429	(−) 0.006
2.5	□	7.5	69.52º	44.35º	25.17º	0.444	(+) 0.009
7.5	□	7.5	60.74º	40.90º	19.84º	0.469	(+) 0.034
□	0.5	7.5	90.42º	58.27º	32.15º	0.495	(+) 0.060
□	2.5	7.5	106.9º	77.62º	29.30º	0.455	(+) 0.020
□	7.5	7.5	134.5º	97.52º	37.93º	0.248	(−) 0.187

[a]The increase (+) or decrease (-) was calculated on the basis of the K (0.435) of pure lactose at 7.5% (w/v).

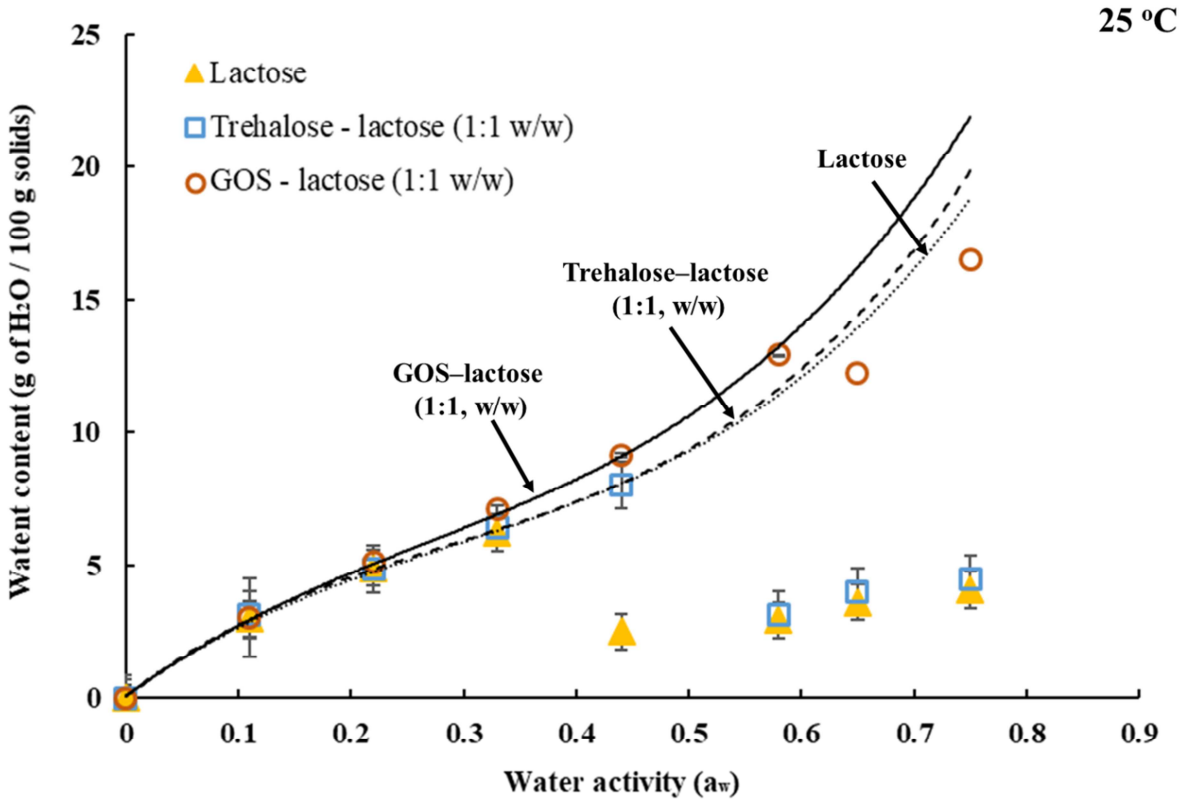


Figure 1. **Experimental (symbols) and GAB calculated (lines) water content of lactose, GOS-lactose (1:1 w/w) and trehalose-lactose (1:1 w/w) over the range of water activity.** The thick solid line (GOS-lactose 1:1 w/w), dashed-dotted line (lactose), and long dashed lines (trehalose-lactose 1:1 w/w) correspond to the GAB sorption isotherms.

