Water sorption and plasticization of an amorphous galacto-oligosaccharide mixture

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ABSTRACT

Food-grade galacto-oligosaccharides (GOS) are commercially available as transparent syrups or dried powders. Food powders can be found in an amorphous metastable state which is very sensitive to changes in temperature and moisture content. In this work the impact of water content on thermal behavior and relative humidity on water sorption behavior of amorphous GOS powders were studied. Results from differential scanning calorimetry (DSC) and sorption isotherms suggest that GOS mixture studied, with high content of oligosaccharides, has low ability to crystallize. A dramatic decrease in the stability of GOS powders occurred above critical water content (12–14 g/100 g) and corresponding critical water activity (0.55–0.62). Above these conditions GOS powder lost its amorphous character, collapsed and shrank, as the powder became a transparent "solution-like" material. The knowledge about the physicochemical changes, acquired during the present study, should be used to a proper control of processing and storage conditions to achieve and maintain optimum powder quality with desired properties.

1. Introduction

Galacto-oligosaccharides (GOS) are mainly formed by enzymatic catalysis from lactose using the transgalactosylation catalytic activity of glycoside hydrolases (EC 3.2.1) to produce several oligomers of different chain lengths (De Roode, Franssen, van der Padt, & Boom, 2003; Prenosil, Stuker, & Bourne, 1987; Tzortzis & Vulevic, 2009). Food-grade GOS are commercially available as transparent syrups or dried powders. In both cases they are mixtures containing oligosaccharides of different degrees of polymerization (galactose$_n$–glucose), un-reacted lactose and generated monomer sugars (Playne & Crittenden, 2009; Tzortzis & Vulevic, 2009). Purified products with more than 90% (w/w) of GOS are commercially available from some manufacturers.

Food powders can be found in an amorphous metastable state which is very sensitive to changes in temperature and moisture content. This amorphous matrix may exist either as a very viscous glass, or as a more liquid like "rubbery" amorphous structure. The change from the glassy state to the rubbery one occurs at glass transition temperature (T_g) which is specific for each material, but also depends on experimental variables (Roos & Karel, 1991b). Above T_g the molecular mobility is greatly increased and many amorphous compounds crystallize. Therefore, the onset of crystallization may take place if either the temperature or the moisture content is increased (Roos & Karel, 1991b).

Sorption isotherms show the amount of water adsorbed as a function of steady state water activity (a_w), at a constant temperature (Roos, 1995b). It is known that plasticizers like water cause a dramatic decrease in T_g (Roos, 1995b; Roos & Karel, 1991b). The knowledge of the material sorption isotherm is extremely important to predict stability and quality changes during the process and storage of a food product.

Stickiness and caking are phenomena that may occur when amorphous food are heated or exposed to high humidity. These phenomena can be a consequence of the softening of powders above their T_g (Adhikari, Howes, Bhandari, & Truong, 2001; Roos, 1995a). Furthermore, stickiness and caking are frequently

undesirable during manufacturing operations causing lower product yields and operational problems.

The aim of this work is to study the impact of process variables (temperature and relative humidity) on the thermal and water sorption behavior of amorphous GOS powders in order to define processing and storage conditions to achieve and maintain optimum powder quality with desired properties.

2. Materials and methods

2.1. Amorphous powders

Lactose was purchased from Hi-Media (>99.5% w/w) and GOS were provided by Biotempo, Biotechnology Consulting Ltd. (Portugal). The GOS mixture was produced in a bioreactor by enzymatic transgalactosylation during yeast growth in a lactose containing medium. The chemical characterization of the sample was previously conducted (unpublished data). Briefly, it has about 97% (w/w) of GOS (47% trisaccharides, 42% tetrasaccharides and 8% pentasaccharides), mainly, $\beta 1 \rightarrow 4$ and $\beta 1 \rightarrow 6$ linked products.

Amorphous powders were obtained by freeze-drying. For that purpose, lactose and GOS were dissolved in distilled water (15% w/v). Both solutions were frozen immediately after preparation at $-196\,°C$, in liquid nitrogen, for 15 min. Afterwards, samples were freeze-dried at below 0.1 mbar. Then, samples were immediately pulverized using mortar and pestle and transferred into a vacuum desiccator, and dried over phosphorus pentoxide (P_2O_5) for at least seven days.

