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Fig. S1 **One-dimensional** loading plots in PC1 performed on the NIR spectra of GOS Cup Oligo H-70®, GOS Biotempo and lactulose equilibrated at different RH ([Fig. 3](#)).



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# Determination of amorphous/rubbery states in freeze-dried prebiotic sugars using a combined approach of near-infrared spectroscopy and multivariate analysis

Q3 Mauricio I. Santos<sup>a</sup>, Cuauhtemoc Araujo-Andrade<sup>b</sup>, E. Elizabeth Tymczyszyn<sup>a</sup>, Andrea Gómez-Zavaglia<sup>a,\*</sup>

<sup>a</sup> Center for Research and Development in Food Cryotechnology, CCT-La Plata, RA-1900, Argentina

<sup>b</sup> Unidad Académica de Física de la Universidad Autónoma de Zacatecas, Zacatecas, Mexico

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## ABSTRACT

Galacto-oligosaccharides (GOS) and lactulose are well-recognized prebiotics widely used in functional food and pharmaceutical products, but there is still a lack of knowledge regarding their physical-chemical properties. In this study, a physical-chemical approach on two GOS of different composition (GOS Cup Oligo H-70® and GOS Biotempo) and lactulose was assessed. Mid infrared and Raman spectra of the freeze-dried sugars allowed their structural characterization in the amorphous state, lactulose, showing the main spectral differences. Freeze-dried sugars were then equilibrated at 4 °C at relative humidity (RH) ranging from 11% to 80%. Near-infrared reflectance spectra were registered in each condition in the 900- to 1700-nm region. A principal component analysis (PCA) was performed on the three sugars equilibrated at different RH. In all the three sugars, the groups observed explained more than 95% of the variance and were related with the RH of the samples. According to the loading plots of PC1, the main differences related with RH were observed in the 1380- to 1500-nm region.

As the amorphous states are very sensitive to changes in temperature and moisture content, and the moisture content is related with the parameter T-Tg (T: storage temperature; Tg: vitreous transition temperature), an effort was made to determine this parameter directly from the NIR spectra. To this aim, a partial least square model (PLS) was defined. Tg values obtained by differential scanning calorimetry (DSC) were used to calculate the T-Tg values of reference. The model was validated with an independent set of data. The mean of predicted values fitted nicely T-Tg obtained from DSC (correlation = 0.966;  $R^2 = 0.934$ ), thus supporting the use of the PLS model to investigate unknown samples. The stability of amorphous sugars in foods and pharmaceuticals is of practical and economical importance because it affects different quality attributes of foods, including texture, aroma retention and shelf life. Therefore, predicting T-Tg, a parameter that is independent on the sugar investigated, directly from their NIR spectra is of utmost importance to determine the shelf life of food and food-related products and up to our knowledge has never been determined hereto.

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## 1. Introduction

Prebiotics are defined as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving the host health” (Gibson & Roberfroid, 1995). From a chemical point of view, most of prebiotics are polyhydroxy compounds, including certain disaccharides and oligosaccharides. Among them, galacto-oligosaccharides (GOS) and lactulose have conclusively proven their efficiency as prebiotics (Barreateau, Delattre, & Michaud, 2006).

GOS are composed by a variable number of galactose units (usually from two to ten) and a terminal glucose unit, linked mostly by  $\beta$ 1-4 and  $\beta$ 1-6 bonds (Casci & Rastall, 2006; Vera, Guerrero, Illanes, & Conejeros, 2011). Their prebiotic effect is mostly associated with trisaccharides [GOS with degree of polymerization equal to 3 (DP3)] and tetrasaccharides (GOS DP4) (Tymczyszyn, Gerbino, Illanes, & Gómez-Zavaglia, 2011). Lactulose is a disaccharide (4-O- $\beta$ -D-galactopyranosyl-D-fructose) produced by isomerization of lactose during heat treatment of lactose containing foods (Marconi et al., 2003) or by chemical or enzymatic isomerization of lactose (Aider & de Halleux, 2007; Guerrero, Vera, Plou, & Illanes, 2011; Kim & Oh, 2012; Zokaee, Kaghazchi, Zare, & Soleimani, 2002).

The stabilizing effect of GOS and lactulose have been recently explained on the basis of their ability to form glassy matrices of high

\* Corresponding author at: Calle 47 y 116 La Plata, Buenos Aires, (1900), Argentina. Tel.: +54 221 4890741; fax: +54 221 4249287. E-mail address: [angoza@qui.uc.pt](mailto:angoza@qui.uc.pt) (A. Gómez-Zavaglia).

## Nomenclature

GOS	galacto-oligosaccharides
DP	degree of polymerization
T <sub>g</sub>	vitreous transition temperature
RH	relative humidity
DSC	differential scanning calorimetry
NIR	near-infrared spectroscopy
PCA	principal component analysis
PLS	partial least squares
RMSEC	root mean square error of calibration
RMSEP	root mean square error of prediction

viscosity and low molecular mobility where molecular interactions are restricted (Golowczyc et al., 2013; Santos, Gerbino, Araujo-Andrade, Tymczyszyn, & Gómez-Zavaglia, 2014; Tymczyszyn et al., 2011, 2012). The stabilization of these glassy matrices occurs below the glass transition temperatures (T<sub>g</sub>) (Tymczyszyn et al., 2012). However, the glassy state mobility of small molecules (i.e., water, gases and small organic molecules) is still possible and may lead to crystallization, browning, etc., finally resulting in destabilization of amorphous compounds or formulations (Franks, 1999; Le Meste, Champion, Boudaut, Blond, & Simatos, 2002; Roos, 2009).

