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Infrared spectroscopy as an alternative methodology to evaluate the effect of structural features on the physical-chemical properties of inulins

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ABSTRACT

Two types of inulins of different composition were investigated in the glassy and in the crystalline states, at relative humidities within 11 and 97%. The melting and glass transition temperatures (Tm, Tg), and their crystallinity indexes (CI) were determined by modulated differential-scanning calorimetry (MDSC) and wide-angle X-ray scattering (WAXS), respectively. In parallel assays, Fourier transform-infrared spectroscopy (FTIR) coupled to principal component analysis (PCA) enabled a physical-chemical and structural characterization of samples, explaining 90% of the total variance. Finally, partial least square (PLS) models were defined to determine Tg, Tm, and CI directly from the FTIR spectra, using the MDSC and WAXS results as reference methods. In all cases, the mean of predicted values fitted very well those of the reference methods ($R^2 > 0.961$), thus supporting the use of the PLS models to investigate unknown samples. The robustness of the models underlines the usefulness of FTIR to easily determine physical-chemical parameters, otherwise requiring complex preparation of samples and prolonged times of analysis.

1. Introduction

Inulin is a natural polysaccharide present in the leaves, fruits and/or roots of many plants (Jerusalem artichoke, chicory, garlic, asparagus, salsify, dandelion, onion, leek, banana, wheat, rye, barley) (Kaur & Gupta, 2002). As it belongs to the dietary fiber, its incorporation in the formulation of functional foods is very valuable (Sirbu & Arghire, 2017; Tighe-Neira et al., 2017).

From a chemical viewpoint, inulin belongs to the fructan family of carbohydrates and is composed of fructose units linked by β-(2→1) glycosidic bonds and a terminating D-glucosyl moiety (Romano, Schebor, Mobili, & Gomez-Zavaglia, 2016). The length of the fructose chains ranges from 2 to 60 monomeric units, and inulins containing maximally 10 fructose units are also known as oligofructose (Romano et al., 2016). Commercial inulins are mixtures of oligo and polysaccharides of different degrees of polymerization (DP).

From a physical perspective, inulin can exist in the glassy or in the crystalline phase. The glass transition temperature (Tg) is the range of temperatures at which amorphous materials pass from the amorphous to the rubbery state (Santos, Araujo-Andrade, Tymczyszyn, & Gomez-Zavaglia, 2014). Glass transition occurs at a given temperature which is dependent on the water content and also a key parameter to set-up storage conditions (Romano et al., 2016; Santos et al., 2014). When the storage temperature is above Tg, amorphous solid matrices are more susceptible to caking (tendency to form lumps or masses rather than being smoothly flowable), leading to undesirable effects on storage (Aguilera, del Valle, & Karel, 1995). In this context, the molecular weight of inulins has a strong influence on the stability of the amorphous states. Although inulins of high average DP are generally more stable (Ronkart et al., 2006; Zimeri & Kokini, 2002) their solubility is much lower (Franck, 2002).

Crystalline states of inulin can be obtained by cooling aqueous solutions with concentrations within 10 and 50% w/v (Glibowski, Pikus, Jurek, & Kotowoda, 2014). The crystallinity of inulins is an important physical-chemical parameter because it determines their stability during storage and their technological properties (Ronkart et al., 2009;

Abbreviations: DP, degree of polymerization; Tg, glass transition temperature; CI, crystallinity index; DSC, differential scanning calorimetry; FTIR, Fourier transform infrared spectroscopy; Tm, melting temperature; MDSC, modulated differential scanning calorimetry; PCA, principal component analysis; PLS, partial least square analysis; HP, inulin ≥23 (containing oligosaccharides with DP ≥23); IQ, inulin 3-60 (containing oligosaccharides with DP from 3 to 60); HP-a, amorphous HP inulin; HP-c, crystalline HP inulin; IQ-a, amorphous IQ inulin; IQ-c, crystalline IQ inulin; RH, relative humidity; HPAEC, high performance anion exchange chromatography; A, area under the band at $10^\circ < 2\theta < 15^\circ$; RMSEC, root mean square error of calibration; RMSEP, root mean square error of prediction; ANOVA, analysis of variance; WAXS, wide-angle X-ray scattering

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Ronkart, Deroanne, et al., 2010; Ronkart, Paquot, et al., 2010). In this sense, the hydrogen bonds established between hydrated (or hemi-hydrated) crystalline forms of inulins and water molecules act as stabilizing forces (André et al., 1996).

