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Review

Inulin, a flexible oligosaccharide I: Review of its physicochemical characteristics

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ABSTRACT

Inulin, a fructan-type polysaccharide, consists of (2→1) linked β-D-fructosyl residues (n = 2–60), usually with an (1↔2) α-D-glucose end group. The applications of inulin and its hydrolyzed form oligofructose (n = 2–10) are diverse. It is widely used in food industry to modify texture, replace fat or as low-calorie sweetener. Additionally, it has several applications in other fields like the pharmaceutical arena. Most notably it is used as a diagnostic agent for kidney function and as a protein stabilizer. This work reviews the physicochemical characteristics of inulin that make it such a versatile substance. Topics that are addressed include morphology (crystal morphology, crystal structure, structure in solution); solubility; rheology (viscosity, hydrodynamic shape, gelling); thermal characteristics and physical stability (glass transition temperature, vapor sorption, melting temperature) and chemical stability. When using inulin, the degree of polymerization and processing history should be taken into account, as they have a large impact on physicochemical behavior of inulin.

Contents

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1. Introduction

Inulin was discovered over two centuries ago by Rose (Fluckiger & Hanbury, 1879) and since then its presence in many plants became apparent (Livingston, Hincha, & Heyer, 2007). Some examples of plants containing large quantities of inulin are Jerusalem artichoke, chicory root, garlic, asparagus root, salisfy and dandelion root (Kaur & Gupta, 2002). More commonly consumed vegetables and fruits containing inulin are onion, leek, garlic, banana, wheat, rye and barley. Daily intakes have been estimated to range from 1 to 10 g per day in the Western diet (Coussement, 1999; Van Loo et al., 1995). The average American diet contains between 1.3 and 3.5 g of inulin per day, with an average of 2.6 g (Coussement, 1999). The European consumption of inulin appears to be substantially higher at 3–11 g per day, which is below reported tolerances of at least 10–20 g per day (Bonnema, Kolberg, Thomas, & Slavin, 2010; Carabin & Flamm, 1999). Inulin has also been used safely in infant nutrition (Closa-Monasterolo et al., 2013). This has led to the American Food and Drug Administration to issuing a Generally Recognized As Safe notification for inulin in 1992 (Kruger, 2002). Inulin is also used pharmaceutically, most notably as a diagnostic agent for the determination of kidney function (Orlando, Floreani, Padrini, & Palatini, 1998; The editors of Encyclopaedia Brittanica, 2015).

Over the past decades, a lot of research has been done showing that inulin is a versatile substance with numerous promising applications. Several reviews have been published on inulin, its characteristics and functionality in food (Boeckner, Schnepf, & Tungland, 2001; Kelly, 2008, 2009; Seifert & Watzl, 2007) and pharma (Imran, Gillis, Kok, Harding, & Adams, 2012). This review aims to provide an overview of the relevant physicochemical properties of inulin, which make it such a useful excipient in food and pharma.

1.1. Chemical structure

Inulin, depending on its chain length, is classified as either an oligo- or polysaccharide and it belongs to the fructan carbohydrate subgroup. It is composed of β-D-fructosyl subgroups linked together by (2→1) glycosidic bonds and the molecule usually ends with a (1↔2) bonded α-D-glucosyl group (Kelly, 2008; Ronkart, Blecker, et al., 2007). The length of these fructose chains varies and ranges from 2 to 60 monomers. Inulin containing maximally 10 fructose units is also referred to as oligofructose (Flamm, Glinsmann, Kritchevsky, Prosky, & Roberfroid, 2001). In food, oligofructose is more commonly used a sweet-replacer and longer chain inulin is used mostly as a fat replacer and texture modifier (Kelly, 2008). Both inulin and oligofructose are used as dietary fiber and prebiotics in functional foods. Its longer chain length makes inulin more useful pharmaceutically than oligofructose.

Before processing, the degree of polymerization of inulin depends on the plant source, time of harvest, and the duration and conditions of post-harvest storage (Kruger, 2002; Ronkart, Paquot, et al., 2006; Saengthongpinit & Sajjaanantakul, 2005). Processing itself also has a great influence on degree of polymerization of the obtained product as will be discussed in Section 1.2. Table 1 provides an overview of the structure and size of some carbohydrates frequently used in the pharmaceutical arena. The structures of a selection of those carbohydrates are shown in Fig. 1.

Like many oligosaccharides, inulin is heterodisperse. High performance anion exchange chromatography (HPAEC) with pulsed amperometric detection can be used to determine the number average degree of polymerization (DPn) and the weight average DP (DPw) of inulin (Timmermans, van Leeuwen, Tournois, Wit, & Vliegenthart, 1994). Several chromatographic methods have been described, but HPAEC has a superior sensitivity and resolution (Barclay, Ginic-Markovic, Cooper, & Petrovsky, 2010; Timmermans et al., 1994). The ratio between DPw and DPn is a measure of the molecular weight distribution (polydispersity) of a sample (Stepto, 2009). The DP and polydispersity of an oligo- or polysaccharide influence the physicochemical properties to a large extent (Blecker et al., 2003; Kim, Faqih, & Wang, 2001).

Inulin is a unique oligo- or polysaccharide because its backbone does not incorporate any sugar ring, which can be seen in Fig. 1. The backbone is in essence polyethylene oxide (Barclay et al., 2010). This translates into a greater freedom to move and thus more flexibility of the molecule. Furthermore, inulin is built up mostly from furanose groups, which are more flexible than pyranose rings (French, 1988; Livingston et al., 2007).

1.2. Isolation and production

Inulin is predominately isolated from chicory root. The isolation process basically consists of three steps: (1) extraction of water-soluble components, including inulins, from chicory root (2) purification to remove impurities and optionally low DP inulins and (3) finally spray drying. Sometimes the extracted product is partially hydrolyzed to reduce the DP of the final product (Franck, 2007). Here isolation and purification are only discussed briefly, for further reading on this topic the reader is directed to the review of Apolinário et al. (2014).

Inulin extracted from chicory root contains up to 10% of sugars (mono-, di- and small oligosaccharides) (Coussement, 1999). Typically, extraction is done by boiling the cleaned and cut or ground up roots in water. Process conditions such as pH of the water, water–root ratio, boiling time, etc., may vary (Panchev, Delchev, Kovacheva, & Slavov, 2011; Ronkart, Blecker, et al., 2007; Toneli, Mürr, Martinelli, Dal Fabbro, & Park, 2007). As will be described in Section 2.6, pH and boiling time could affect the DP of the produced inulin. After extraction, the obtained mixture is condensed through evaporation.

Purification of inulin is mostly done by making use of the solubility difference of the DP fractions present in extracts. Heating and cooling in combination with filtration, decantation and (ultra)centrifugation have been described to produce different molecular weight fractions of inulin (European Patent No. EP 120302881, 2001; Leite, Martinelli, Murr, & Jin, 2004; Toneli et al., 2007; Toneli, Park, Murr, & Martinelli, 2008; U.S. Patent No. 6,419,978, 2002; World Patent No. WO/2000/011967, 2000). Alternatively (organic) co-solvents, such as methanol, ethanol and acetone, can be used to selectively precipitate long chain (DPn 25–40) inulin (Moerman, Van Leeuwen, & Delcour, 2004). Inulin that has not been precipitated in these processes can be turned into a solid by (spray) drying. Optimization of the spray drying process, by varying inlet air, solution temperature and feed pump speed, based on microstructure of the produced inulin and rheological behavior of concentrated inulin solutions have been described (Toneli et al., 2008; Toneli, Park, Negreiros, & Murr, 2010).

Table 1
Some carbohydrates used frequently in food and pharma, their structure and size. Glc*p* = Glucopyranosyl, Fru*f* = Fructofurananosyl, Gal*p* = Galactopyranosyl (IUPAC-IUBMB Joint Commission on Biochemical Nomenclature, 1997).

Carbohydrate	Building blocks and linkages	Molecular weight (Da)	Backbone	Article cited
Glucose	α-D-Glc	1.8×10^2	–	National Center for Biotechnology Information (2015)
Trehalose	α-D-Glc*p*-(1↔1)-α-D-Glc*p*	3.4×10^2	Linear	National Center for Biotechnology Information (2015), Tarantino (2000)
Sucrose	α-D-Glc*p*-(1↔2)-β-D-Fru*f*	3.4×10^2	Linear	National Center for Biotechnology Information (2015)
Lactose	β-D-Gal*p*-(1→4)-D-Glc	3.4×10^2	Linear	National Center for Biotechnology Information (2015)
Maltodextrin	[4)-α-D-Glc*p*-(1→]$_n$	1.8×10^2 to 3.2×10^3	Linear	Council of Europe (2005)
Amylose (α-Glucan)	[4)-α-D-Glc*p*-(1→]$_n$	5×10^5 to 2×10^6	Linear	National Center for Biotechnology Information (2015), Potter and Hassid (1948), Suortti, Gorenstein, and Roger (1998)
Dextran (α-Glucan)	[6)-α-D-Glc*p*-(1→]$_n$ (Main) α-D-Glc*p*-(1→3)-α-D-Glc*p* (also (1→2) and (1→4) (Branches)	1.0×10^3 to ~10^7	Branched	Kim, Robyt, Lee, Lee, and Kim (2003), Naessens, Cerdobbel, Soetaert, and Vandamme (2005), National Center for Biotechnology Information (2015)
Cellulose (β-Glucan)	[4)-β-D-Glc*p*-(1→]$_n$	3×10^5 to 2×10^6	Linear	Klemm, Schmauder, and Heinze (2005)
Inulin (Fructan)	[1)-β-D-Fru*f*-(2→]$_n$ (Main) α-D-Glc*p*-(1↔2)-β-D-Fru*f* (End, usually)	5.0×10^2 to 1.3×10^4 [a]	Linear	Barclay et al. (2010), Kelly (2008), Ronkart, Blecker, et al. (2007), Vereyken, Chupin, et al. (2003)
Levan (Fructan)	[6)-β-D-Fru*f*-(2→]$_n$ (Main) β-D-Fru*f*-(2→1)-β-D-Fru*f* (Branches)	1×10^4 to 1×10^8	Branched	French and Waterhouse (1993), French (1988), Tanaka, Oi, and Yamamoto (1980), Vereyken, Chupin, et al. (2003)

[a] Bacterially produced inulin has been reported to be branched and have a significantly higher molecular weight than plant derived inulin, see also Table 2 (Wolff et al., 2000).

