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CHARACTERISATION OF DIFFERENT INULIN SAMPLES BY DSC
Influence of polymerisation degree on melting temperature

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Abstract

Melting behaviour of powder inulin has been studied by differential scanning calorimetry. DSC curves show two endothermic peaks, relative to water elimination and to inulin melting, respectively. The second peak is dependent on inulin type and a shift to a higher temperature is observed with increasing average polymerisation degree (*DP*) of the sample. For similar crystallinity index, linear relations have been underlined and so predicting inulin mean *DP* can be done by DSC analysis. The study shows that a relatively high heating rate (25°C min^{-1}) can be used and brings a supplementary interest by an important reduction of analysis time.

Keywords: degree of polymerisation, differential scanning calorimetry, inulin, melting temperature

Introduction

Inulin, often called by the generic name of oligofructose, is a mixture of polysaccharides composed of a chain of fructose units (linked by β-(2→1) *D*-fructosyl-fructose bonds) with generally a terminal glucose unit (linked by an α-*D*-glucopyranosoyl bond). Only a small fraction depending essentially on hydrolysis rate does not contain any glucose units at all [1, 2]. The general formula may be depicted as GF_n and F_m, with G being a terminal glucose unit, F representing the fructose residue and *n* or *m* characterising the number of fructose units.

A lot of attention is paid to inulin because it is classified as a prebiotic dietary fibre. This aspect and other nutritional interests are broadly discussed in literature [3]. More information on structure, conformation, solubility and the numerous texturing properties offered by this functional food is reported in a recent review [4].

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Inulin is mainly found in chicory roots, Jerusalem artichokes and dahlia tubers. Composition depends on the plant source, harvesting date but also on extraction and post extraction process [1]. The various inulin chains can be characterized according to molecular size or degree of polymerisation (*DP*); *DP* expressing the total number of residues and being equal to n+1 or m for the formula GF_n and F_m, respectively.

As inulin is a mixture of polysaccharides with different *DP*, the chain distribution may be determined by chromatographic techniques such as high performance anion exchange chromatography (HPAEC) with pulsed amperometric detection which allows the separation of the different chain lengths and hence to calculate the average degree of polymerisation in number (*DPn*) and the average degree of polymerisation in mass (*DPw*) [5]. The ratio between *DPw* and *DPn* gives the polydispersity of the chain distribution.

From a technological point of view, the average distribution of inulin chains is very important as it implicates different applications based mainly on reduced solubility and increased gel strength with the increase of inulin *DP*. Inulin concentration, heating temperature, pH and particule size also affect the degree of gel formation [6].

Several authors have shown an interest in DSC to study some of the characteristics of inulin. Hébette *et al.* [7] used this analytical technique to compare melting profiles of different molecular mass inulin in aqueous state. DSC curves display melting profiles with three to four partly overlapping endotherms in the 40–100°C range. Their shape is found to vary as function of concentrations, molecular mass distribution, cooling rate during crystallisation and storage time of the crystallite suspension. From inulin powder, Heyer *et al*. [8] quote some melting temperatures in unhermetic pans varying from 173 to 183°C with inulin source and melting enthalpies near 8 J g^{-1} while in hermetic pans, Zimeri and Kokini [9] show several curves characterised by comparable melting temperatures but having melting enthalpies of 25–47 J g^{-1} for samples stored at different humidity.

DSC can also be employed to study glass transition properties of dry inulin [5, 10]. It is established that glass transition of inulin is directly related to the average chain length but also to moisture content, likely for other biopolymers such as starch, ...

The main objective of this paper is to study the influence of inulin mean polymerisation degree on the DSC profile. In particular, relationship between the fusion temperature of inulin in dry powder state and its *DPn* or *DPw* is investigated.

Experimental

Materials

Different kinds of inulin were studied. First, Fibruline® LCHT, a commercial product from Cosucra (Fontenoy, Belgium) has been considered. This product is a fraction of native inulin obtained by a physical separation process containing essentially long chain polysaccharides. Other samples were obtained in laboratory by fractionation of Fibruline® LCHT. The general process of fractionation is the following: inulin was dispersed in hot water, this dispersion was then stirred, equilibrated at room tempera-

ture during two h and centrifuged at 20°C using a Beckman J2-21 with a JA14 rotor, supernatant and pellet were recovered and freeze-dried [11]. So, different ranges of polymerisation degree were achieved by varying initial inulin concentration (from 20 to 30%), water temperature (from 20 to 95°C) and/or centrifugation conditions (5 to 20 000 g for 15 min). Humidity of all prepared samples was checked and proven to be between 94 and 96% dry matter. Experimental fractions F1 to F9 correspond to various supernatants obtained by variation of this fractionation process while fractions with higher *DP* (F10 to F13) are relative to the pellet after centrifugation, maximum *DP* values being obtained by several applications of this method; pellet of first extraction becoming the inulin dispersed for the second extraction, etc…