2.2. Moisture determination

The initial water content of the samples was determined using a Karl-Fisher coulometer 737 (Methrohm, Switzerland). Samples were dissolved in dimethyl sulfoxide (DMSO) and the water content of the pure solvent was used as blank. Each sample was analyzed six times and the average value was taken as being the initial water content.

2.3. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to determine T_g and crystallization temperature (T_c) as a function of the water content. The instrument used was a DSC 141, SETARAM (Caluire, France). Hermetically sealed steal cells were used in all measurements. An empty (air) steal cell was used as reference sample. The DSC was calibrated chemically for temperature and heat flow at a scan rate of 3 °C/min, by use of tin (mp 231.9 °C, ΔH_m 60.2 J g^{-1}), indium (mp 156.6 °C, ΔH_m 28.4 J g^{-1}), benzoic acid (mp 122.4 °C, ΔH_m 147.9 J g^{-1}), naphthalene (mp 80.2 °C, ΔH_m 148.7 J g^{-1}), and o-terphenyl (mp 58.0 °C, ΔH_m 74.6 J g^{-1}).

The Setaram software, SETSOFT, was used for determination of T_g, T_c and transition enthalpy (ΔH).

2.4. Sample preparation for differential scanning calorimetry

Completely dry samples were placed in DSC cells (15–20 mg) and hermetically sealed. Samples of varying water contents were prepared by the following method: 15–20 mg of dried material was weighted into DSC pans in a Microbalance Mettler–Toledo (readability 0.1 μg). The open cells were stored over a saturated MgCl$_2$ solution (equilibrium relative humidity of air (RH) of 33% at 25 °C), for varying periods at room temperature. With this procedure, samples with different moisture contents were obtained. At a given time cells were sealed and reweighted. Water content was determined from weight gain after equilibration. This procedure was repeated twice with independent samples.

2.5. DSC measurements

The samples were heated at 3 °C/min from T_g -30 to 200 °C. Thermograms were analysed in order to obtain T_g (determined as the temperature of onset of an endothermic shift in apparent specific heat), and T_c (determined as the temperature of onset of an exothermic peak).

The Gordon and Taylor equation (Gordon & Taylor, 1952) was fitted to the experimental T_g data according to Eq. (1):

$$T_g = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2} \tag{1}$$

where w_1 and w_2 are the weight fraction of the solute and water respectively; T_{g1} is the T_g of anhydrous solute; T_{g2} is the T_g of amorphous water; and k is a constant. A T_g of $-135\,°C$ was used for water (Johari, Hallbrucker, & Mayer, 1987). The Gordon and Taylor (Eq. (1)) is generally used for predicting the water plasticization in binary systems. Therefore, Eq. (1) was used in the present study to predict the water plasticization of lactose and GOS, considering that all solid components contributing to the observed glass transition were miscible and formed a single phase (Roos, 2009).

Root mean square deviation (in percentage) (RSM) was calculated as an indication of the goodness of fit of the model to the experimental data according to Eq. (2),

$$\%RSM = \sqrt{\frac{\sum_i^N \left[(y_i - y_i^*)/y_i \right]^2}{N}} \times 100 \tag{2}$$

where y_i is the experimental value; y_i^* is the calculated value according to Eq. (1) and N is the number of experimental points.

2.6. Sorption isotherms

Measurement of water uptake by the initially dried sugars was determined periodically from changes in weight of the samples kept at room temperature (20–21 °C) in different saturated salt solutions to maintain a constant relative humidity (Table 1). Approximately 0.1 g of dry sample was weighted into a constructed aluminium foil pan (20 mm diameter × 15 mm height). The samples were weighted every 12 h. Water content was determined from weight gain after equilibration. This procedure was performed in triplicate.

During the determination of the sorption isotherms, it is assumed that, at a steady state, the ratio of the vapour pressure in food, p, and that of pure water, p_0, equals water activity, a_w. The relative vapour pressure of water in food and in the surrounding atmosphere is also equal, and therefore water activity may be obtained from RH of air ($p/p_0 = a_w = RH/100$) (Roos, 1995b).