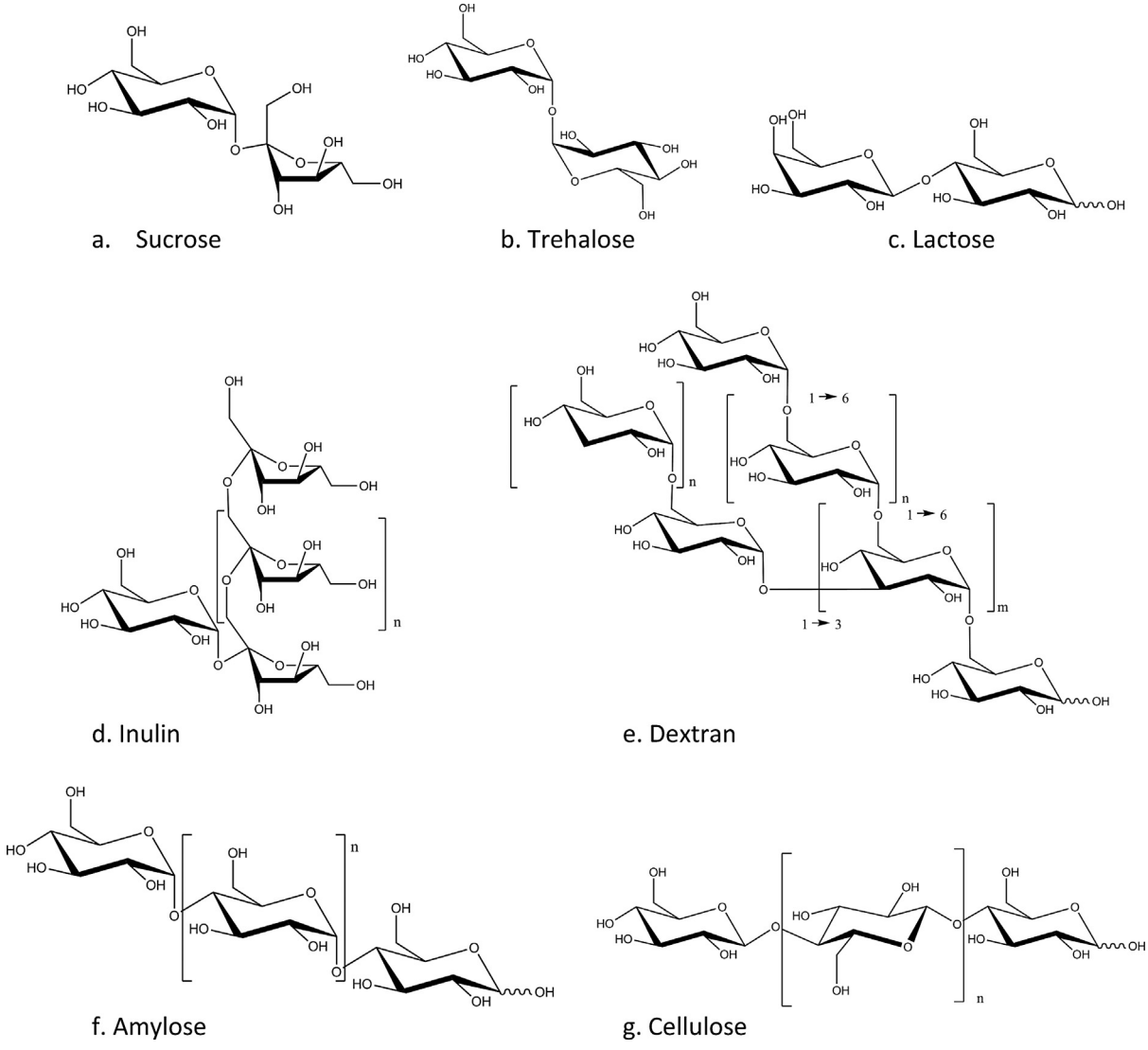

Fig. 1. Chemical structures from a selection of the carbohydrates listed in Table 1.

Ronkart, Deroanne, et al. (2007) investigated several aspects of the isolation and purification of inulin, with emphasis on the physical characteristics of the produced inulin. They investigated the influence of several parameters, such as feed and inlet temperature during spray-drying on the physicochemical characteristics of the produced inulin. It was found that at a feed temperature of 80 °C and higher, the produced inulin was completely amorphous. A high air inlet temperature (230 °C compared to 120–170 °C) also increased the amount of amorphous inulin produced. Next to that, they characterized oligofructose produced by hydrolysis of inulin from globe artichoke by endo-inulinase (Ronkart, Blecker, et al., 2007).

Apart from extraction from plants, inulin can also be produced enzymatically. Inulosucrase type fructosyltransferase can synthesize inulin from sucrose by catalyzing both transglycosylation and hydrolysis of sucrose (Ozimek, Kralj, van der Maarel, & Dijkhuizen, 2006). Several procedures to do so have been described, these mostly involve enzymes derived from bacteria. Enzymes from *Bacillus* species ^{217}C–^{11} have been used to produce inulin on a large scale (Wada, Sugatani, Terada, Ohguchi, & Miwa, 2005) and *Escherichia coli* and *Streptococcus mutans* derived fructosyltransferase can produce very high molecular weight inulins (Heyer et al., 1998). Both these studies reported remarkably low polydispersity (around 1.1) of the produced inulin. Inulin producing fructosyltransferases from several *Lactobacillus* strains have also been characterized (Anwar et al., 2010; Ozimek et al., 2006). Inulosucrase from *Leuconostoc citreum CW* 28 was shown to produce different molecular weight inulin when it was cell associated compared to when it was free in solution. The cell associated enzyme predominately produced inulin with a molecular weight between 1.35–1.60 × 10^6 Da and the free enzyme produced more inulin with a molecular weight between 2600 and 3400 Da (Ortiz-Soto, Olivares-Illana, & López-Munguía, 2004).

Isolation of two plant derived fructosyltransferases from *Helianthus tuberosus* and the production of inulin with those purified enzymes was described by Lüscher et al. (1996). The fungus *Aspergillusi oryzae* KB is also able to produce inulin type oligofructoses from sucrose, but additionally possesses another enzyme which simultaneously hydrolyzes sucrose. The first enzyme produces 1-kestose, nystose and fructosyl nystose, whereas the second one produces glucose and fructose (Kurakake et al., 2008). Oligofructoses can be produced by partial enzymatic hydrolysis of polyfructoses. Enzymes from *Aspergillus niger* can produce oligofructose from both hydrolysis of inulin (by inulinase) and synthesis from sucrose (by β-fructosyltransferase) and its inulinases provided higher yields than inulinases from *Kluyveromuces marxianus* (Silva et al., 2013). Beghin-Meiji, a commercial supplier of oligofructose, use β-fructo-furanosidase from *A. niger* to synthesize, rather than to hydrolyze, oligofructose from sucrose (Beghin-Meiji, 2015). For more information on microbial enzymatic production of oligofructoses either from synthesis from sucrose or from hydrolysis of inulin, the reader is directed to a recent review of Mutanda, Mokoena, Olaniran, Wilhelmi, and Whiteley (2014). To the best of our knowledge, high molecular weight inulin from synthetic source is not yet commercially available on a large scale, most likely because of the high production costs.

Finally, a completely different method of production is the genetic modification of a potato to make it produce inulin like globe artichoke. However the inulin yield is low (5%) and inulin production goes at the cost of starch production (Hellwege, Czapla, Jahnke, Willmitzer, & Heyer, 2000). Van Arkel et al. (2013) recently published a review on plants that were genetically modified to produce inulin. They named modified sugar beet, sugarcane and rice as potential candidates for production of inulin, with possibilities to control certain characteristics (e.g. chain length) of the produced inulin by selectively controlling the expression of specific synthesizing enzymes.

1.3. Uses

Inulin is widely applied in the food industry and it serves many purposes. It has been used as a (low calorie) sweetener, to form gels, to increase viscosity, to improve organoleptic properties, and as a non-digestible fiber. Mostly it is used as a sugar and fat replacer in dairy products and as a prebiotic (Meyer, Bayarri, Tárrega, & Costell, 2011). Examples of use in dairy are application in cheese, milk, yogurt and ice cream (Meyer et al., 2011). Some examples of use of inulin in non-dairy food are use in bread, biscuits, cereal and meat products (González-Herrera et al., 2015; Karimi, Azizi, Ghasemlou, & Vaziri, 2015; Kuntz, Fiates, & Teixeira, 2013; Rodriguez Furlán, Pérez Padilla, & Campderrós, 2015). Previous reports have already extensively reviewed the food applications of inulin (Barclay et al., 2010; Boeckner et al., 2001; Franck, 2007; Kelly, 2008, 2009; Kruger, 2002; Meyer et al., 2011; Tungland & Meyer, 2002), as well as its prebiotic effects (Kelly, 2008, 2009; Kolida, Tuohy, & Gibson, 2007; Roberfroid & Delzenne, 1998; Seifert & Watzl, 2007).