The low dissolution of dehydrated inulins, as well as their hygroscopicity, are clearly undesirable effects when intended to be incorporated in the formulation of functional foods (Aguilera et al., 1995). The solubility of dehydrated inulins is directly related to their DP and their physical state (Bot, Erle, Vreeker, & Agterof, 2004). The higher the DP the lower the solubility (Franck, 2002; Mensink, Frijlink, Voort Maarschalk, & Hinrichs, 2015). Furthermore, at the same temperature, amorphous inulins are less dispersible than crystalline ones (Bot et al., 2004).

Inulin powders are usually analyzed by determining their wide-angle X-ray scattering (WAXS) and their thermal transitions by differential scanning calorimetry (DSC) (Barclay et al., 2016). Although the reliability of such methods is doubtless, they require expensive equipment, determinations are time-consuming and involve specialized operators. Other techniques such as Fourier transform infrared spectroscopy (FTIR), could provide physical and chemical information, including structural aspects, in an easy, quick and reliable way. As bands in the FTIR originate in changes in the dipole moments of the studied compounds, and hydrogen bonds are polar interactions, this technique is particularly useful for studying processes involving the formation and breaking of such intermolecular interactions. In this sense, it must be underlined that the glassy state is characterized by weak hydrogen bonds between the carbohydrates hydroxyl groups. Contrarily, the rubbery state is dominated by stronger hydrogen bonds, established between the carbohydrate hydroxyl groups and water (Wolkers, Oliver, Tablin, & Crowe, 2004). These structural features are useful for their physical characterization and can be determined by FTIR (Santos et al., 2014). The significant disorder of the glassy phases leads to broad spectral bands, typical from this phase. In contrast, the characteristic organization of the crystalline phases originates much narrower bands (Gomez-Zavaglia & Fausto, 2003). Considering that FTIR does not require exogenous chemical reagents, samples need almost no preparation and analytical testing does not generate hazardous waste, this technique appears as an adequate and sensitive approach for the characterization of amorphous and crystalline phases of inulin.

Taking into account that the physical states of inulins (crystalline, glass, rubbery) directly determine their stability during storage, a quick and reliable analysis of these properties would be useful for an adequate selection of inulins, aiming at technological processing. For this reason, a deep investigation of two types of inulins [one of them composed of high DP carbohydrates (HP-inulin), and the other one, of low DP carbohydrates (IQ-inulin)] was carried out in this work. To this aim, amorphous and crystalline inulins were equilibrated at relative humidities (RH) within 11 and 97%. Their melting and glass transition temperatures (Tm, Tg), as well as their crystallinity index (CI), were determined by modulated differential-scanning calorimetry (MDSC) and WAXS, respectively. In parallel, a physical-chemical and structural analysis of all samples was carried out by FTIR. Principal component analysis (PCA) carried out on the FTIR spectra enabled the grouping of samples on the basis of structural and physical aspects. Partial least square (PLS) models were defined to determine Tg, T and CI directly from the FTIR spectra, using the MDSC and WAXS results as references.