Previously, the Guggenheim–Anderson–de Boer (GAB) (van den Berg & Bruin, 1981) water sorption model (Eq. (3)) has proved to fit to water sorption data of most food materials. This model can be used to determine the sorption isotherms, providing the relationship between a_w and moisture content (m).

$$\frac{m}{m_m} = \frac{CK'a_w}{(1 - K'a_w)(1 - K'a_w + CK'a_w)} \tag{3}$$

Table 1
Equilibrium relative humidity (RH) of saturated salt solutions at 20 °C (Greenspan, 1977; 6).

	RH (%)		RH (%)
P$_2$O$_5$	0.0	NaBr	59.1
LiCl	11.3	SrCl$_2$	72.5
MgCl$_2$	33.1	NaCl	75.5
K$_2$CO$_3$	43.2	(NH$_4$)$_2$SO$_4$	81.3
Mg(NO$_3$)$_2$	54.4	KCl	85.1

Additionally, a second-order polynomial model (Eq. (4)) was suggested by other authors (Bizot, 1983; Roos, 1993).

$$\frac{a_w}{m} = \alpha a_w^2 + \beta a_w + \gamma \qquad (4)$$

where $\alpha = \frac{K'}{m_m((1/C)-1)}$, $\beta = \frac{1}{m_m(1-(2/C))}$, and $\gamma = \frac{1}{CK'm_m}$.

The GAB isotherm parameters were determined by plotting a_w/m against a_w. The constants α, β, and γ in Eq. (4) were calculated by quadratic minimum squares regression analysis. The experimental sorption data in a_w range from 0.113 to 0.432 for amorphous lactose, and 0.113 to 0.850 for amorphous GOS, were used to obtain the constant values. Constants C and K' and monolayer value (m_m) were calculated from Eqs. (5)–(7), respectively.

$$C = \frac{1}{m_m \gamma K'} \qquad (5)$$

$$K' = \frac{\beta - (1/m_m)}{-2\gamma} \qquad (6)$$

$$m_m^2 = -\frac{1}{4\alpha\gamma - \beta^2} \qquad (7)$$

RMS values (Eq. (2)) were calculated as an indication of the goodness of fit of the tested models to our data.

2.7. Statistics

The *t test* was used to compare moisture content of GOS and lactose at different a_w. The level of confidence required for significance was selected as $P \leq 0.05$.

3. Results and discussion

3.1. Effect of moisture content on glass transition temperature

Lactose showed a typical thermal behaviour of amorphous materials, and therefore the glass transition and crystallization parameters could be determined. However, in the thermogram of the GOS mixture, no exothermic peak was observed at any moisture content (Fig. 1). This observation suggests that the crystallization rate of GOS mixture is very slow or negligible in the studied experimental conditions. The GOS sample is a mixture containing mainly linear tri-, tetra-, and pentasaccharides (as described). As a time-dependent phenomenon, crystallization can be impaired by higher molecular sizes that contribute to higher viscosity and slow diffusion (Roos, 1995a). There is a lack of studies in the literature
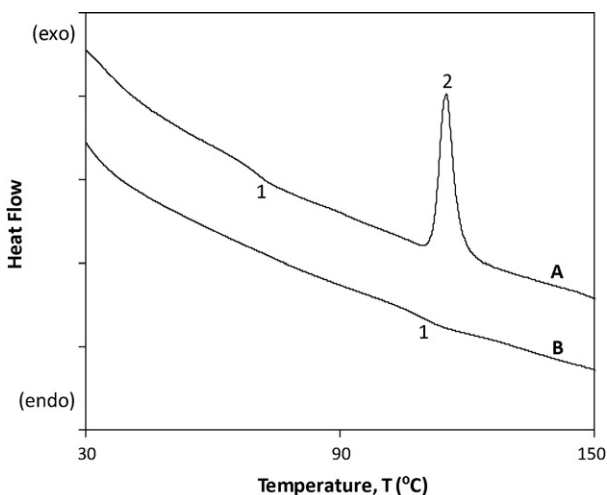
Fig. 1. Typical DSC thermograms for amorphous lactose (A) and amorphous GOS (B) 1, glass transition; 2, crystallization. Scanning rate was 3 °C/min.
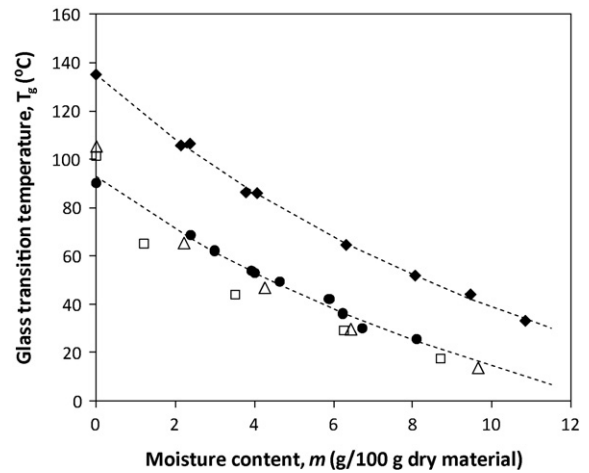
Fig. 2. Effect of moisture content of amorphous food materials on glass transition temperatures. ●, Amorphous lactose (this work); ♦, amorphous GOS (this work). Dashed lines represent the fitting of the Gordon and Taylor equation to the experimental data. Other data for amorphous lactose was extracted from the literature, △ (Roos & Karel, 1990) and □ (Haque & Roos, 2004) and is superimposed for comparison.