Applications of inulin as pharmaceutical excipient are even more diverse and range from stabilization of protein-based pharmaceuticals (Hinrichs, Prinsen, & Frijlink, 2001), through solid dispersions to increase dissolution rate (Visser et al., 2010), to targeted colon delivery (Imran et al., 2012). Moreover, as mentioned earlier, inulin itself is used as a diagnostic tool for measuring the kidney function (glomerular filtration rate) (Orlando et al., 1998; The editors of Encyclopaedia Brittanica, 2015). Inulin is injected intravenously, after which it is excreted renally. As inulin is not naturally present in the body and it is not metabolized in circulation, the amount of inulin secreted in the urine provides information on kidney function. Less widespread is the use of inulin for industrial and chemical purposes. Stevens, Meriggi, and Booten (2001) reviewed the derivatization of inulin and applications of these chemically modified inulins for a wide range of applications, from inhibiting calcium carbonate crystallization industrially to use in hair gel.

Section 2 will address the physicochemical characteristics of inulin. These characteristics are what make inulin such a versatile substance. For example, inulin is used in food as a texture modifier and fat replacer because of its DP-dependent gel forming and viscous behavior (see Section 2.4). The (2→1) glycosidic bonds of inulin make it indigestible to humans and it can therefore be used as a low-calorie sweetener, fat replacer and dietary fiber (Barclay et al., 2010). Colonic microorganisms such as lactobacilli, however, are capable of breaking down this bond, making inulin suitable for colonic targeting. The relatively high glass transition temperature of amorphous inulin (Section 2.5) in combination with its flexible backbone makes it a good stabilizer of proteins applied both pharmaceutically (Tonnis et al., 2015) and in food (Rodriguez Furlán, Lecot, Pérez Padilla, Campderrós, & Zaritzky, 2012). Lastly, specific crystalline morphologies (Section 2.2) make inulin suitable as an adjuvant for vaccines (Honda-Okubo, Saade, & Petrovsky, 2012).

2. Physicochemical characteristics

2.1. Chain length

As mentioned in the introduction the DP of inulin determines its physicochemical characteristics to a substantial extent. Table 2 provides an overview of the reported degrees of polymerization of different types of inulin to serve as a frame of reference. It is, however, to be noted that the degree of polymerization alone oversimplifies reality, as it does not take into account the distribution of the different fractions. Also, in many cases no distinction is made between the DPw and DPn (thus nor between the weight and number based molecular weights (Mw and Mn)), which are only

Table 2
Overview of size and origin of different inulins.

Manufacturer	Product name	Source	Size DP	Molecular weight	DPw/DPn	Article cited
Orafti	Raftilose P95	Chicory	DPn 4–5	*Mn* 624–679		Blecker et al. (2002), De Gennaro et al. (2000)
	Raftiline ST	Chicory	DPn 10–12	*Mn* 1250		De Gennaro et al. (2000), Schaller-Povolny et al. (2000)
	Raftiline HP	Chicory	DPn 21–26, DPw 31	*Mn* 2499		Ronkart, Paquot, et al. (2006), Schaller-Povolny et al. (2000), Vereyken, van Kuik, et al. (2003), Wada et al. (2005)
	RS	Chicory	DPn 14.2; DPw 19.4		1.13	Hinrichs et al. (2001)
Cosucra	Fibrulose F97	Chicory	DPn 5.5			Blecker et al. (2002)
	Fibruline Instant	Chicory	DPn 9			Blecker et al. (2002)
	Fibruline LCHT	Chicory	DPn 20–22, DPw 26.4		1.3	Blecker et al. (2003, 2002)
	Fibruline XL	Chicory	DPn 20–23, DPw 27–30			Ronkart, Paquot, et al. (2006), Ronkart, Deroanne, et al. (2007), Ronkart, Paquot, et al. (2010)
Imperial Sensus	SC 95	Chicory	DPn 5.5, DPw 6.0		1.09	Hinrichs et al. (2001)
	Frutafit CLR	Chicory	DPn 7–9			Gonzalez-Tomás et al. (2008)
	Frutafit	Chicory	DPn 9	*Mn* 832		Schaller-Povolny et al. (2000)
	Frutafit IQ	Chicory	DPn 8–12			Bouchard et al. (2008), Gonzalez-Tomás et al. (2008)
	Frutafit Tex!, EXL	Chicory	DPn ≥23, DPw 26.2		1.3	Gonzalez-Tomás et al. (2008), Hinrichs et al. (2001)
Sigma	Inulin	Chicory	DPn 25	*Mn* 4450, *Mw* 4620–6200		Azis et al. (1999), De Gennaro et al. (2000), Naskar et al. (2010b), Wada et al. (2005)
	Inulin	Jerusalem Artichoke	DPn 29	*Mw* 3400 ± 150		Azis et al. (1999), Wada et al. (2005)
	Inulin	Dahlia	DPn 26–35			Vereyken, van Kuik, et al. (2003), Wada et al. (2005)
N.C.P.[*]	n/a	Jerusalem Artichoke		*Mw* 7200 ± 100 *Mn* 6100 ± 500	1.18	Eigner et al. (1988)
N.C.P.[*]	n/a	Jerusalem Artichoke	DPn 28–33	*Mn* 4900–5600 ± 500		Panchev et al. (2011)
Beghin-Meiji	Actilight 950P	*Aspergillus niger*	DPn 3	*Mn* 579		Blecker et al. (2002), De Gennaro et al. (2000)
N.C.P.[a]	n/a	*Bacillus* sp. 217C-1	DPn 16–18			Wada et al. (2005)
N.C.P.[a]	n/a	Globe artichoke	DPn 80			Ronkart, Blecker, et al. (2007)
N.C.P.[a]	n/a	*Aspergillus sydowi*		*Mw* 1.49×10^4–5.29×10^6	1.13–3.01	Kitamura et al. (1994)
N.C.P.[a]	n/a	*Aspergillus sydowi*		*Mw* $26–28 \times 10^6$	1.7	Wolff et al. (2000)
N.C.P.[a]	n/a	Synthetic FTF *Streptococcus mutans*		*Mw* $30–90 \times 10^6$	1.1	Heyer et al. (1998), Wolff et al. (2000)

[a] N.C.P. = non-commercial product, purified or produced by the authors; n/a = does not apply.

identical when the material is monodisperse. Where a degree of polymerization without further specification was reported, it was assumed to be the number based variety. For inulin the DPn can be converted into the average molar mass using the following formula: Mn = 180 + 162 × (DPn-1), similar can be done for DPw by substituting DPn by DPw and Mn by Mw. Table 2 contains reported DP and molecular weight values of inulin from various sources as reported in literature, it was not completed with calculated values for clarity purposes.

Wada et al. (2005) reported that the main difference between the inulin they synthesized enzymatically and plant-derived inulin was the polydispersity. Synthetic inulin had a lower polydispersity, which they illustrated with chromatograms from HPAEC with pulsed amperometric detection. Unfortunately, however, the polydispersity was not quantified.

2.2. Morphology

2.2.1. Crystal morphology

Lis and Preston (1998) patented the production of obloid and needle-like shaped crystals of inulin. The needle-like crystals were 1–20 μm in length with the other axes being 10–30% of that (U.S. Patent No. 5,840,884, 1998). The obloid crystals were of the same length, yet the other axes were sized at 50–80% of the length. The different types of crystals were produced by cooling an aqueous liquid containing 10–50% of Fibruline Instant (DP 6–12). The crystal transition temperature of the two crystals was approximately 75–95 °C. If the solution was cooled form a temperature higher than the crystal transition temperature obloid crystals would be produced, if lower (given all inulin was previously dissolved) needle-like crystals were obtained (U.S. Patent No. 5,840,884, 1998). It was argued that the mouth feel of the obloid shaped crystals is better than that of the needle shaped crystals. Viscosity could be altered by varying the ratio and sizes of the two types of crystals. Needle-like crystals predominately increased viscosity while obloid ones improved lubricity.

Hébette et al. (1998) investigated the influence of cooling rate, molecular weight, concentration, and storage time on the crystallization of inulin using Raftiline ST (DP 10–12) and fractions thereof. The crystallization produced obloid, or more accurately eight-shaped, crystals which were 5–20 μm in size if they started forming at a high temperature (77 °C) and up to a tenfold smaller if

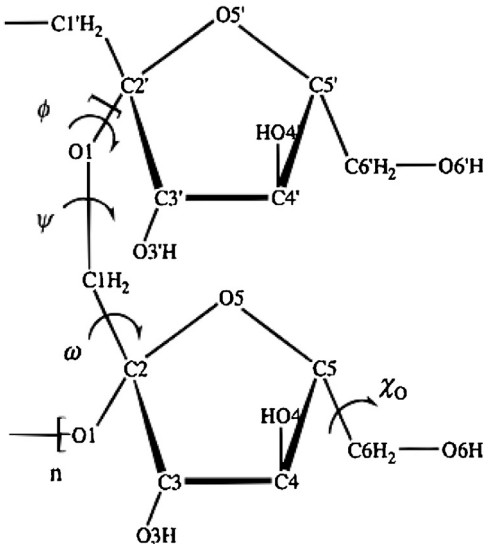

Fig. 2. Representation of the atomic labeling scheme for the inulin chain. Reprinted with permission (André, Mazeau, et al., 1996). Copyright 1996 American Chemical Society.