Determination of polymerisation degree

Determination of the molecular mass distribution of the samples was done by high performance anion exchange chromatography coupled with pulse amperometric detection (HPAEC-PAD) on a Dionex DX500 chromatographic system operating at 1 mL min^{-1}. 25 μL of a 0.8 g L^{-1} solution were injected. Separation of the various chain lengths was achieved on PA100 column. 160 mM sodium hydroxide was used as eluant. Sodium acetate gradient was applied and amperometric detection was used (Dionex application note). Relative response factors of the various species were taken from Timmermans *et al.* and Kang [12, 13]. The average degree of polymerisation number and mass were calculated as proposed by Timmermans *et al.* [12].

Determination of water content

Total moisture analysis were performed on a Mettler-Toledo Karl-Fischer DL31 titrator. Hydranal® solvent and titrant 5 were used. About 200 mg inulin samples were weighed to an accuracy of 1 mg and poured in the titration reactor. Prior to the titration, analysis grade formamide was added to the solvent to help solubilization of inulin.

Differential scanning calorimetry (DSC)

Inulin products were analysed by differential scanning calorimetry with a DSC 2920CE TA Instrument (USA). DSC curves were recorded during heating of the sample from 20 to 200°C. Two different heating rates were tested: 5 and 25°C min^{-1}. Indium and dodecane were used to calibrate the temperature and enthalpy reading. All these DSC experiments were made using unhermetic aluminium pans of matched mass, i. e. the empty sample and reference pans were of equal mass to within ±0.10 mg. The analysed sample mass was generally of 2.50±0.50 mg. The DSC cell was purged with 70 cm^3 min^{-1} dry nitrogen.

X-ray analysis

All samples were analysed in powder form. X-ray diffraction patterns at room temperature were registered on a Philips PW 1120/00/60 diffractometer using a Ni-filtered CuK_α

radiation, generated by an anode device operating at 40 kV and 24 mA, in conjunction with a proportional detector. The patterns were recorded with a fixed time of 0.4 s per step of 0.02° in the range $4° \leq 2\theta \leq 30°$.

Results and discussion

In aqueous solution, Hébette *et al.* observed different DSC profiles depending notably on inulin chain distribution [7]. They have shown that, for inulin with a very disparate *DP*, there is a shift of the highest temperature endotherm towards higher temperatures with the increase of the proportion of high molecular mass polymers but no relation has been established between these parameters. The present study is focused on the influence of mean polymerisation degree on the melting temperature of powder sample determined by DSC.

DSC profiles of inulin

Firstly, DSC curve of a commercial inulin 'Fibruline® LCHT' (*DPn*=20.5 and *DPw*=26.4) is presented in Fig. 1. Two endothermic peaks are observed. The first is very large (enthalpy of 200 J g^{-1}) and is mainly due to water evaporation while the second peak is attributed to the melting of the sample (enthalpy of 9 J g^{-1}). This second peak spanning over a range of 25°C is not symmetric and is characterized by different temperatures: temperature at the first increase in heat flow (157.8°C), onset (165.1°C), maximum (172.8°C) and end (181.2°C).

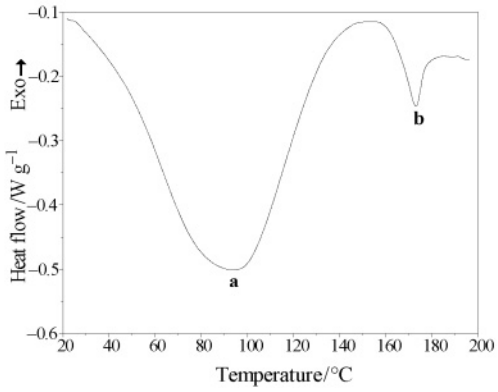

Fig. 1 DSC profile of Fibruline® LCHT (*DPn*=20.5–*DPw*=26.4). a – water elimination peak, b – inulin melting peak

X-ray analysis has been used in order to confirm the fusion phenonenom. Some samples were heated to an intermediate temperature and immediately cooled down. X-ray diffraction patterns were then recorded at room temperature. After heating up to 140°C, X-ray diagrams display principal *d*-spacing bands at 8.35, 7.24 and 4.90 Å. Previous studies [14, 15] indicated that those bands correspond to a pseudo-hexagonal unit cell

(semi-hydrated form of inulin). After heating at 165°C, X-ray *d*-spacing intensity are reduced in comparison to those observed at 140°C. After heating at 190°C, i.e. above the second endotherm, no X-ray diffraction band appeared at all, corresponding to the total melting of crystals. Moreover, Dionex analysis on a sample heated at 190°C shows that melting phenomenon is accompanied by a degradation of the inulin sample which explains the irreversibility of this melting as mentioned by Heyer *et al.* [8].