relating the polymerization degree with the crystallization ability of oligosaccharides. However, it was observed a reduction in the growth rate of sucrose crystals when different oligosaccharides from corn syrup were mixed with sucrose (Tjuradi & Hartel, 1995).

The effect of moisture content in T_g for both materials is shown in Fig. 2 along with the glass transition temperatures obtained for lactose that have been reported in the literature. It was observed a drastic decrease of T_g with the increasing water content. This is a typical behaviour of water plasticization that has also been reported in other studies (Haque & Roos, 2004; Roos & Karel, 1991b; Zimeri & Kokini, 2002). The experimental T_g within the moisture content range from 0 to 8.1 g of water/100 g dry material for lactose; and from 0 to 10.8 g of water/100 g dry material for GOS was successfully predicted by the Gordon and Taylor equation as shown in Fig. 2. The data suggest that a similar water plasticization effect occurs in binary systems water/lactose and water/GOS. The determined constant k was 5.3 (RSM, 4.6%) and 5.5 (RSM, 1.9%) for lactose and GOS, respectively.

Furthermore, T_g values of GOS were considerably higher than the ones observed for lactose (Fig. 2). Completely dry lactose and GOS have T_g values of 90.3 and 135.1 °C, respectively. T_g value for GOS mixture is between the T_g values reported to maltotetraose and maltopentose (Roos & Karel, 1991a). An increase in T_g of glucose homopolymers (maltodextrins) with increasing molecular weight has been reported (Roos & Karel, 1991a). This behaviour is described by the relation previously established by Fox and Flory for polystyrene homopolymers (Fox & Paul, 1950). It is important to notice that in this study it was not possible to determine a relation between molecular weight and T_g of GOS, because of the polydispersity of the sample. However, since GOS are homopolymers of aldohexoses, a similar effect of its molecular weight on T_g is expected to occur.
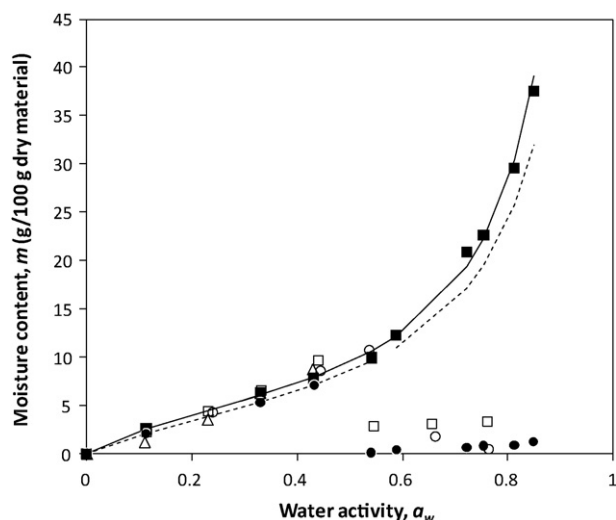
3.2. Water sorption

The moisture content, at various a_w, sorbed by amorphous lactose and GOS at equilibrium after 144 h of storage, is given in Table 2. The amount of water sorbed by lactose is significantly lower ($P \leq 0.05$), comparing to that of GOS, at a_w from 0.331 to 0.851.