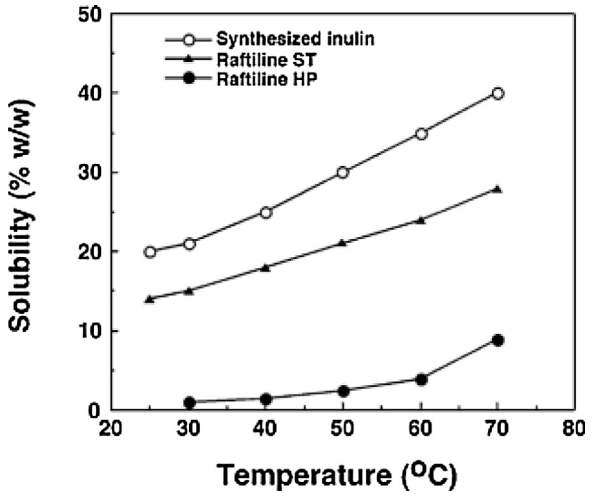

Fig. 3. Differences of aqueous solubility between plant-origin (DPn 10–12 and 23–25) and enzymatically synthesized inulin (DPn 16–18).
Reprinted with permission (Wada et al., 2005). Copyright 2005 American Chemical Society.

they were formed at lower temperatures (65 °C). The thickness and perfection of the formed crystalline lamellae was inversely related to the amount of undercooling. By small angle X-ray scattering (SAXS), they found that the crystal structure was the same as the monohydrate form (see Section 2.2.2) (André, Putaux, et al., 1996). The periodicity of the crystals produced at higher temperatures was 110 Å and at lower temperatures 90 Å.

2.2.2. Crystal structure

Marchessault, Bleha, Deslandes, and Revol (1980) investigated the three-dimensional crystal structure of inulin. They reported it to have a 5-fold helix, being either left- or right-handed with a space of 2.16 Å per monomer and thus 10.8 Å per loop. Reported bond angles were $\Psi = 130°$, $\varphi = 75°$ and $\omega = 60°$ (right-handed) or $\omega = 180°$ (left-handed), see Fig. 2 for an illustration of which bond-angles are described. Large differences in crystal structure were shown between polyethylene glycol and inulin, which were explained by steric interactions between the substituents and the exo-anomeric effect.

André, Putaux, et al. (1996) claimed Marchessault's findings of an unusual 5-fold helix to be based on limited data and in fact incorrect and that the crystals they produced actually contained a 6-fold helix. They reported the formation an orthorhombic hemihydrate crystal with dimensions of $a = 16.70$ Å, $b = 9.65$ Å, $c = 14.4$ Å per 6 units and a pseudo-hexagonal monohydrate crystal with $a = 16.70$ Å, $b = 9.80$ Å, $c = 14.7$ Å per loop. The hemi-hydrate contained one water molecule per two fructosyl residues while the mono-hydrate had one per fructosyl residue. The helical conformation of the hemi-hydrate was characterized by $\varphi = 66°$, $\psi = 154°$, and $\omega = -82°$ and the monohydrate's dimensions were very similar with the following bond angles $\varphi = 68°$, $\psi = 159°$, and $\omega = -87°$. André thus concluded that the progress per loop was 14.4 or 14.7 Å as opposed to 10.8 Å (André, Mazeau, & Tvaroska, 1996; André, Putaux, et al., 1996). It should however be noted that the methods used to produce the crystals by André and Marchessault were not identical and the inulin used was not characterized apart from crystal structure. As described in Section 2.2.1, the method of production is of influence on the morphology of the produced crystals and thus it is possible that different isoforms might have been produced. Further down several isoforms of inulin monohydrate will be discussed based on classifications of solubility and size.

2.2.3. Structure in solution

French (1988) calculated the theoretically allowed conformations for inulin in solution and concluded that the allowed conformations were similar to those of dextran. Of course the reported conformations are merely the allowed conformations based on specific assumptions, French also noted that there are a lot of factors influencing the favored structure of oligosaccharides. Vereyken, van Kuik, Evers, Rijken, and de Kruijff (2003) also found many possible conformations for inulin in their models, including a zigzag conformation with the ω angle at 180° which stayed stable in their simulations. This multitude of possible conformations shows the molecular flexibility of inulin.

Several reports have described the behavior of a broad range of inulins in solution. Models and measurements by Oka, Ota, and Mino (1992) and Liu, Waterhouse, and Chatterton (1994) indicate that a helical conformation is possible for oligofructose of DP 5. This conformation would however not be possible for higher molecular weight inulins due to steric hindrance. Liu et al. (1994) reported that for inulins sized up to DP 9 simple helical structures are not the predominant structure and Oka et al. (1992) found that for a DP of 8 and higher the backbone would reach a more rigid conformation. It thus seems that an organized three-dimensional structure does not occur for oligosaccharides with a DP smaller than about 8 or 9.

2.3. Solubility

Wada et al. (2005) investigated the aqueous solubility at various temperatures of three different types of inulin, two Raftiline inulins which differed in size and an enzymatically produced synthetic inulin. Their results are depicted in Fig. 3, Raftiline HP (DPn 23–25) displays lowest solubility, followed by Raftiline ST (DPn 10–12). What is remarkable, however, is that the enzymatically produced synthetic inulin (DPn 16–18) had a higher solubility than Raftiline ST despite its higher DP. Normally the solubility of polymers decreases with increasing DP. As mentioned, the average DP of a polymer only tells part of the story and it is also relevant to consider the molecular weight distribution of the different DP fractions. The reader is directed to the cited article for molecular weight profile chromatograms of these inulins. The absence of highly polymerized fractions (no fraction with a DP larger than 30) in the enzymatically produced synthetic inulin could explain the higher solubility of the synthetic inulin (Wada et al., 2005). Unfortunately, the method by which solubility was established was not

Table 3
Aqueous solubilities of different sizes of inulin at various temperatures.

DPn or Mw (g/mol)	Solubility	Temperature (°C)	Source
4	>75% (w/v)	25	Franck (2007)
12	12% (w/v)	25	Franck (2007)
25	2.5% (w/v)	25	Franck (2007)
4468	~10% (w/w)	30	Naskar et al. (2010a)
8–12	17.4% (w/w)	37	Bouchard, Hofland, and Witkamp (2007)

described. Kim et al. (2001) also investigated the solubility of Raftiline HP over a temperature range and also found a low solubility up to 50 °C from where on the solubility drastically increased until 35% at 90 °C. Reported aqueous solubilities of some other inulines are listed in Table 3.

Bot, Erle, Vreeker, and Agterof (2004) reported hazing when dissolving Raftiline ST inulin in water. This was presumably the result of a small, high-DP crystalline fraction of inulin which did not dissolve readily. It was found that this fraction did not dissolve at room temperature, but typically would do so at temperatures of 60 °C and higher.

Cooper and Carter (1986) and Cooper and Petrovsky (2011) initially identified four polymorphs of crystalline inulin (α, β, γ and δ) based on their dissolution behavior. β inulin, which was produced by addition of ethanol or by freeze-thawing, is readily soluble in water at room temperature. The other polymorphs, which could be interconverted into more stable versions (in the order β, α, γ to δ), required higher temperatures to dissolve. All polymorphs could be interconverted by re-dissolution. The γ polymorph was made up only out of inulin with a molecular weight >8000 g/mol, where the α and β forms also contained lower molecular weight inulin fractions (Cooper & Carter, 1986). More recently the list of polymorphs was expanded to seven plus the amorphous form (Cooper, Barclay, Ginic-Markovic, & Petrovsky, 2013). All the polymorphs, which differed in chain length, were monohydrate inulin crystals described earlier (André, Putaux, et al., 1996; Cooper, Barclay, Ginic-Markovic, Gerson, & Petrovsky, 2014). The monohydrate and hemi-hydrate only differ in the amount of water associated to the inulin, not in their crystal structures (André, Mazeau, et al., 1996; Ronkart, Deroanne, Paquot, Fougnies, & Blecker, 2010). As suggested by André, Putaux, et al. (1996), the fructose units of inulin formed helices with a 6-unit repeat. Cooper et al. (2014) found that the different polymorphs increased in size by steps of 6 fructose units and concluded that these units formed additional helical turns. Surprisingly, these polymorphs were characterized by a degree of polymerization of 6n + 1, rather than 6n. This additional fructosyl residue was shown to be able to link to glucose of another molecule through hydrogen bonding, allowing formation of tertiary structures of inulin (Cooper et al., 2015).

Ronkart et al. (2007b) found that increasing the feed temperature during spray drying reduced crystallinity and increased the Tg of the produced samples. As a higher Tg is correlated with a higher molecular weight (see Section 2.5.1), this too indicates that the crystals that dissolve at higher temperatures are made up out of higher molecular weight inulins.

In summary, inulin is poorly soluble in water, with decreasing solubility for higher molecular weight fractions. Solubility increases at higher temperatures for all different inulins. These characteristics enable a controlled production of several isomorphs, allowing modification of product characteristics such as rheology. Glibowski (2010) however reported difficulties in controlling inulin crystallization.

Inulin is hardly soluble in ethanol (Bouchard et al., 2008), explaining the use of ethanol in precipitating inulin (Cooper & Carter, 1986), it is freely soluble in dimethyl sulfoxide (DMSO) and very poorly to sparingly soluble in isopropanol (Azis, Chin, Deacon, Harding, & Pavlov, 1999; Dan, Ghosh, & Moulik, 2009; Naskar, Dan, Ghosh, & Moulik, 2010a, 2010b). Phelps (1965) reported that crystals produced using ethanol-recrystallization contained more low DP inulin compared to water-recrystallized samples. Considering that ethanol reduces the solubility of inulin so drastically, one would indeed expect that lower DP fractions of inulin are also affected and separate from solution.