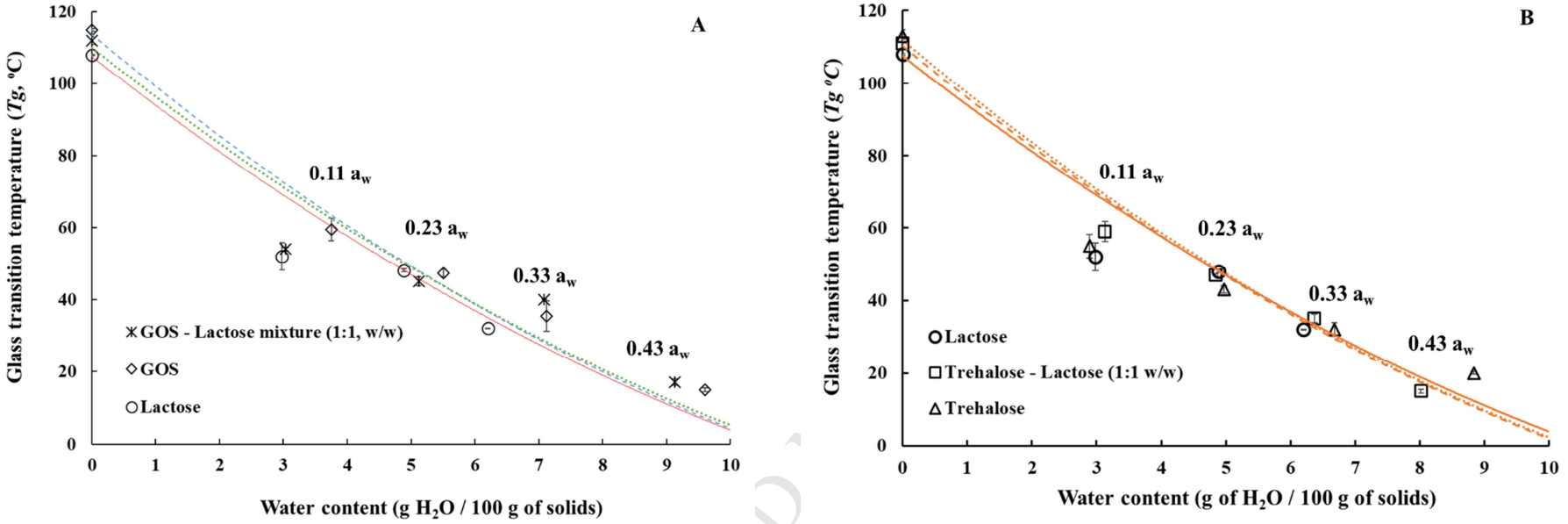


Figure 2. Experimental T_g (symbols) and Gordon-Taylor calculated T_{gmix} (lines) of amorphous lactose, trehalose and trehalose-lactose (1:1 w/w) plotted against water content. (A) The thick solid (lactose), dashed-dotted (GOS) and long dashed lines (GOS-lactose 1:1 w/w) correspond to the Gordon-Taylor predicted models; (B) The thick solid (lactose), dashed-dotted (trehalose) and long dashed lines (trehalose-lactose 1:1 w/w) correspond to the Gordon-Taylor predicted models.