2. Materials and methods

2.1. Materials

Inulin 3–60 (IQ inulin) (Frutafit IQ®, Sensus, Roosendaal, The Netherlands), containing oligosaccharides with DP from 3 to 60. Inulin ≥23 (HP inulin) (Raftilose HP, Orafti Beneo, Germany), containing oligosaccharides with DP ≥ 23. LiCl, CH_3CO_2K, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $NaNO_2$, NaCl, KCl, CH_3CO_2Na and K_2SO_4 were obtained from Anedra, (Buenos Aires, Argentina). 1-kestose, nystose and 1^F-fructofuranosylnystose were obtained from Wako Chemicals (Richmond, VA, USA), and sucrose, glucose and fructose, from Sigma Chemical (St. Louis, MO, USA).

2.2. Methods

2.2.1. Composition of IQ and HP inulins

The composition of IQ and HP inulins was determined by high performance anion exchange chromatography (HPAEC), using a Dionex ICS-3000 HPLC system equipped with a pulse amperometric detector (Ronkart et al., 2006). A CarboPac PA-100 ion-exchange analytical column (4 × 250 mm) (Dionex™, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a guard column CarboPac PA-100 (4 × 50 mm) (Dionex™, Thermo Fisher Scientific, Waltham, MA, USA) was eluted with a linear gradient from 0 to 500 mM CH_3CO_2Na in 100 mM NaOH at a flow rate of 0.9 mL/min at 25 °C during 60 min. External standards of fructose, glucose, sucrose, 1-kestose (DP3), nystose (DP4) and 1^F-fructofuranosylnystose (DP5) were used to determine their retention times and check the linear range of the measurements.

2.2.2. Obtaining of amorphous and semi-crystalline inulins

20% w/v suspensions of both IQ and HP inulins were heated at 95 °C under shaking (100 rpm, 15 min) to eliminate the initial crystalline structures and allow a complete dissolution. Scheme 1 shows the experimental protocol.

2.2.2.1. Amorphous inulin samples. The 20% w/v inulin solutions were spray-dried at a pilot scale on an Armfield FT80 spray-dryer (DY, United Kingdom) at a constant air inlet temperature of 160 °C and an outlet temperature of 65 ± 5 °C, to avoid spontaneous crystallization. The resulting powder was named “amorphous inulin” (HP-a and IQ-a), as it appears in the following sections.

2.2.2.2. Semi-crystalline samples. In parallel assays, the 20% w/v solutions of inulin were stored at 4 °C for 72 h under shaking (100 rpm). Crystallization was monitored by registering the absorbance at 700 nm in a UV–vis spectrophotometer (Shimadzu, Kyoto, Japan), according to Cooper et al. (2015). The obtained crystals were centrifuged at 10000 ×g for 30 min. The supernatants were named “non-crystalline fractions” and were not further used in the present work. The pellets were resuspended in milli Q-water to the original volume and then, spray-dried at a constant air inlet temperature of 160 °C and an outlet temperature of 65 °C. The resulting powder was named “crystalline inulin” (HP-c and IQ-c), as it appears in the following sections, even when in rigor it consists in semi-crystalline samples presenting different degrees of crystallinity.

2.2.3. Equilibration procedure

The amorphous and crystalline spray-dried samples, obtained as explained in Section 2.2.2, were equilibrated for 30 days at 20°C in atmospheres of the following saturated salts: LiCl, CH_3CO_2K, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $NaNO_2$, NaCl, KCl and K_2SO_4, giving relative humidities (RH) of 11, 22, 33, 40, 54, 65, 75, 84 and 97%, respectively (Scheme 1), according to Romano et al., 2016 and Tymczyszyn et al., 2012.

2.2.4. Determining of Tg and Tm

Tg and Tm of amorphous and crystalline samples, equilibrated at RH within 11–97% (Section 2.2.3), were determined by DSC using a Q100 calorimeter (TA Instruments, New Castle, DE, USA), calibrated for the baseline on an empty oven and for temperature and enthalpy with indium (T_{onset}: 156.55 °C; ΔH: 28.82J/g) (Romano et al., 2016). Hermetically sealed 40 μL medium pressure pans containing approximately 5 mg of dehydrated inulins were used (an empty pan served as reference). Thermograms were carried out from −100 °C to 220 °C, at