For this reason, T<sub>g</sub> itself is important only as a macroscopic parameter, and for an adequate preservation of food and food-related products, a low molecular mobility is also required. In practice, the low molecular mobility is attained at low water contents and low storage temperatures (Schoonman, Ubbink, Bisperink, Le Meste, & Karel, 2002; Tromp, Parker, & Ring, 1997; Tymczyszyn et al., 2012). Therefore, the parameter T-T<sub>g</sub> (T being the temperature of storage) is more appropriate to assess the correctness of storage conditions. This indicates that the best condition will be the one in which the temperature of storage is as far as possible below T<sub>g</sub>. As T-T<sub>g</sub> is independent on the sugar analyzed, the determination of T-T<sub>g</sub> represents a useful tool to investigate the stability of amorphous sugars in foods and pharmaceuticals, which is of practical and economical importance because it affects different quality attributes of foods (i.e., texture, aroma retention and shelf life) (Franks, 1999; Le Meste et al., 2002; Roos, 2009).

Even when the usefulness of differential scanning calorimetry (DSC) to determine T<sub>g</sub> is doubtless, vibrational spectroscopy-based methods, including near-infrared spectroscopy (NIR), provide fast and valuable physico-chemical information due to its sensitivity to hydrogen bondings (Alander, Bochko, Martinkauppi, Saranwong, & Mantere, 2013; Bonanno, Olinger, & Griffiths, 1992; Small, 2006). For this reason, NIR spectroscopy is particularly useful to determine changes related with water structure and to analyze physical changes associated with amorphous states (Buckton, Yonemochi, Hammond, & Moffat, 1998; Buckton, Yonemochi, Yoon, & Moffat, 1999; Campbell, Campbell, Campbell, Fontana, & Ozanich, 2006; Czarnik-Matusewicz & Pilorz, 2006; Czarnik-Matusewicz, Pilorz, & Hawranek, 2005; Delwiche, Pitt, & Norris, 1992; Giangiacomo, 2006; Grohgan et al., 2010; Izutsu, Hiyama, Yomota, & Kawanishi, 2009). It has been recently used to investigate the stabilizing effect of GOS and lactulose on freeze-dried lactic acid bacteria (Santos et al., 2014).

Considering the relevance of T-T<sub>g</sub> to predict the storage stability of dehydrated products and the accuracy of NIR to quickly provide reliable information about amorphous states, the aim of this work was to develop a multivariate model to predict T-T<sub>g</sub> directly from the NIR spectra. For this purpose, two GOS of different composition and the disaccharide lactulose were equilibrated at 4 °C at different RH, and T<sub>g</sub> values were determined using DSC. In parallel, the NIR spectra were registered at all RH and used to define a partial least squares (PLS) model. The information provided by DSC was used to calculate T-T<sub>g</sub> values of reference.

## 2. Materials and methods

## 2.1. Galacto-oligosaccharides and lactulose

Two types of GOS consisting in mixtures of carbohydrates containing galactose and glucose as monomers were studied: a commercial syrup Cup Oligo H-70® (Kowa Company, Tokyo, Japan) kindly donated by Kochi S.A. (Santiago, Chile) and GOS Biotempo synthesized and kindly donated by Biotempo S.L. (Guimarães, Portugal). Cup Oligo H-70® contained 75% GOS of different DP: 4% of high-molecular-weight oligosaccharides (DP ≥ 5), 21% of tetrasaccharides (DP4), 47% of trisaccharides (DP3), 23% of disaccharides (DP2) and lactose and 5% of monosaccharides, including glucose and galactose (Tymczyszyn et al., 2011). GOS Biotempo contained 97% GOS of different DP: 8% of pentasaccharides (DP5), 42% of tetrasaccharides (DP4) and 47% of trisaccharides (DP3) (Torres, Bastos, Goncalves, Teixeira, & Rodrigues, 2011).

A commercial lactulose [(4-O-β-D-galactopyranosyl-(1 → 4)-β-D-fructofuranose)] was provided by Discovery Fine Chemicals (Wimborne, UK).

## 2.2. Freeze-drying procedure

One-milliliter solutions of 20% w/v sugars were transferred into 5 mL glass vials and frozen at -80 °C for 48 h. Freeze drying was carried out on a Heto FD4 equipment (Heto Lab Equipment, Denmark) operating with the condenser at -50 °C at a chamber pressure of 0.04 mbar. The freeze-drying process lasted for 48 h.

## 2.3. Structural analysis

FTIR and Raman spectra of the three sugars immediately after freeze-drying were registered. The FTIR spectra were registered in the 4000- to 500-cm<sup>-1</sup> range on KBr pellets, prepared with a ratio of 1 mg sugar/200 mg KBr. They were recorded in a transmission mode by co-adding 64 scans with 4-cm<sup>-1</sup> spectral resolution, on a Thermo Nicolet iS10 spectrometer (Thermo Scientific, MA, USA).

The Raman spectra were measured on a Renishaw 1000B Raman spectrometer. The wavelength of excitation was 830 nm. The laser power irradiation over the samples was 45 mW. Each spectrum was registered with an exposure of 30 s, two accumulations, and collected in the 1800- to 200-cm<sup>-1</sup> region with 2-cm<sup>-1</sup> spectral resolution.

The fluorescence contribution was removed by approximating a polynomial function to the spectra and then subtracting it from the spectra.

## 2.4. Humidification procedure

Freeze-dried sugars were equilibrated for 15 days at 20 °C in atmospheres of the following saturated salts: LiCl, KCH<sub>3</sub>COO, MgCl<sub>2</sub>, K<sub>2</sub>CO<sub>3</sub>, Mg(NO<sub>3</sub>)<sub>2</sub>, NaCl and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, giving RH 11%, 22%, 33%, 44%, 52%, 75% or 80%, respectively.