2.4. Rheology

2.4.1. Viscosity

Multiple reports have appeared on the intrinsic viscosity of several inulins in different media, the results of which have been summarized in Table 4.

The intrinsic viscosity decreases by addition of salts and increases with increasing DMSO concentration and molecular weight. The dynamic viscosity of several types of inulin at specific concentrations and temperatures has also been reported, an overview can be found in Table 5.

Like Table 4, Table 5 also shows an increase in viscosity with increasing molecular weight. With increasing temperature, the viscosity is reduced. Wada et al. (2005) reported a slightly lower viscosity for enzymatically produced synthetic inulin (DPn 16–18) than for two commercial Raftiline samples (ST with a DPn of 10–12 and HP with a DPn of 23–25) despite the fact that it has a higher average molecular weight than Raftiline ST. However, as explained in Section 2.3 the average molecular weight does not provide information about the size distribution. The enzymatically produced synthetic inulin lacks highly polymerized fractions, which could be an explanation for this difference in viscosity. Wada et al. (2005) only presented the viscosity data graphically and they were thus not added to Table 5.

2.4.2. Hydrodynamic shape

The Mark–Houwink equation (Eq. (1)) defines the relationship between intrinsic viscosity ([η]) and molecular weight (*M*) for polymers, with two constants (*K* and *a*) (Dan et al., 2009; Wolff et al., 2000).

$$[\eta] = K \times M^a \quad (1)$$

The constant *a* in this equation is indicative for the shape of the polymer in the solution. The *a*-value for compact spheres is 0, whereas an *a*-value below 0.5 indicates branched structures, an *a*-value between 0.5 and 0.9 is associated with a random coil, and an *a*-value over 2.0 with a rod structure (Wolff et al., 2000). Intermediate *a* values represent intermediate shapes.

The plots in Fig. 4 from the publication of Wolff et al. (2000) show linear correlations between Mw and intrinsic viscosities for inulin species with a $Mw > 5.0 \times 10^4$ and for species with a $Mw < 5.0 \times 10^4$. They found that $a = 0.71$ for the 'small' inulins, showing a random coil structure and $a = 0.02$ for the high molecular weights, indicative of a compact sphere. Remarkably, these results are similar to those reported for levan, which does not have a polyethylene glycol-like flexible backbone. Apparently, these bacterially produced fructans have similar characteristics, despite differences in their backbone structure, branching may explain the found similarities (Wolff et al.,

Table 4
Intrinsic viscosity ([η]) of inulin in several media at various temperatures (T).

Medium	[η] (mL/g)	Kh (–)	T (°C)	Mw (g/mol)	Source (manufacturer)	Article cited
Water	4.92	1.13	30	4450	Chicory root (Sigma)	Naskar et al. (2010b)
Water	4.49	1.10	30	4478	Chicory root (Sigma)	Dan et al. (2009)
Water	5.85	n.r.	25	1.49×10^4	*A. sydowi*	Kitamura et al. (1994)
Water	6.97	n.r.	25	1.87×10^4	*A. sydowi*	Kitamura et al. (1994)
Water	8.26	n.r.	25	2.38×10^4	*A. sydowi*	Kitamura et al. (1994)
Water	10.5	n.r.	25	3.37×10^4	*A. sydowi*	Kitamura et al. (1994)
Water	12.8	n.r.	25	7.52×10^4	*A. sydowi*	Kitamura et al. (1994)
Water	16.3	n.r.	25	16.6×10^4	*A. sydowi*	Kitamura et al. (1994)
Water	16.5	n.r.	25	60.4×10^4	*A. sydowi*	Kitamura et al. (1994)
Water	16.5	n.r.	25	97.4×10^4	*A. sydowi*	Kitamura et al. (1994)
Water	18.6	n.r.	25	178×10^4	*A. sydowi*	Kitamura et al. (1994)
Water	19.1	n.r.	25	529×10^4	*A. sydowi*	Kitamura et al. (1994)
Water	18.0	n.r.	25	54×10^6	FTF from *S. Mutans*	Wolff et al. (2000)
Water:DMSO (3:1)	5.86	2.12	30	4450	Chicory root (Sigma)	Naskar et al. (2010b)
Water:DMSO (2:1)	6.63	1.50	30	4450	Chicory root (Sigma)	Naskar et al. (2010b)
Water:DMSO (1:1)	7.96	1.27	30	4450	Chicory root (Sigma)	Naskar et al. (2010b)
Water:DMSO (1:2)	11.0	1.09	30	4450	Chicory root (Sigma)	Naskar et al. (2010b)
Water:DMSO (1:6)	14.9	1.75	30	4450	Chicory root (Sigma)	Naskar et al. (2010b)
DMSO	18.8	1.30	30	4450	Chicory root (Sigma)	Naskar et al. (2010b)
DMSO	15.2	0.48	30	4478		Dan et al. (2009)
DMSO	9.1 ± 0.2	n.r.	25	3400 ± 150	Jerusalem artichoke (Sigma)	Azis et al. (1999)
DMSO	10.7 ± 0.2	n.r.	25	6200 ± 200	Chicory root (Sigma)	Azis et al. (1999)
0.5 M NH_4SCN (in water)	3.65	2.40	30	4478	Chicory root (Sigma)	Dan et al. (2009)
0.5 M NaCl (in water)	4.30	2.16	30	4478	Chicory root (Sigma)	Dan et al. (2009)
0.5 M Na_2SO_4 (in water)	4.21	2.24	30	4478	Chicory root (Sigma)	Dan et al. (2009)

Kh = Huggins constant (if the Huggins formula was used to calculate the intrinsic viscosity), n.r. = not reported.

2000). In addition, it should be noted that levan is still quite flexible compared to other polysaccharides like amylose, as it is linked via the C6 carbon (a primary alcohol) and not directly to the ring.

Next to viscosity, static light scattering was also used to determine the influence of molecular weight on the radius of gyration of the bacterially produced inulins. Those results too indicated a compact globular shape for high *Mw* inulin, but more importantly showed that there might be a difference in branching architecture for inulins of different origins (Wolff et al., 2000). Using small angle X-ray scattering, Eigner, Abuja, Beck, and Praznik (1988) showed that inulin from Jerusalem artichoke with a *Mw* of 7200 had a rod-like formation in aqueous solution. This is not consistent with the above-mentioned conclusions for bacterially produced inulins. The most likely explanations for this are the enormous difference in molecular weight between bacterially produced and natural inulin (see Table 2) combined with the amount of branching of the bacterially produced inulins and the lack thereof in natural inulins.

Azis et al. (1999) investigated characteristics of inulin extracted from Jerusalem artichoke and chicory root (*Mw* 3400 ± 150 and 6200 ± 200, respectively) in DMSO. They differed significantly in size, but a lot less in intrinsic viscosity, indicating a conformation between a random coil and a compact sphere in that solvent. Naskar et al. (2010b) concluded that inulin forms globular aggregates in aqueous solutions and rod-like or spindle-like assemblies in DMSO. In summary hydrodynamic shape and behavior of inulin are influenced by molecular weight, solvent and branching (depending on the inulin source).

De Gennaro, Birch, Parke, and Stancher (2000) investigated the hydrodynamic behavior of several inulins (ranging from oligofructose with *Mn* 579 to inulin with *Mn* 4620) by looking at apparent specific volume (ASV), isentropic apparent specific compressibility [$K_{2(s)}$] and spin-lattice relaxation times (T_1). ASV, a measure of hydrostatic packing with water molecules, was found to increase with degree of polymerization, indicating that low DP inulin had better hydrostatic packing and interacted with water more. Isentropic compressibility values can be interpreted as a measure for the compatibility between water and inulin. $K_{2(s)}$ increased with DP and concentration, showing reduced solute-water affinity. Inulin was found to be more water compatible than other tested carbohydrates except at high concentrations (>15% (w/w)) and/or for a DP of 9 or higher. In the light of the discussion above the latter could mean that the formation of three-dimensional helical structures reduces inulin's water compatibility. Lastly, due to an increased order of protons and reduced water mobility, T_1 values decreased with increasing *Mn* and concentration (De Gennaro et al., 2000).

2.4.3. Gelling

In general inulin gels are based on the interactions occurring between dissolved inulin chains. However, inulin gels may also still contain undissolved microcrystals. These microcrystals can be interconnected, forming a network that is able to interact with both the solvent and other inulin particles thereby increasing gel strength (Bot et al., 2004; Franck, 2007; Kim et al., 2001; Ronkart, Paquot, et al., 2010; Van Duynhoven, Kulik, Jonker, & Haverkamp,

Table 5
Reported dynamic viscosities of several sizes of inulin in water.

Viscosity (mPa s)	T (°C)	Concentration (%)	DPn	Article cited
<1.0	10	5	4	Franck (2007)
1.6	10	5	12	Franck (2007)
2.4	10	5	25	Franck (2007)
1.21 ± 0.06	25	5	28	Panchev et al. (2011)
1.27 ± 0.08	25	5	30[a]	Panchev et al. (2011)
1.29 ± 0.09	25	5	30[a]	Panchev et al. (2011)
1.31 ± 011	25	5	33	Panchev et al. (2011)
1.12	37	10	8–12	Bouchard et al. (2007)

[a] Samples are from two different subspecies of Jerusalem artichoke.