It is worth noting that DSC analyses are very repeatable. Standard deviation of inulin melting temperature measured at the peak maximum is 0.1°C. The other characteristic temperature present a maximal standard deviation of 1.2°C. Consequently, temperature measured at peak maximum has been preferred in this study. Moreover, this temperature is not affected by variation of the sample mass from 0.5 to 5 mg (standard deviation of melting temperature is also 0.1°C), nor by one or several preheatings to 110 or 130°C. Figure 2 illustrates the non-influence of preheating on the fusion peak and shows that the water peak is completely eliminated from the second and third scan.

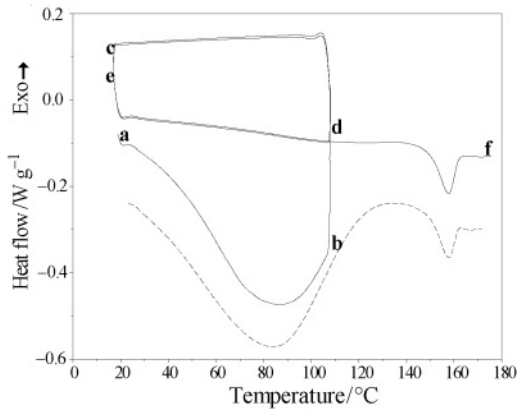

Fig. 2 DSC profile of sample F2 with preheating (full line) and without (discontinuous line). a–b – first preheating to 110°C, b–c – first cooling to 20°C, c–d – second preheating to 110°C, d–e – second cooling to 110°C and e–f – final heating to 180°C with inulin melting peak. The 2 traces are displaced vertically for better visualisation

Influence of water content has also been envisaged on samples comprised between 90 and 99% dry matter. These samples have been prepared by equilibration in a closed enclosure with a $MgCl_2$ saturated solution or P_2O_5 powder during 1 to 3 weeks. Study of this humidity range is sufficient because commercially available inulin have a minimum dry matter of 95%. Results confirm that melting temperature is independent on humidity content (standard deviation of inulin melting temperature is 0.2°C). For these samples, water activity varies between 0.25 and 0.48 but does not influence the melting temperature. The non-influence of water content and water activity is easily understandable if we consider the range of observed fusion temperature that are all higher than 150°C. At these temperature, water content is below of 1.0%.

Relation between mean polymerisation degree and the inulin melting temperature

In this part, numerous inulin samples with different *DP* have been characterized. All correspond to experimental fractions obtained from Fibruline® LCHT aqueous solutions by applying a well defined heat treatment followed by a centrifugation step.

Firstly, all experimental fractions have been characterized by Dionex analysis. Detailed description is reported in Table 1. The main difference between samples is the percentage of chains with a *DP* higher than 40 that permits an easy distinction between supernatant and pellet. Note that the sample polydispersity decreases regularly with the increase of average polymerisation degree and reaches a minimum of 1.1. So our fractionation process can lead to unusually weak value of polydispersity for inulin. On the contrary, in term of rate of non-glucosiled chains, no significant change has been observed.

For each sample, the observed melting temperatures are reported in Table 2. As for the initial product (Fibruline® LCHT), temperature at the peak maximum has been chosen in the rest of this study because it gives a more accurate relation with inulin mean *DP*. Maximum peak temperatures span over a much wider range (151 and 186°C) than that reported in literature [8, 9]. This is probably due to the wide range of average *DP* investigated in the present study.

The few DSC curves reported in Fig. 3 show the impact of *DP* on melting temperature. Each sample was analysed at a rate of 5°C min^{-1}. These five particular samples present a similar DSC profile as Fibruline® LCHT but the second endothermic peak position is dependent on inulin sample, a shift towards higher temperatures being observed in function of an increasing of the average *DP*. Also, peak width varies slightly but not significantly with inulin chain distribution (*DPn* or *DPw*).