## 2.5. Glass transition temperatures

Glass transitions of the freeze-dried lactulose were determined by DSC (onset values, heating rate: 10 °C/min) using a Q100 calorimeter (TA Instruments, USA), calibrated with indium, lead and zinc. Hermetically sealed 40-μL medium pressure pans were used (an empty pan served as reference). An average value of at least two replicates is reported. The standard deviation for glass transition temperatures measurement was ± 1 °C.

For GOS Cup Oligo H-70® and GOS Biotempo, the values reported by us in Tymczyszyn et al. (2012) and in Torres et al. (2011), respectively, determined in the same conditions as those described in this work for lactulose, were used for the analysis in Section 2.7.

## 176 2.6. Near-infrared spectroscopy

177 NIR reflectance spectra corresponding to all humidified samples prepared as stated in Section 2.4 were collected in the 900– to 1700-nm spectral region. They were registered in an Ocean Optics spectrophotometer model NIRQuest 512 equipped with a thermoelectrically cooled CCD in a linear array of 512 pixels at 20 °C with an optical resolution of 3 nm FWHM and a signal to noise ratio of 15000:1. A reflection fiber-optic probe with low OH content, 400 μm core diameter and numerical aperture of 0.22 was used to illuminate the sample and collect the backscattered light in a 45° configuration (probe/sample). A Tungsten-Halogen lamp, model LS-1 from Ocean Optics Company, was used as radiation source. The power output of the lamp was of 6.5 W, working in the visible/NIR range (360–2500 nm). The spectrometer, connected to a computer, was controlled via SpectraSuite™ software from Ocean Optics. The integration time was set at 600 ms, and five spectra were taken non-consecutively for each sample in order to evaluate the spectral reproducibility during the collection stage.

## 193 2.7. Data analysis

194 Multivariate analysis and data pre-processing as mean centering and extended multiplicative scatter correction were performed on the NIR spectra, using The Unscrambler® software (version 9.8, CAMO, Norway).

198 In order to evaluate the spectral differences among sugars equilibrated at different RHs, a principal component analysis (PCA) was performed over NIR spectra covering the whole range of RH investigated (from 11% to 80% RH). Taking into account the spectral differences associated to RH in the PCA scores plot, a PLS model was calibrated to determine T-Tg values (Esbensen, 2005; Martens & Næs, 1989). To calibrate the model, a group of 34 NIR spectra covering the whole range of T-Tg, including spectra obtained from independent experiments and repetitions, was used. Tg values obtained by DSC (Section 2.5) were used to calculate the parameter T-Tg used as reference. The reliability and robustness of the calibrated model was determined as a function of their coefficient of determination,  $R^2$ , BIAS and their calibration and prediction errors (RMSEC and RMSEP, indicating the average difference between predicted and experimentally obtained results). High correlation values and low errors indicate a good capacity to correctly predict or classify unknown samples. A set of 34 spectra collected independently from those used for calibration (and in the same conditions of T-Tg) were used to validate the model. All the information regarding the set up of PLS model is shown in Table 1.

## 217 3. Results and discussion

## 218 3.1. Structural analysis of sugars. FTIR and Raman spectra

219 As vibrational spectroscopic techniques, infrared and Raman spectroscopies provide information on both the chemical and physical states of samples. Figs. 1A and B depict the FTIR and Raman spectra of GOS Cup Oligo H-70®, GOS Biotempo and lactulose after freeze drying. In contrast with the spectra of crystalline sugars, where the organized structure of the unit cells originates narrow and highly resolved bands

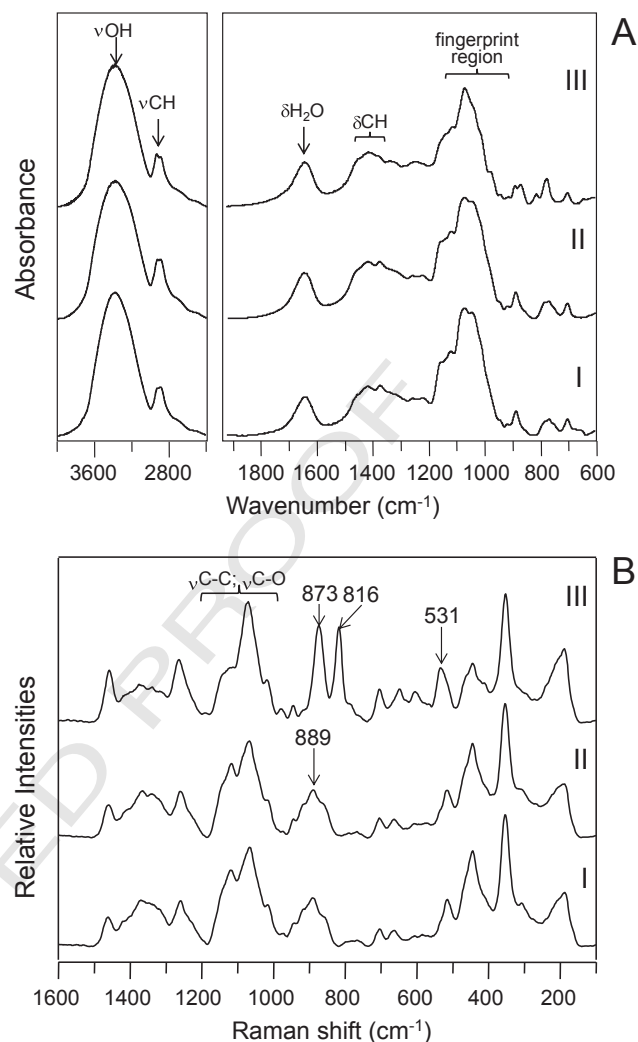


Fig. 1. (A) FTIR spectra of GOS Biotempo (I), GOS Cup Oligo H-70® (II) and lactulose (III). (B) Raman spectra of GOS Biotempo (I), GOS Cup Oligo H-70® (II) and lactulose (III).