Lactose sorption isotherm shows a dramatic decrease in sorbed water when $a_w > 0.432$ (Table 2 and Fig. 3). As observed by other authors, this phenomenon is a result of lactose crystallization

Table 2
Moisture contents (g/100 g dry material) of lactose and GOS stored for 144 h at various a_w at room temperature (mean ± standard deviation).

a_w	Lactose	GOS
0.113	2.09 ± 0.19*	2.55 ± 0.15*
0.331	5.34 ± 0.16	6.24 ± 0.07
0.432	7.10 ± 0.07	7.81 ± 0.09
0.544	0.12 ± 0.09	9.94 ± 0.08
0.591	0.41 ± 0.29	12.29 ± 0.18
0.725	0.65 ± 0.06	20.87 ± 0.58
0.755	0.82 ± 0.29	22.68 ± 0.80
0.813	0.91 ± 0.12	29.58 ± 0.17
0.851	1.21 ± 0.04	37.59 ± 0.69

* Not statistically different ($P = 0.056$). Significant differences ($P \leq 0.05$) were observed between moistures content of lactose and GOS stored at $a_w > 0.113$.

Fig. 3. Water sorption isotherms of lactose and GOS stored for 144 h at room temperature (20–21 °C) using experimental data from the present work (●, lactose and ■, GOS). Full and dashed lines represent the fitting of the GAB model to the experimental data of GOS and lactose, respectively. Other experimental data for amorphous lactose was extracted from the literature and superimposed for comparison: △ amorphous lyophilized lactose stored for 24 h at room temperature (Roos & Karel, 1991b); □, amorphous, spray dried lactose (coarse powder) stored for 144 h at room temperature (22–23 °C) (Haque & Roos, 2004); ○, amorphous lyophilized lactose stored for 24 h at 24 °C (Jouppila & Roos, 1994).

and consequent release of adsorbed water (Haque & Roos, 2004; Jouppila & Roos, 1994; Roos & Karel, 1991b). When $a_w > 0.432$ changes in structural properties of lactose powder were also observed, namely, collapse and shrinkage.

Sorbed water by GOS increased within the full range of tested a_w, suggesting that crystallization rate of GOS mixture is very slow or negligible in the studied experimental conditions. It is known that the time needed for crystallization depends on the material char-

acteristics, including molecular size. As described, GOS mixture is mainly composed by tri-, tetra- and pentasaccharides. Probably, higher molecular sizes contribute to higher viscosity and slow diffusion, impairing crystallization. At $a_w \geq 0.591$, GOS powder lost its amorphous character, collapsed and shrinked, and became a transparent "solution-like" material. It is important to stress that the sorption behaviour at $a_w > 0.432$ was the major difference observed between the two materials tested.

Comparing the results obtained in this study for the sorbed water content by lactose with other published works (Haque & Roos, 2004; Jouppila & Roos, 1994; Roos & Karel, 1991b), small differences were observed, probably due to the drying procedures, particles size, storage time, and temperature.

The experimental water sorption data for amorphous lactose and amorphous GOS powders, in the a_w range shown in Table 3, were successfully modelled with the GAB isotherm model (Fig. 3). The materials showed the typical sigmoid water sorption isotherm (Roos, 1995b). The GAB monolayer value determined for GOS powder was slightly higher than the one for lactose powder. The monolayer value for lactose was 5.40 g/100 g dry solids, which was close to the water content of 5.34 g/100 g dry solid when stored at a_w 0.331 at room temperature. The monolayer value for GOS was 5.49 g/100 g dry solid, which was close to the water content of GOS when stored at a_w 0.296 at room temperature. The GAB monolayer value for lactose reported in this study is higher than values reported for amorphous spray dried lactose (coarse powder) stored for 144 h at room temperature (22–23 °C) (Haque & Roos, 2004) and for 3 weeks at 20–38 °C (Bronlund & Paterson, 2004); and lower than monolayer value for spray dried lactose stored for 3 weeks at 12 °C (Bronlund & Paterson, 2004) (Table 3). Hence, the values obtained fall well within other ones reported in the literature (Bronlund & Paterson, 2004; Haque & Roos, 2004; Jouppila & Roos, 1994).