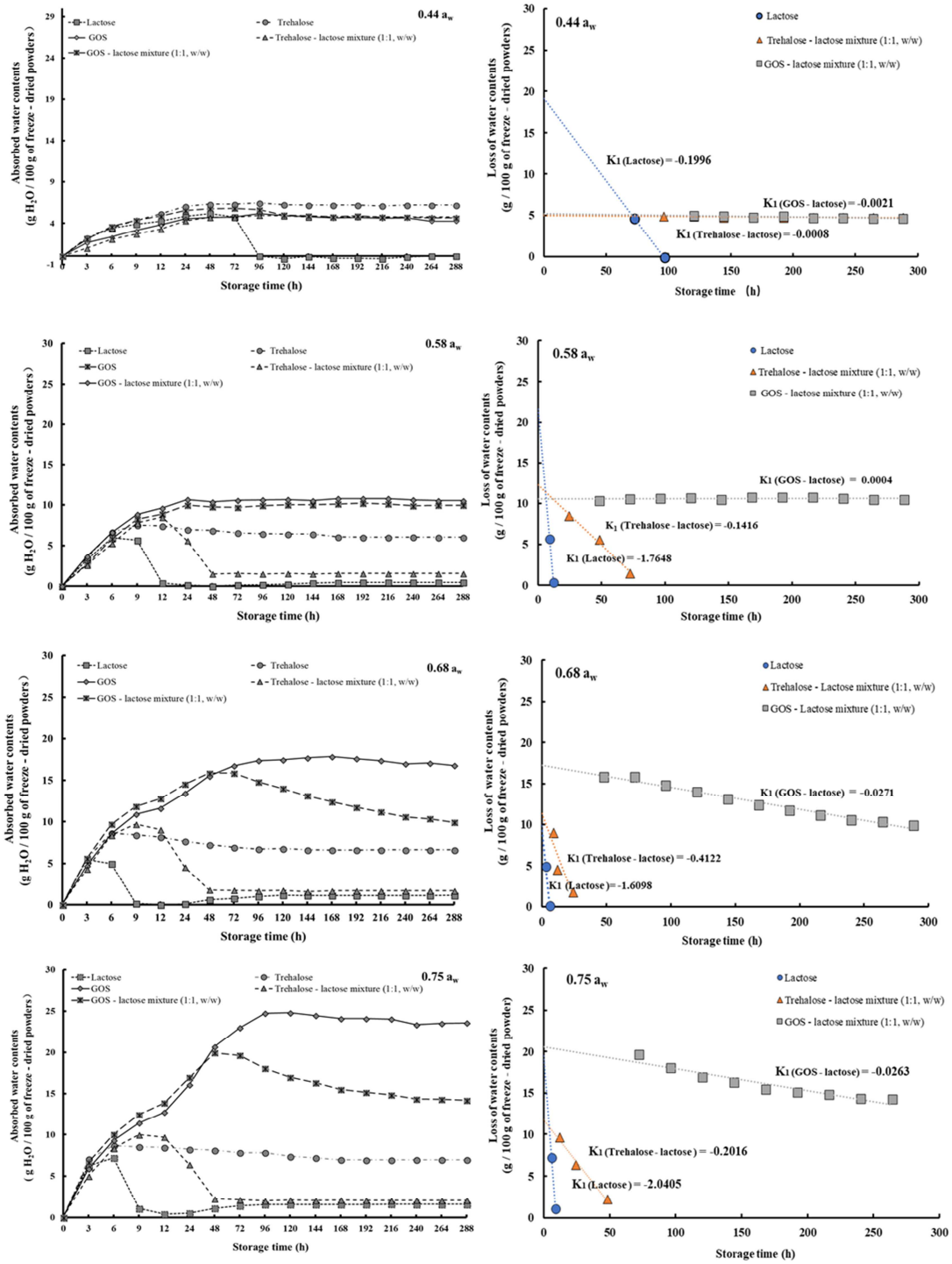
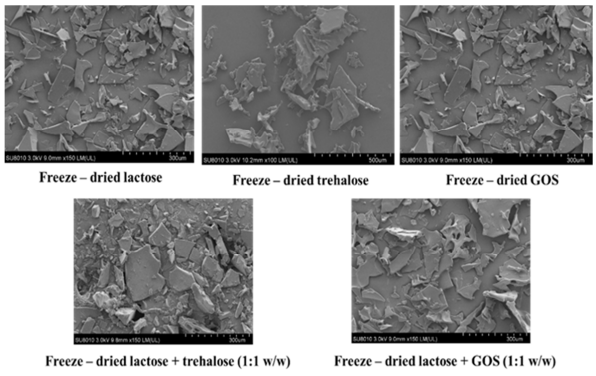
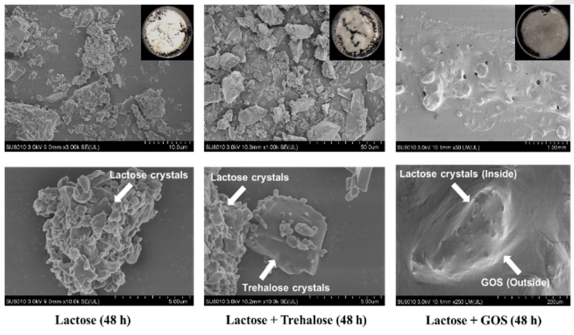