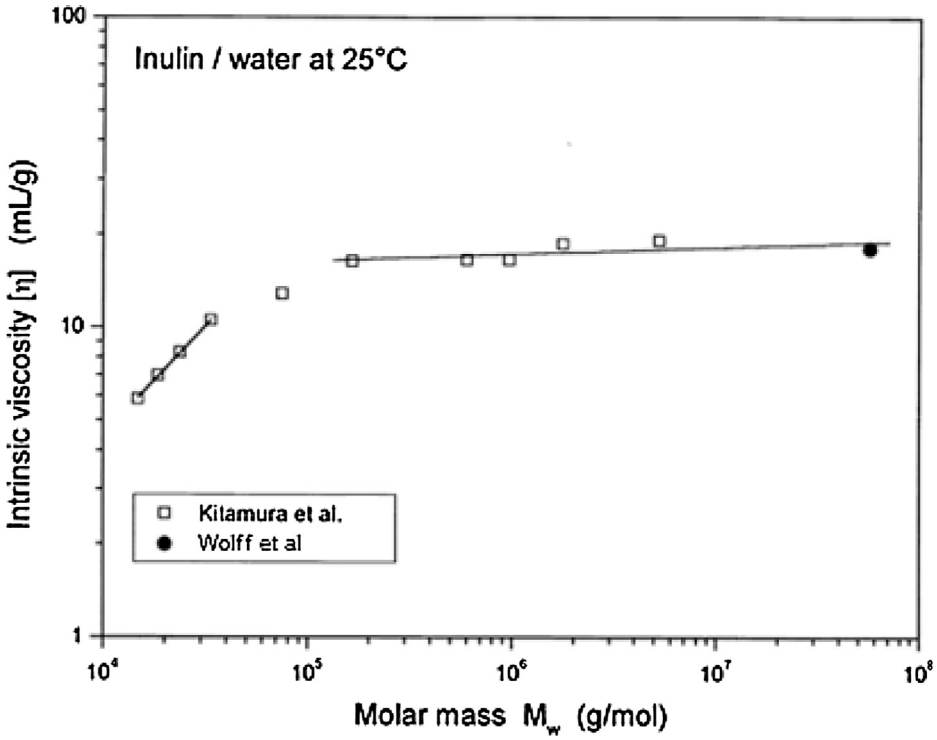

Fig. 4. Molar mass dependence of intrinsic viscosity for high Mw bacterially produced inulin, data from Kitamura, Hirano, and Takeo (1994) and Wolff et al. (2000). Lines represent the linear regression of the Mark–Houwink equation (Eq. (1)).

1999). As described earlier, temperature and molecular weight influences the formation of microcrystals and thereby also gel formation. Based on this and their higher viscosities, high molecular weight inulins are better gel formers than their lower molecular weight counterparts. This also explains why hydrolysis, which reduces the degree of polymerization, reduces gel formation by disturbance of the network (Kim & Wang, 2001). Using nuclear magnetic resonance spectroscopy, Van Duynhoven et al. (1999) showed that lower inulin concentrations lead to lower concentrations of crystalline material. This results in a reduction in the network formation, explaining lower mechanical strength of the gel.

Inulin gels can be formed either thermally, through heating and cooling, or by applying shear forces (Kim et al., 2001). Kim et al. (2001) and Kim and Wang (2001) have investigated both methods of gel production extensively. Thermally produced gels were found to be stronger and smoother than shear induced ones. Gel production was dependent on temperature, heating time, concentration, pH and addition of other solvents. Addition of other solvents (ethanol or glycerol) reduced polarity of the solution causing less solvent–inulin interactions, resulting in faster gel formation but with similar gel strengths. The minimal concentration of inulin needed for gel formation differed with temperature. The solution needed to be heated up to at least 40 °C to achieve gelling. However, heating to temperatures of 80 °C and higher, and acidic conditions (pH < 3) lead to substantial hydrolysis of inulin, resulting in reduced gel formation (Kim et al., 2001). In these studies, only Raftiline HP (DPn 23–25) was used, the influence of molecular weight was thus not taken into account. Meyer et al. (2011) did investigate the influence of DP and concentration on gel strength. They found that higher molecular weight inulins produce stronger gels and are able to form gels at lower concentrations as can be seen in Fig. 5.

Chiavaro, Vittadini, and Corradini (2006) specifically investigated the influence of DP on thermal gelation and found that by using inulin of different molecular weight gels could be produced with different characteristics due to a difference in balance between solid–solid and solid–liquid interactions. Using texture profile analysis, higher molecular weight inulins were found to form gels that were harder, more adhesive and less cohesive both after production and after storage at 4 °C for 4 weeks. This means that higher molecular weight gels required more force to be deformed, would stick to surfaces more and had weaker internal bonds between components (Szczesniak, 1963). The gels prepared from higher molecular weight inulin had more freezable water than gels prepared from low molecular weight inulin (Chiavaro et al., 2006). These observations were ascribed to an increase in inulin–inulin interactions and a decrease in inulin–water interaction with increasing molecular weight. As solid–solvent interactions were needed for storage stability, lower molecular weight inulin gels maintained their textural characteristics better during storage. Here too, the average DP does not tell the complete story and the polydispersity should be taken into account as well. It seems that for a stable gel a fraction of the

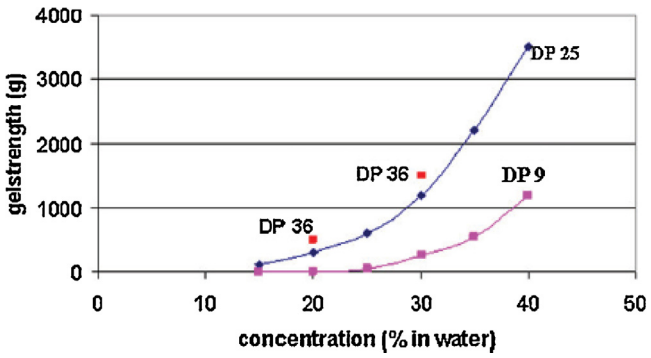

Fig. 5. Gel strength in relation to concentration of different inulin types. The gels were prepared by heating the solutions at given concentrations to 85 °C and allowing them to cool overnight at 4 °C (Meyer et al., 2011).

inulin needs to be of high enough DP for micro-crystallization and solid–solid interactions to form a network, and another part needs to be smaller to interact with the solvent (Chiavaro et al., 2006). This is in line with the findings of Glibowski, Pikus, Jurek, and Kotowoda (2014) that addition of low concentrations (≥0.02%) of seeding crystals allowed heated inulin solutions to form gels instead of precipitating during cooling. At a higher concentration of seeding crystals (≥0.4%) stronger and more stable gels were obtained.

Shear-induced gels were reported to become smoother when the applied shear stress was increased (Kim et al., 2001). This is because low shear caused the formation of larger aggregates; at higher shear stresses a better dispersion was achieved. In comparison to thermally produced gels, shear gels contain larger particles with a broader particle size distribution and with that the gels have a reduced yield stress. Ronkart, Paquot, et al. (2010) found that repeated application of high shear stress reduced particle size, facilitating the formation of a finer network of particles and textural modifications. In addition, the reduction in particle size might have resulted in more inulin dissolving, increasing viscosity and also modifying gel behavior. Bot et al. (2004) investigated how several methods of crystallization influenced the large deformation rheology of inulin gels and found that shape and size of the produced crystals play an important role in the formed network and thus the texture of the produced gel.

Using high-pressure homogenization, Alvarez-Sabatel, de Marañón, and Arboleya (2015) related gel characteristics to pressures used during this process. It is important to note here that the product temperature increases during processing and that this temperature increase is much larger for higher processing pressures. Caution should therefore be taken in relating processing pressures to gel characteristics directly, as this heating also influences the characteristics of the formed gel (Glibowski, 2010). Nonetheless, by varying this pressure and therewith the product temperature, inulin gels with specific characteristics can be produced.

Gelling and texture modifying properties of inulin in more complex systems have been reported. Some reports suggest that inulin has a synergistic effect on gelation with other gelling agents (e.g. gelatin, alginate, maltodextrins and starch) and proteins whilst others actually report inulin competing with them (Franck, 2007; Gonzalez-Tomás, Coll-Marqués, & Costell, 2008; Meyer et al., 2011; Tseng, Xiong, & Boatright, 2008). It seems that for some excipients a competition for water occurs whilst with others a combined network is formed, but it goes beyond the scope of this review to discuss this behavior in detail here.

Lastly, several reports described the synthesis (Maris et al., 2001; Vervoort & Van den Mooter, 1997) and behavior of (meth)acrylated inulin gels for controlled release of drugs in the colon (Castelli et al., 2008; Fares, Salem, & Khanfar, 2011; Pitarresi, Giacomazza, Triolo, Giammona, & San Biagio, 2012; Tripodo, Pitarresi, Palumbo, Craparo, & Giammona, 2005; Van den Mooter, Vervoort, & Kinget, 2003). Gels of these chemically modified inulins were produced by formation of covalent cross-links between the added side-chains using free radical polymerization. In terms of rheological behavior, a higher degree of substitution resulted in a faster gelation process and higher rigidity of the obtained gels for methylacrylated inulin due to more inter-molecular crosslinking (Vervoort et al., 1999). Different cross-linkers were investigated and found to modify rate of crosslinking and elasticity of produced gels differently, allowing for control of mechanical properties of these gels (Pitarresi et al., 2012). Controlling the amount of swelling of the hydrogels is critical. High swelling of the gel is needed to allow degradation in the colon by bacteria (Van den Mooter et al., 2003), however, to prevent premature drug release before the colonic environment is reached, low swelling is key (Maris et al., 2001). Recently, chemically crosslinking of inulin molecules using divinyl sulfone was used to produce microgels intended for controlled release in the stomach (Sahiner, Sagbas, Yoshida, & Lyon, 2014).