(Ahlqvist & Taylor, 2002), the spectra depicted in Figs. 1A and B are typical of the amorphous phases. They are constituted by broad and poorly resolved bands resulting from unordered structures.

The three spectra look similar in most of the FTIR regions (Fig. 1A). The strong band at 3380 cm<sup>-1</sup> corresponds to the νOH vibrational mode, those at 2935 and 2886 cm<sup>-1</sup>, to νCH vibrational modes, the group of bands at ~1400 cm<sup>-1</sup>, to the δCH (Fig. 1A) (Sekkal, Dincq, Legrand, & Huvenne, 1995). The band at 1643 cm<sup>-1</sup> corresponds to water molecules embedded in the amorphous sugar matrix. The main differences in the FTIR spectra were observed in the so-called "fingerprint region" of sugars (1200–900 cm<sup>-1</sup>) (Sekkal et al., 1995). This region is rich in bands that collectively provide a pattern of bands characteristic of the compound. The main vibrational modes absorbing in this region are related with the C-O-C glycosidic linkage, the δCOH and the νC-C. The narrower band of lactulose in this region can be explained considering the simpler structural nature of this sugar. It is a disaccharide with only one glycosidic linkage. The FTIR spectra of other disaccharides (maltose and cellobiose) also depict narrower fingerprint bands than those of higher polymerized structures (Sekkal et al., 1995).

Raman spectroscopy also provides rich information about sugars. However, it has been scarcely used to assess the structure of amorphous sugars (Connolly, Patapoff, Wang, Moore, & Kamerzell, 2010; Söderholm et al., 1999). Even when the absorption of the Raman vibrational modes does not differ significantly from that of the FTIR modes,

t1.1 Table 1

t1.2 Data set preparation and performance results of partial least squares analysis.

t1.3	Sample	
t1.4	No. total of spectra collected	68
t1.5	No. spectra used for calibration	34
t1.6	No. spectra used for validation	34
t1.7	No. spectra removed from the calibration (outliers)	0
t1.8	Validation method	independent
t1.9	Mathematical treatment	raw
t1.10	PLS factors for prediction	8

the Raman spectra showed more noticeable differences between lactulose and GOS (Fig. 1B).

The bands observed in the 1200- to 1000- $\text{cm}^{-1}$  region can be ascribed to the  $\nu\text{C-C}$  and  $\nu\text{C-O}$  vibrational modes (Söderholm et al., 1999). The main differences between lactulose and GOS were observed in the 900- to 800- $\text{cm}^{-1}$  region. This region takes part of the so-called “anomeric region.” In this regard, Söderholm et al. (1999) reported that amorphous  $\alpha$  and  $\beta$  glucose can be distinguished in this region. These bands were ascribed to skeletal modes involving the anomeric carbon (Söderholm et al., 1999). This also explains that these bands occur at different wave numbers for lactulose (816 and 873  $\text{cm}^{-1}$ ) and GOS (a broader band centered at 889  $\text{cm}^{-1}$ ). The bands occurred in the 550- to 300- $\text{cm}^{-1}$  region can be ascribed to the  $\delta\text{C-C-C}$ ,  $\delta\text{C-C-O}$ ,  $\delta\text{C-O}$  and  $\tau\text{C-C}$  vibrational modes. Mathlouthi, Luu, Meffroy-Biget, and Luu (1980) reported that the  $\nu\text{C-C-O}$  vibrational mode of fructose occurs at 531  $\text{cm}^{-1}$ . Therefore, the band at 531  $\text{cm}^{-1}$  observed in the Raman spectrum of lactulose, a disaccharide composed by fructose and galactose, can be ascribed to this vibrational mode (Fig. 1BIII).

In summary, small changes were observed in the positions, widths and intensities of the bands in the spectra of the three sugars. The greater differences observed in lactulose can be explained considering both its structure of disaccharide and the occurrence of a fructose furanose ring instead of the pyranose rings of both GOS.

### 3.2. Effect of RH on the NIR spectra

The sensitivity of infrared spectroscopy to water molecules supports the use of IR-based methods to assess amorphous states of sugars. As the near-infrared is a cleaner region than the mid infrared, and NIR features originate from combinations and overtones of fundamental vibrations that may be involved in hydrogen bonds (OH, NH, CH and SH), this technique was selected to get an insight on the physical properties of GOS and lactulose.

Fig. 2 depicts the spectra of GOS Cup Oligo H70, GOS Biotempo and lactulose at different RH. The main changes observed in the spectra were the following: (a) a clear decrease of the shoulder at  $\sim 1571$  nm and (b) a decrease in the intensity of the band at 1204 nm. PCA performed on the three sugars equilibrated at different RH is shown in Fig. 3. In all the three sugars, the groups observed explained more than 95% of the variance and were related with the RH of the samples. According to the loading plots in PC1 (Fig. S1), the main differences related with RH were observed in the 1380- to 1500-nm region. This region was recently related with the amorphous or rubbery state of samples (Santos et al., 2014). The capacity of infrared spectroscopy to discriminate both states is directly related with the type of hydrogen bondings. These hydrogen bondings are predominantly weaker in the amorphous state (established between sugar and water) than those in the rubbery state (predominantly established among water molecules). This explains the occurrence of the first overtone of the symmetric valence vibration ( $2\nu\text{OH}$ ) at lower wave numbers in the rubbery state (Gergely & Salgo, 2003; Oldenhof, Wolkers, Fonseca, Passot, & Marin, 2005; Wolkers, Oliver, Tablin, & Crowe, 2004).