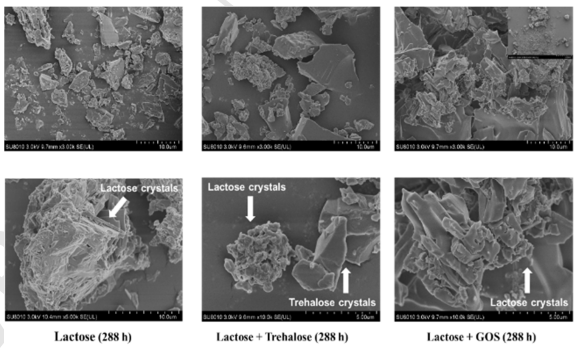
Figure 3. Dynamic water sorption of lactose, GOS, GOS-lactose mixture (1:1, w/w), trehalose, and trehalose-lactose mixture (1:1 w/w) stored at a water activity of 0.44, 0.58, 0.68, and 0.75 at 25 ^{o}C for 288 h (left graphs) and rate constants (K_1; left graphs) and loss of water (right graphs) absorbed in lactose, GOS-lactose mixture (1:1 w/w), and trehalose-lactose mixture (1:1 w/w) under 0.44, 0.58, 0.68, and 0.75 a_w at 25 ^{o}C for 288 h (right graphs)



(A) Morphological characteristics of freeze-dried amorphous powders



(B) Morphological characteristics of crystallized lactose, trehalose-lactose (mass ratio1:1) and GOS-lactose (mass ratio1:1) after 48 h storage under RH 75% and 25 ^{o}C



(C) Morphological characteristics of crystallized lactose, trehalose-lactose (mass ratio1:1) and GOS-lactose (mass ratio1:1) after 288 h storage under RH 75% and 25 ^{o}C

Figure 4. Scanning electron microscopy images of amorphous lactose, trehalose-lactose (1:1 w/w), and GOS-lactose (1:1 w/w) powders (A) and lactose crystals in crystallised pure lactose, trehalose-lactose mixture (1:1 w/w), and GOS-lactose mixture (1:1 w/w) after storage under RH 75% at 25 ^{o}C for 48 h (B) and 288 h (C).