2.5. Thermal characteristics and physical stability

2.5.1. Glass transition temperature (Tg)

Most commercially available types of inulin are amorphous and can thus be characterized by a glass transition temperature (T_g). Above the glass transition temperature molecular mobility is strongly increased and crystallization can occur. Molecular weight influences the T_g of anhydrous carbohydrates and the T_g of the maximally freeze concentrated fraction (T_g') of carbohydrates.

The T_g' is of interest when freeze-drying is used as a production process. The T_g' should not be surpassed during the first part of freeze-drying (primary drying) in order to achieve an amorphous product. The Fox–Flory equation (Eq. (2)) describes the relationship between T_g and molecular weight (Fox & Flory, 1950).

$$T_g = T_{g,\infty} - \frac{C}{M} \tag{2}$$

With $T_{g,\infty}$ being the T_g at infinite molecular weight, M molecular weight, and C a constant.

The $T_{g,\infty}$ and constant C were calculated for inulin using data of Hinrichs et al. (2001) and unpublished data. The maximal T_g ($T_{g,\infty}$) was 175 °C, with a fitting constant of 75 kDa. The maximal T_g' ($T'_{g,\infty}$) was −14 °C with a fitting constant of 11.3 kDa. Compared to smaller carbohydrates like sucrose and fructose, inulin has a much higher T_g. At similar molecular weights glucans have even higher T_g values. For the T_g' values the same trends apply (Kawai, Fukami, Thanatuksorn, Viriyarattanasak, & Kajiwara, 2011).

Water acts as a plasticizer on amorphous carbohydrate samples, meaning it decreases the T_g. The Gordon–Taylor equation (Eq. (3)) describes T_g of an ideal mixture of two amorphous components, in this case a mixture of water and inulin. Water has a very low T_g of approximately 165 K, explaining why even small amounts strongly decrease the T_g (Giovambattista, Angell, Sciortino, & Stanley, 2004; Velikov, Borick, & Angell, 2001).

$$T_{g,\mathrm{mix}} = \frac{f_a * T_{g,a} + K * f_b * T_{g,b}}{f_a + K * f_b} \tag{3}$$

(Gordon & Taylor, 1952) f_x is the weight fraction of component x (with x either a or b), and K is usually considered as a fitting parameter.

Several papers have reported measurements of the influence of the water content on the T_g of inulin. Fig. 6 shows the results of water uptake of up to 12% on the T_g of inulins of various molecular weights. The Gordon–Taylor equation was used to fit the curves. For all inulins, a water content of just 2% decreased the T_g with around 30 K and at a moisture content of 10% the T_g of the mixture had gone down by nearly 100 K.

2.5.2. Vapor sorption

Knowing that water can strongly reduce the T_g of a mixture, it is important to determine the water sorption of inulin in relation to relative humidity in the atmosphere. Using dynamic vapor sorption, water uptake of several inulins and trehalose was studied as a function of relative humidity (RH) (Hinrichs et al., 2001). Water sorption was similar for all sizes of inulin and was similar to that of other amorphous carbohydrates. Trehalose crystallized at a RH above 50%, whereas the inulin samples remained amorphous on the timescale of the dynamic vapor sorption experiments (hours), even though they all surpassed their T_g during the measurement. This shows that inulin crystallizes less easily than trehalose. Incidental (short term) exposure to high relative humidity of amorphous inulin does therefore not necessarily lead to immediate crystallization. In two other studies where inulin was stored at controlled

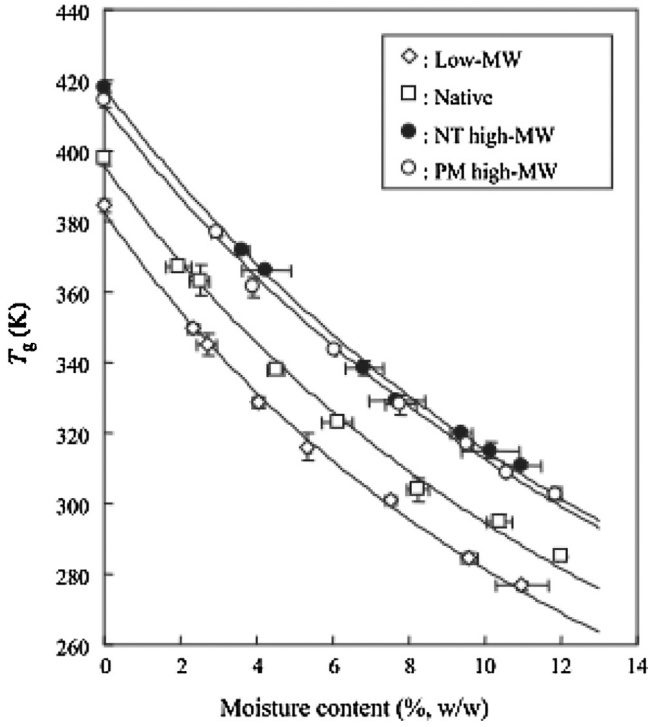

Fig. 6. Effect of moisture content on T_g of several inulin samples. The low molecular weight, native and high molecular weight samples had degrees of polymerization of 7, 13 and 27, respectively. PM denotes pre-melted, meaning the sample had been heated in solution, quench-cooled and subsequently freeze-dried to make the sample completely amorphous, NT denotes not treated (Kawai et al., 2011).

relative humidities for weeks, crystallization was found (Schaller-Povolny, Smith, & Labuza, 2000; Zimeri & Kokini, 2002).

Ronkart, Blecker, et al. (2006), Ronkart et al. (2008) and Ronkart, Paquot, Fougnies, Deroanne, and Blecker (2009) described the consequences of moisture sorption for inulin samples with different degrees of crystallinity. Depending on the molecular weight of the inulin, the amorphous particles fused at RH of >56% (Ronkart, Blecker, et al., 2006) or at RH over >75% at 20 °C (Ronkart et al., 2008) (corresponding to a water uptake of 12–15 g/100 g dry inulin at >75% RH). This lead to caking, i.e. sticking together of the powder particles resulting in reduced flowability. The presence of crystals in the amorphous matrix limited the caking (Ronkart et al., 2008). This behavior is not uncommon for polysaccharides.

They then defined three regions based on water uptake and crystallinity at 20 °C, as shown in Fig. 7 (Ronkart et al., 2009). In region I inulin remained completely amorphous, in region III inulin was completely crystallized (and caked). Region II represents an intermediate region where inulin's macroscopic and thermal properties were changing. In region I the T_g of the samples was at least 10 °C above storage temperature, in region III the T_g was room temperature or lower. This shows that if the T_g drops below storage temperature +10 °C, mobility will increase and lead to crystallization and caking, which is nearly always undesirable. Therefore, storage conditions should be carefully chosen and exposure to high relative humidities and temperatures should be avoided.

Similarly, Schaller-Povolny et al. (2000) defined a critical moisture content (and corresponding critical relative humidity) based on macroscopic changes to inulin morphology, above which inulin would be crystalline. These large macroscopic changes are only truly apparent crystallization is widespread and are therefore not a good measure for determination of a critical moisture content (Ronkart et al., 2009). The study does however show that inulins of different molecular weight pass through this critical point at different amounts of water uptake. Inulins with a higher

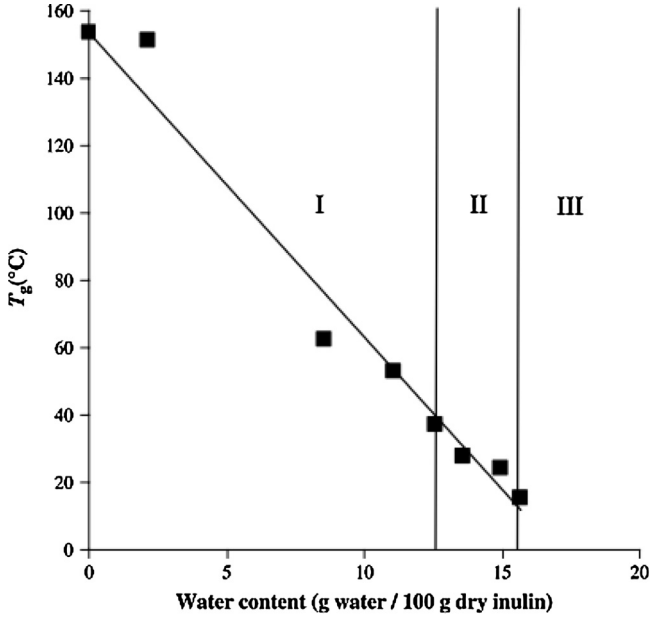

Fig. 7. Glass transition temperature-water content relationship for inulin DPn 23/DPw 30 with three regions of different crystallinity (Ronkart et al., 2009).

molecular weight can withstand more water uptake before they reach the critical point and thus be stored at higher RH. Higher molecular weight inulins may therefore be used to improve processability and storage stability in food or other products (Schaller-Povolny et al., 2000).