Water plasticization is an important factor contributing to the characteristics and storage stability of the GOS powders. A dramatic decrease in the stability of GOS powders occurred above critical water content and corresponding critical water activity. These values of critical water content (approximately, 14 g/100 g) and water activity (approximately 0.62) correspond to those at which the estimated glass transition of GOS occurs at the storage temperature (Fig. 4). However, during storage at room temperature, lower a_w values should be used, since it was observed that when $a_w \geq 0.591$ (water content in equilibrium was 12.3 g/100 g, see Table 2) the GOS powder lost its amorphous character, collapsed and shrank as the powder became a transparent "solution-like" material.

Based on these results, it was possible to conclude that glass transition region at room temperature occurs for a_w between 0.55 and 0.62 and a water content between 12 and 14 g/100 g. Using the same methodology for amorphous lactose, the glass transition region at room temperature occurred for a_w between 0.45 and 0.50 and a water content between 8.0 and 8.7 g/100 g. Another study

Table 3
Monolayer value (m_m), and constants K' and C for the GAB isotherms.

| Material	a_w	n	m_m	K'	C	%RMS	Ref.
Lactose	0.113–0.432	3	5.40	0.99	4.14	3.9	This study
Lactose	0.115–0.444	4	4.91	1.18	4.33		[a]
Lactose	0.114–0.441	4	4.77	1.59	2.93	0.29	[b]
Lactose	0.114–0.441	4	6.93	0.77	3.39		[c]
Lactose	0.114–0.441	4	4.88	1.16	3.23		[d]
GOS	0.113–0.851	9	5.49	1.02	5.42	4.6	This study

n, Number of experimental points. In this study each experimental point was made in triplicate.

[a] Amorphous lyophilized lactose stored for 24 h at 24 °C (Jouppila & Roos, 1994).
[b] Amorphous, spray dried lactose (coarse powder) stored for 144 h at room temperature (22–23 °C) (Haque & Roos, 2004).
[c] Amorphous, spray dried lactose stores for 3 weeks at 12 °C (Bronlund & Paterson, 2004).
[d] Amorphous, spray dried lactose stores for 3 weeks at 20–38 °C (Bronlund & Paterson, 2004).
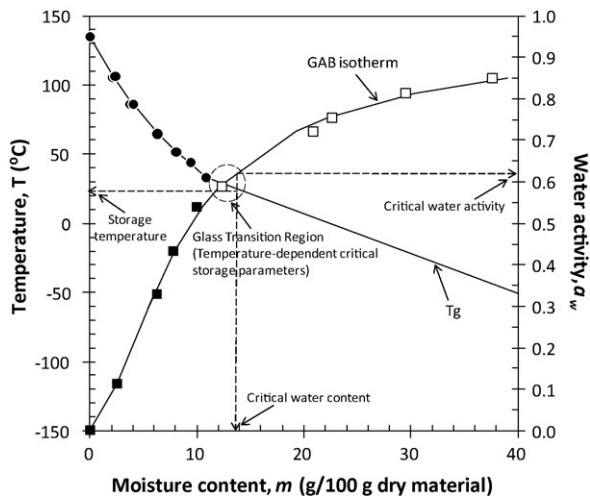
Fig. 4. Water plasticization and T_g of the characterized GOS mixture, at various water contents. Depression of the T_g with water content was predicted by the Gordon and Taylor equation (1). The critical water content and water activity correspond to plasticization, depressing T_g to room temperature.

conducted for amorphous lactose reported a critical a_w around 0.37 and a critical water content around 7.5 g/100 g (Roos, 2009). Again, the differences observed can be due to differences in drying procedures, particles size, storage time, and temperature.

4. Conclusions

Unlike amorphous lactose, that exhibits an exothermic peak during DSC measurements, no crystallization was observed during GOS heating at 3 °C/min until 200 °C. Furthermore, sorbed water by GOS was found to increase all the way within the range of a_w used, suggesting that GOS mixtures with high content of oligosaccharides have low ability to crystallize. This observation has an important impact in GOS applications and product development, since these ingredients can replace lactose or other sugars whenever crystal formation is undesirable.

Water plasticization is a key factor contributing to the characteristics and storage stability of the GOS powders. A dramatic decrease in the stability of GOS powders occurs above critical water content and corresponding critical water activity. GOS powder lost its amorphous character, collapsed and shrank as the powder became a transparent "solution-like" material. In summary, it is possible to conclude that the critical storage a_w is between 0.55 and 0.62 and the water content is between 12 and 14 g/100 g. These values are considerably higher than the critical a_w and the critical water content found for amorphous lactose.

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