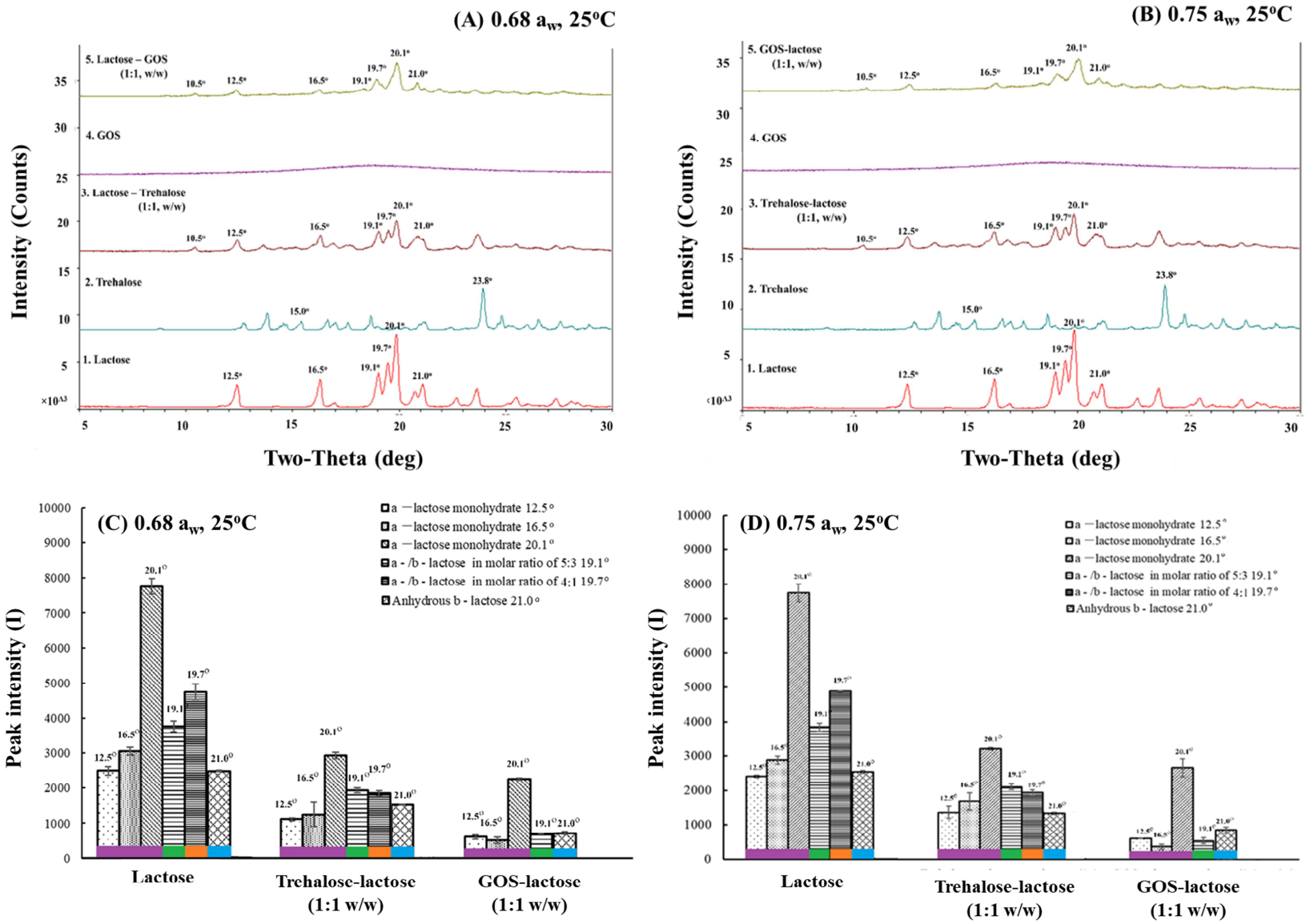


Figure 5. XRD patterns of crystallised GOS-lactose (1:1 w/w), GOS, trehalose-lactose (1:1 w/w), trehalose, and lactose stored at an RH of 68% (0.68 a_w) (A and C) and 75% (0.75 a_w) (B and D) at 25 °C for 288 h.

Highlights

- Addition of galacto–oligosaccharides (GOS) to lactose altered water sorption isotherm and increased the hygroscopicity of the mixture
- Minor change in glass transition temperature (T_g) by adding GOS to lactose
- Reduction of crystallization rates and degree of crystallinity of lactose in the presence of GOS by modification of lactose mutarotation
- As comparing to trehalose, GOS has a stronger ability to prevent lactose from crystallization in hydrous matrices