Considering the relevance of amorphous and rubbery states in the storage of food and food-related products and the sensitivity of NIR to determine them, an effort was made to calibrate an NIR-based PLS model to assess the correctness of storage conditions. As low molecular mobilities and low temperatures of storage are required for an adequate long-term preservation, the calibration of a PLS model on the basis of T-Tg (T: 4 °C) instead of on the bases of Tg becomes a more adequate approach. This way, the correctness of storage conditions can be evaluated directly from the spectra regardless the sugar under analysis (Schoonman et al., 2002; Tromp et al., 1997; Tymczyszyn et al., 2012).

The values of Tg, necessary to calculate T-Tg and construct the model, were determined by DSC and are shown in Table 2. The vitrification capacity of the three sugars investigated follows the order GOS Biotempo > GOS Cup Oligo H-70® > lactulose (Santos et al., 2014).

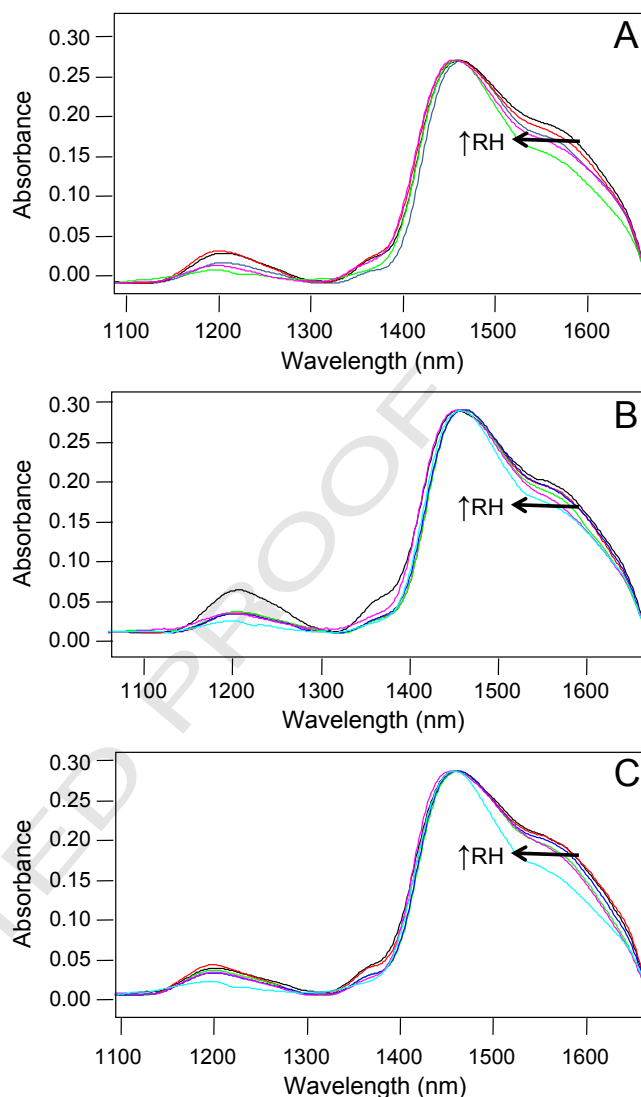
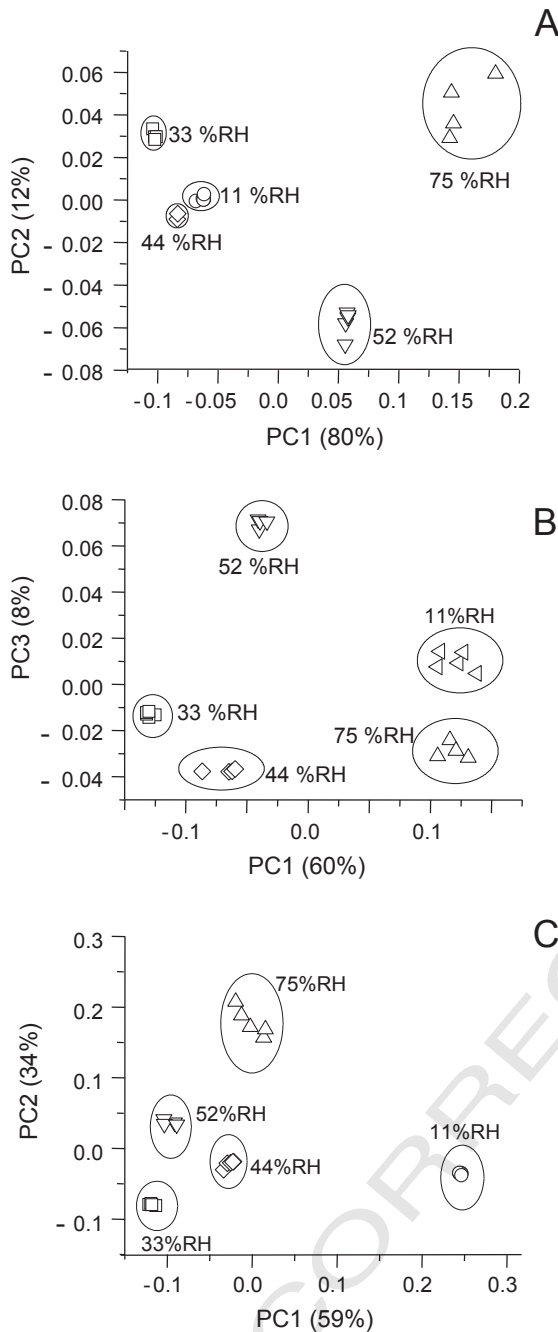


Fig. 2. NIR spectra of freeze-dried sugars equilibrated at different RH. (A) GOS Cup Oligo H-70®, (B) GOS Biotempo, (C) lactulose. Arrows indicate increase of RH.