2.5.3. Melting temperature

Melting temperatures of fractions of Fibruline LCHT with different degrees of polymerization were determined and are shown in Fig. 8 (Blecker et al., 2003). Two groups with different degree of crystallinity could be distinguished. The higher DP fractions were insoluble in water (obtained by precipitation in aqueous solutions at various temperatures), while the low DP fractions were produced by freeze-drying water soluble fractions (Blecker et al., 2003). Low DP fractions had a lower melting enthalpy, which is indicative for crystallinity, of 7–9 J/g and the higher fractions 17–19 J/g (Blecker et al., 2003). Even higher melting enthalpies ranging up to 47.6 J/g have also been reported (Zimeri & Kokini, 2002). Melting temperatures reported elsewhere were similar to the ones shown in Fig. 8, with melting temperatures being reported between 165 and 183 °C (Dan et al., 2009; Heyer et al., 1998; Panchev et al., 2011; Zimeri & Kokini, 2002). The melting temperature of a enzymatically produced synthetic inulin as determined by Heyer et al. (1998) was

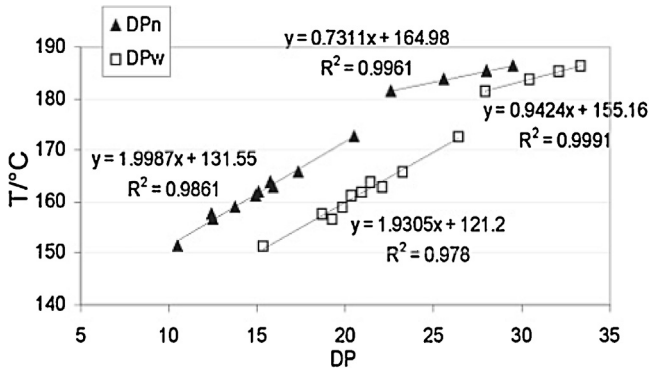

Fig. 8. Relations between degree of polymerization (DP) and inulin's melting temperature (Blecker et al., 2003).

only 183 °C despite its much larger size (70×10^6 g/mol), which is common for polymers (Flory & Vrij, 1963). Inulin started degrading after melting, when heated above 200–225 °C (Dan et al., 2009; Heyer et al., 1998; Ronkart, Deroanne, et al., 2010).

The hemi-hydrate of inulin (produced by water sorption of amorphous inulin) had a melting temperature of around 155–160 °C and the mono-hydrate (seeding crystals) had a melting point between 170 and 180 °C (Ronkart, Deroanne, et al., 2010). Similar melting temperatures were reported for the different monohydrate polymorphs described in Section 2.3, which differed from each other in molecular weight (Cooper et al., 2013). It is therefore likely that the two different fractions shows in Fig. 8 are mono-hydrate and hemihydrate forms of inulin.

2.6. Chemical stability

Inulin with a glucose end group does not have or form any reactive aldehyde or ketone groups and is therefore non-reducing. However, inulin molecules lacking this glucose end group, thus ending with a fructose group, is reducing (BeMiller, Steinheimer, & Allen, 1967). Furthermore, as discussed previously, inulin is a polydisperse mixture and can also contain mono- and disaccharides which are more reactive. These inulins without glucose end group can thus take part in reactions with other components, such as the amino group of proteins in the Maillard reaction. In the light of the above, it could be useful to distinguish between inulin with and without glucose end groups. If reducing groups are present and the Maillard could potentially occur, formulation modifications such as the addition of sulfite, or adjusting the pH could be used to reduce the risk of the Maillard reaction occurring (Martins, Jongen, & Boekel, 2001; McWeeny, Biltcliffe, Powell, & Spark, 1969).

Several reports discussed the amount of reducing groups of inulin, some supplied more details than others (De Gennaro et al., 2000; Hinrichs et al., 2001; Stevens et al., 2001). Stevens et al. (2001) found a residual reducing activity of 0.5–2.5% after removal of mono- and disaccharides from 'native inulin'. Hinrichs et al. (2001) found that the percentage of carbohydrate units containing reducing groups was much higher for small inulins than for larger inulins. Oligofructose synthesized from sucrose contains fewer reducing groups than oligofructose produced by hydrolysis of inulin (De Gennaro et al., 2000). Hydrolyzed inulin will contain fructose chains both with and without glucose end group, whereas inulin synthesized from sucrose only contains fructose chains with a glucose end group. The relative abundance of fructose chains without glucose can explain the difference in amount of reducing groups between these two production methods.

Influence of several processing parameters on the amount of reducing groups of inulin were reported (Kim et al., 2001; Kim & Wang, 2001). Reducing sugar content of aqueous inulin solutions increased with increasing temperature and with lower pH due to hydrolysis of inulin (Kim et al., 2001). At neutral pH, the percentage of reducing groups increased from <0.1% to only 1.2% after heating a concentrated solution to 100 °C for 5 min. At pH values of 3 or lower the amount of reducing sugars formed increased drastically, up to 25% at pH 1 (Kim et al., 2001). Reducing groups were formed as a result of hydrolysis, which followed pseudo first-order kinetics with reducing activity increasing continuously over time during heating (Kim & Wang, 2001). Since hydrolysis was the cause of the increase in reducing activity, it was indirectly indicative of a reduction of DP. This is because hydrolysis cleaves the end fructosyl group of inulin, reducing its DP. Which, as explained above, in turn influences several other characteristics of inulin (Kim et al., 2001).

For oligofructose, the influence of various processing parameters on hydrolysis have also been reported (Barclay, Ginic-Markovic, Johnston, Cooper, & Petrovsky, 2012; Blecker, Fougnies, Van Herck, Chevalier, & Paquot, 2002; L'homme, Arbelot, Puigserver, & Biagini, 2003; Matusek, Merész, Le, & Örsi, 2008; Vega & Zuniga-Hansen, 2015). Hydrolysis of oligofructose also follows pseudo first-order kinetics (Barclay et al., 2012; Blecker et al., 2002; L'homme et al., 2003). Little hydrolysis was found up to 60 °C, this changed at 70 °C and above (Matusek et al., 2008). Hydrolysis mainly took place at acidic rather than neutral or alkaline conditions, where low molecular weight oligofructose reacted faster than high molecular weight ones (L'homme et al., 2003). It was also found that fructose was produced at a higher rate than glucose (Barclay et al., 2012). Sucrose, containing only a (1↔2) linked β-D-glucosyl and β-D-fructosyl group, reacted more slowly than the oligofructose carbohydrates. Combined, these results indicate that the terminal β-D-fructosyl-(2→1)-β-D-fructosyl glycosidic bond is most susceptible to acidic hydrolysis (Barclay et al., 2012; Blecker et al., 2002; L'homme et al., 2003). At lower degrees of polymerization this terminal bond is relatively more abundant and they thus have a lower chemical stability. At a pH of around 3, changes in pH of 0.3 units were found to have a large impact on hydrolysis (Matusek et al., 2008). At pH 2.7 and a temperature of 90–100 °C nearly complete degradation of oligofructose into monomers was achieved in 30–40 min (Matusek et al., 2008). At a pH ≥ 5, relevant for food applications, no degradation was found regardless of thermal processing (up to 100 °C for 55 min) (Glibowski & Bukowska, 2011).

Inulin and oligofructose thus show similar trends with respect to pH, temperature and molecular weight dependent hydrolysis (Blecker et al., 2002). The kinetics of the reactions are however different (Barclay et al., 2012). For higher molecular weight inulins, the rate of hydrolysis is initially low, but increases as hydrolysis progresses (Blecker et al., 2002). An explanation for this could be the amount of end-chain fructosyl groups. Initially, they are scarce, meaning hydrolysis of mid-chain glycosidic bonds will be more pronounced. Mid-chain hydrolysis in turn increases the amount of more reactive end chain fructosyl groups, resulting in an increase in reaction rate (Blecker et al., 2002). It was also suggested that the helical structure of inulin, or the lack thereof for oligosaccharides, influences their reaction rate and how those are influenced by temperature (Barclay et al., 2012).

3. Overview

Here the physicochemical characteristics of inulin, an oligosaccharide widely used in food and pharma, have been reviewed. The average DP of inulin is often used when describing the physicochemical properties such as solubility and thermal and rheological properties. This generally works well but can potentially also be misleading as the average DP only provides an average and does not provide information on the actual size distribution. Inulin consists of a mixture of polymers of different chain length, its physicochemical properties are to a great extent dependent on the size distribution of this mixture. This means that two different batches of inulin with the same average DP can have different size distributions and therewith different characteristics. Higher DP inulin fractions are less soluble in water, possess higher melting temperatures if crystalline or higher glass transition temperatures if amorphous, are chemically more stable (less sensitive to hydrolysis), form stronger gels and are more viscous when dissolved.

Inulin is used to modify texture or replace fat in food, its DP-sensitive gel forming and viscous behavior make it suitable for that purpose. Additionally, the (2→1) linked fructosyl residues of inulin are not hydrolyzed by the human digestive enzymes, enabling low-calorie replacement of fat. The partially hydrolyzed form of inulin, oligofructose (DP ≤ 10), has this same feature and is more sweet, and is therefore used as a low-calorie sugar replacer. Their indigestibility makes both inulin and oligofructose suitable as dietary

fibers. As microbiota in the colon are capable of breaking down inulin, it is also used as a prebiotic and to prepare gels for targeted drug release in the colon.

Inulin's backbone is relatively flexible compared to other polysaccharides, as it does not incorporate the sugar ring. This combined with a relatively high T_g makes inulin a suitable stabilizer of proteins in the dry state for both food and pharma applications. Some specific pharmaceutical applications are its use as a diagnostic agent for kidney function and as an adjuvant for vaccines. Again, the size distribution of the inulin is relevant for these applications. Therefore, regardless of its application both the average DP and the size distribution of inulin should be taken into account. Information on how the molecular weight of inulin and other factors affect its characteristics relevant for its various application can be found in this review.

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