GOS Biotempo are composed of oligosaccharides with degree of polymerization higher than 3 (Torres et al., 2011), GOS Cup Oligo-H70®, and mono- and disaccharides (Tymczyszyn et al., 2011). It was reported that higher contents of tri and tetrasaccharides in mixtures of oligosaccharides are related with higher Tg (Higl et al., 2007; Miao et al., 2008). Lactulose is mainly known as prebiotic, and its physical chemical properties have never been addressed. The Tgs depicted in Table 2 explain the protective effect of this sugar against bacteria dehydration at low RH (Santos et al., 2014).

### 3.3. PLS model

The PLS model was calibrated using 34 spectra (correlation = 0.994,  $R^2 = 0.989$ , RMSEC = 3.131 °C, bias =  $9.59 \times 10^{-5}$ ) (Tables 1 and 3). The model was validated with an independent set of data composed of 34 spectra collected in the same RH conditions as those used in the calibration set (Table 3). Fig. 4 depicts the independent validation results for the prediction model developed to determine T-Tg. The root mean square error of prediction (RMSEP) is depicted in the Y-error bars. The use of spectra of the three sugars covering a broad range of T-Tg, both in calibration and validation, strengthens the predictive capacity of the model. The mean of predicted values nicely fits the T-Tg obtained from DSC (Table 2), thus supporting the use of the PLS model to



**Fig. 3.** PCA performed on the freeze-dried sugars equilibrated at different RH. (A) GOS Cup Oligo H-70®, (B) GOS Biotempo, (C) lactulose. The loading plots in PC1 are shown in Fig. S1.

**Table 2**

Vitreous transition temperatures of GOS Cup Oligo H-70®, GOS Biotempo and lactulose at different RH.

RH	GOS Cup Oligo H-70® <sup>a</sup> (°C)	GOS Biotempo <sup>b</sup> (°C)	Lactulose (this work) (°C)
11	49.71	105.71	41.95
22	36.77	84.76	33.1
33	36.32	69.08	7.395
44	13.92	51.43	1.715
52	6.36	37.62	-14.055
75	-24.55	n.d.	-47.545
80	-41.87	n.d.	n.d.

t2.1  
t2.2  
t2.3  
t2.4  
t2.5  
t2.6  
t2.7  
t2.8  
t2.9  
t2.10  
t2.11  
t2.12

<sup>a</sup> Values were taken from Tymczyszyn et al. (2012).  
<sup>b</sup> Values were taken from Torres et al. (2011).

**Table 3**

Statistical values of the calibration and validation sets for the PLS model.

Calibration set		Validation set	
Correlation	0.994	Correlation	0.966
$R^{2a}$	0.989	$R^{2a}$	0.934
RMSEC <sup>b</sup>	3.131 °C	RMSEP <sup>c</sup>	8.051 °C
SEC <sup>d</sup>	3.178 °C	SEP <sup>e</sup>	8.148 °C
Bias	$9.58 \times 10^{-5}$ °C	Bias	-0.618 °C

<sup>a</sup>  $R^2$ : coefficient of determination.  
<sup>b</sup> RMSEC: root mean square error of calibration.  
<sup>c</sup> RMSEP: root mean square error of prediction.  
<sup>d</sup> SEC: standard error of calibration.  
<sup>e</sup> SEP: standard error of prediction.

investigate unknown samples. The complete data set of the performance calibration and validation processes are shown in Table 3.

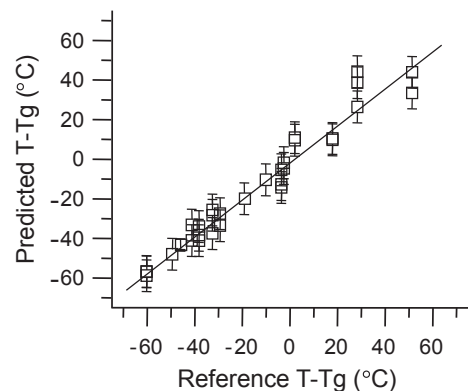
The amorphous states are very sensitive to changes in temperature and moisture content. As mentioned, T<sub>g</sub> is specific for each sugar and also depends on experimental variables (Roos & Karel, 1991). Above T<sub>g</sub>, the molecular mobility is greatly increased and numerous amorphous compounds crystallize. For that reason, crystallization may take place if either the temperature or the moisture content is increased (Roos & Karel, 1991). This underlines the usefulness of the PLS model developed. As the NIR spectra could be correlated with T-T<sub>g</sub>, the model can predict not only the behavior of different sugars but also the behavior of sugars stored at different temperatures or RH.

**4. Conclusions**

This work provided vibrational spectroscopic information about two types of GOS and lactulose in the amorphous states. NIR spectroscopy allowed the discrimination of samples equilibrated at different RH (PCA).

As a low molecular mobility is crucial to avoid sugar crystallization during storage, the determination of T-T<sub>g</sub>, a parameter directly related with water mobility and independent on the sugar considered, is very useful to predict the storage behavior of food components. The sensitivity of NIR to T-T<sub>g</sub> is of great importance to determine the shelf life of food and food-related products. Therefore, the application of the PLS model to predict T-T<sub>g</sub> will contribute to save time in determining the correct storage of food and food-related products, directly by registering NIR spectra.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.foodres.2014.07.040>.



**Fig. 4.** Predicted vs reference values of T-T<sub>g</sub>. T is the storage temperature (4 °C). The values of T<sub>g</sub> depicted in Table 2 (determined by DSC) were used to determine the reference T-T<sub>g</sub>.



## Author's contributions

M.S. and E.T. did the experimental work. M.S. and C.A.-A. did the multivariate analysis, A.G.-Z. coordinated the work (analysis of results, discussion and writing of the manuscript).

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## References

- Ahlqvist, M. U. A., & Taylor, L. S. (2002). Water diffusion in hydrated crystalline and amorphous sugars monitored using H/D exchange. *Journal of Pharmaceutical Sciences*, 91, 690–698.
- Aider, M., & de Halleux, D. (2007). Isomerization of lactose and lactulose production: Review. *Trends in Food Science and Technology*, 18, 356–364.
- Alander, J. T., Bochko, V., Martinkauppi, B., Saranwong, S., & Mantere, T. (2013). A review of optical nondestructive visual and near-infrared methods for food quality and safety. *International Journal of Spectroscopy* (Article ID 341402, 36 pp.).
- Barreteau, H., Delattre, C., & Michaud, P. (2006). Production of oligosaccharides as promising new food additive generation. *Food Technology and Biotechnology*, 44, 323–333.
- Bonanno, A. S., Olinger, J. M., & Griffiths, P. R. (1992). In K. I. Hildrum (Ed.), *Near Infra-Red Spectroscopy* (pp. 19–28). Chichester, UK: Horwood.
- Buckton, G., Yonemochi, E., Hammond, J., & Moffat, A. (1998). The use of near infra-red spectroscopy to detect changes in the form of amorphous and crystalline lactose. *International Journal of Pharmaceutics*, 168, 231–241.
- Buckton, G., Yonemochi, E., Yoon, W. L., & Moffat, A. C. (1999). Water sorption and near IR spectroscopy to study the differences between microcrystalline cellulose and silicified microcrystalline cellulose before and after wet granulation. *International Journal of Pharmaceutics*, 181, 41–47.
- Campbell, G., Campbell, N., Campbell, S., Fontana, A., & Ozanich, R. (2006). Water activity determination using near-infrared spectroscopy. US Pat. No. 20060043300 A1.
- Casci, T., & Rastall, R. A. (2006). Manufacture of prebiotic oligosaccharides. In G. R. Gibson, & R. A. Rastall (Eds.), *Prebiotics: Development and applications* (pp. 29–55). John Wiley & Sons: New York Inc.
- Connolly, B., Patapoff, T. W., Wang, Y. J., Moore, J. M., & Kamerzell, T. J. (2010). Vibrational spectroscopy and chemometrics to characterize and quantitate trehalose crystallization. *Analytical Biochemistry*, 399, 48–57.
- Czarnik-Matusewicz, B., & Pilorz, S. (2006). Study of the temperature-dependent near-infrared spectra of water by two-dimensional correlation spectroscopy and principal components analysis. *Vibrational Spectroscopy*, 40, 235–245.
- Czarnik-Matusewicz, B., Pilorz, S., & Hawranek, J. P. (2005). Temperature-dependent water structural transitions examined by near-IR and mid-IR spectra analyzed by multivariate curve resolution and two-dimensional correlation spectroscopy. *Analitica Chimica Acta*, 544, 15–25.
- Delwiche, S. R., Pitt, R. E., & Norris, K. H. (1992). Sensitivity of near infrared absorption to moisture content versus water activity in starch and cellulose. *Cereal Chemistry*, 69, 107–109.
- Esbensen, K. H. (2005). *Multivariate data analysis—In practice* (5th ed.). Esbjerg, Denmark: CAMO Process AS.
- Franks, F. (1999). Thermomechanical properties of amorphous saccharides: their role in enhancing pharmaceutical product stability. *Biotechnology and Genetic Engineering Reviews*, 16, 281–292.
- Gergely, S., & Salgo, A. (2003). Changes in moisture content during wheat maturation—What is measured by near infrared spectroscopy? *Journal of Near Infrared Spectroscopy*, 11, 17–26.
- Giangiaco, R. (2006). Study of water–sugar interactions at increasing sugar concentration by NIR spectroscopy. *Food Chemistry*, 96, 371–379.
- Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition*, 125, 1401–1412.

- Golowczyc, M., Vera, C., Santos, M., Guerrero, C., Carasi, P., Illanes, A., et al. (2013). Use of whey permeate containing *in situ* synthesized galacto-oligosaccharides for the growth and preservation of *Lactobacillus plantarum*. *Journal of Dairy Research*, 80, 374–381.
- Grohgan, H., Fonteyne, M., Skibsted, E., Falck, T., Palmqvist, B., & Rantanen, J. (2010). Classification of lyophilised mixtures using multivariate analysis of NIR spectra. *European Journal of Pharmaceutics and Biopharmaceutics*, 74, 406–412.
- Guerrero, C., Vera, C., Plou, F., & Illanes, A. (2011). Influence of reaction conditions on the selectivity of the synthesis of lactulose with microbial  $\beta$ -galactosidases. *Journal of Molecular Catalysis B: Enzymatic*, 72, 206–212.
- Higl, B., Kurtmann, L., Carlsen, C. U., Ratjen, J., Forst, P., Skibsted, L. H., et al. (2007). Impact of water activity, temperature, and physical state on the storage stability of *Lactobacillus paracasei* ssp. *paracasei* freeze-dried in a lactose matrix. *Biotechnology Progress*, 23, 794–800.
- Izutsu, K., Hiyama, Y., Yomota, C., & Kawanishi, T. (2009). Near-infrared analysis of hydrogen-bonding in glass- and rubber-state amorphous saccharide solids. *AAPS PharmSciTech*, 10, 524–529.
- Kim, Y. S., & Oh, D. K. (2012). Lactulose production from lactose as a single substrate by a thermotable cellobiose 2-epimerase from *Caldicellulosiruptor saccharolyticus*. *Bioresource Technology*, 104, 668–672.
- Le Meste, M., Champion, D., Boudaut, G., Blond, G., & Simatos, D. (2002). Glass transition and food technology: a critical appraisal. *Journal of Food Science*, 67, 2444–2458.
- Marconi, E., Messia, M. C., Amine, A., Moscone, D., Vernazza, F., Stocchi, F., et al. (2003). Heat-treated milk differentiation by a sensitive lactulose assay. *Food Chemistry*, 84, 447–450.
- Martens, H., & Næs, T. (1989). Methods for calibration. In H. Martens, & T. Næs (Eds.), *Multivariate calibration* (pp. 97). John Wiley & Sons: New York Inc.
- Mathlouthi, M., Luu, C., Meffroy-Biget, A. M., & Luu, D. V. (1980). Laser-Raman study of solute-solvent interactions in aqueous solutions of D-fructose, D-glucose and sucrose. *Carbohydrate Research*, 81, 213–233.
- Miao, S., Mills, S., Stanton, C., Fitzgerald, G. F., Roos, Y. H., & Ross, R. P. (2008). Effect of disaccharides on survival during storage of freeze dried probiotics. *Dairy Science and Technology*, 88, 19–30.
- Oldenhof, H., Wolkers, W. F., Fonseca, F., Passot, S., & Marin, M. (2005). Effect of sucrose and maltodextrin on the physical properties and survival of air-dried *Lactobacillus bulgaricus*: An *in situ* Fourier transform infrared spectroscopy study. *Biotechnology Progress*, 21, 885–892.
- Roos, Y. H. (2009). Solid and liquid states of lactose. In P. L. H. McSweeney, & P. F. Fox (Eds.), *Advanced Dairy Chemistry. Lactose, water, salts and minor constituents*, Vol. 3. (pp. 17–33). New York: Springer Science & Business Media.
- Roos, Y. H., & Karel, M. (1991). Plasticizing effect of water on thermal behavior and crystallization of amorphous food models. *Journal of Food Science*, 56, 38–43.
- Santos, M., Gerbino, E., Araujo-Andrade, C., Tymczynszyn, E., & Gómez-Zavaglia, A. (2014). Stability of freeze-dried *Lactobacillus delbrueckii* subsp. *bulgaricus* in the presence of galacto-oligosaccharides and lactulose as determined by near infrared spectroscopy. *Food Research International*, 59, 53–60.
- Schoonman, A., Ubink, J., Bisperink, C., Le Meste, M., & Karel, M. (2002). Solubility and diffusion of nitrogen in maltodextrin/protein tablets. *Biotechnology Progress*, 18, 139–154.
- Sekkal, M., Dincq, V., Legrand, P., & Huvenne, J. P. (1995). Investigation of the glycosidic linkages in several oligosaccharides using FT-IR and FT Raman spectroscopies. *Journal of Molecular Structure*, 349, 349–352.
- Small, G. W. (2006). Chemometrics and near-infrared spectroscopy: Avoiding the pitfalls. *Trends in Analytical Chemistry*, 25, 1057–1066.
- Söderholm, S., Roos, Y. H., Meinander, N., & Hotokka, M. (1999). Raman spectra of fructose and glucose in the amorphous and crystalline states. *Journal of Raman Spectroscopy*, 30, 1009–1018.
- Torres, D. P. M., Bastos, M., Goncalves, M. P. F., Teixeira, J. A., & Rodrigues, L. R. (2011). Water sorption and plasticization of an amorphous galacto-oligosaccharide mixture. *Carbohydrate Polymers*, 83, 831–835.
- Tromp, R. H., Parker, R., & Ring, S. G. (1997). Water diffusion in glasses of carbohydrates. *Carbohydrate Research*, 303, 199–205.
- Tymczynszyn, E. E., Gerbino, E., Illanes, A., & Gómez-Zavaglia, A. (2011). Galacto-oligosaccharides as protective molecules in the preservation of *Lactobacillus delbrueckii* subsp. *bulgaricus*. *Cryobiology*, 62, 123–129.
- Tymczynszyn, E. E., Sosa, N., Gerbino, E., Hugo, A., Gómez-Zavaglia, A., & Schebor, C. (2012). Effect of physical properties on the stability of *Lactobacillus bulgaricus* in a freeze-dried galacto-oligosaccharides matrix. *International Journal of Food Microbiology*, 155, 217–221.
- Vera, C., Guerrero, C., Illanes, A., & Conejeros, R. (2011). A pseudo steady-state model for galacto-oligosaccharides synthesis with  $\beta$ -galactosidase from *Aspergillus oryzae*. *Biotechnology and Bioengineering*, 108, 2270–2279.
- Wolkers, W. F., Oliver, A. E., Tablin, F., & Crowe, J. H. (2004). A Fourier-transform infrared spectroscopy study of sugar glasses. *Carbohydrate Research*, 339, 1077–1085.
- Zokaee, F., Kaghazchi, T., Zare, A., & Soleimani, M. (2002). Isomerization of lactose to lactulose-study and comparison of three catalytic systems. *Process Biochemistry*, 37, 629–635.