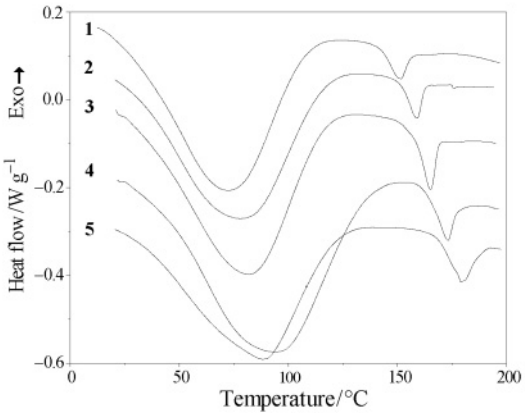

Fig. 3 DSC curves of inulins with different chains distributions. 1 – F1, 2 – F4, 3 – F9, 4 – Fibruline® LCHT and 5 – F10. The different traces are displaced vertically for better visualisation

Table 1 Dionex characterisation of Fibruline® LCHT and various experimental fractions

	DP/% 3–10	*DP*/% 11–20	*DP*/% 21–30	*DP*/% 31–40	*DP*/% 41–50	*DP*/% 51–60	*DPn*	*DPw*	Polydispersity	*Fn*/%
Fibruline® LCHT	3.9	25.1	38.7	23.5	8.2	0.6	20.5	26.4	1.3	0.3
F1	15.6	67.4	15.7	1.3	0.0	0.0	10.5	15.4	1.5	3.2
F2	11.7	49.7	32.3	6.0	0.3	0.0	12.4	18.7	1.5	1.5
F3	11.8	44.3	36.9	6.6	0.4	0.0	12.5	19.2	1.5	2.4
F4	9.5	46.5	34.9	8.3	0.8	0.0	13.8	19.8	1.4	0.8
F5	7.5	43.3	41.2	7.8	0.2	0.0	14.9	20.4	1.4	1.5
F6	5.3	46.1	37.9	10.2	0.5	0.0	15.1	20.9	1.4	1.5
F7	4.4	44.2	39.9	10.9	0.6	0.0	15.8	21.4	1.4	1.7
F8	4.6	41.4	39.1	13.6	1.3	0.0	15.9	22.1	1.4	1.7
F9	5.5	34.3	40.8	16.4	2.9	0.1	17.3	23.2	1.3	0.5
F10	1.8	22.6	38.7	26.4	8.7	1.8	22.6	27.9	1.2	1.3
F11	1.0	16.9	36.7	29.4	12.2	3.8	25.7	30.4	1.2	0.6
F12	0.6	12.6	34.5	32.6	15.1	4.6	28.0	32.1	1.2	0.6
F13	0.5	9.8	32.2	34.8	17.5	5.2	29.5	3.3	1.1	0.5

*DP*3-10, *DP*11-20, ..., *DP*51-60: mass percentage of determined *DP* estimated by HPAEC-PAD
DPn: average polymerisation degree in number estimated by HPAEC-PAD
DPw: average polymerisation degree in mass estimated by HPAEC-PAD
Fn: number of non-glucosiled chains estimated by HPAEC-PAD

Table 2 Characterisation of melting temperature and enthalpy change of various inulin fractions

	T_{onset}/°C	$T_{maximum}$/°C	$\Delta_{melt}H$/J g^{-1}
Fibruline® LCHT	164.5	172.8	9.2
F1	142.8	151.4	6.7
F2	150.2	157.6	7.5
F3	148.4	156.7	7.6
F4	152.0	159.1	7.4
F5	154.2	161.1	7.9
F6	154.4	161.9	8.1
F7	157.9	163.8	7.1
F8	155.6	162.8	8.6
F9	159.2	165.7	9.0
F10	168.3	181.4	18.8
F11	169.6	183.9	17.0
F12	171.5	185.4	17.6
F13	172.6	186.5	16.9

Detailed observation of Fig. 3 permits especially to note that the fraction F4 is completely melted when the fraction F10 has not yet begun to melt. However these 2 samples both contain numerous polysaccharides with a common *DP* range, notably the *DP* 11–30 range (Table 1). Consequently, inulin melting could not be considered as the fusion in an ascending order of each determined *DP* polymers but rather as the melting of a global crystal structure.

It is known that crystallization of inulin from dilute solution yields thin lamellar crystals with relatively large lateral dimensions with respect to their thickness [15]. Inulin crystallizes into an orthorhombic unit cell that contains two antiparallel six-fold inulin helices which are perpendicular to the lamellar basal plane [16]. From concentrated solutions, Hébette *et al.* have shown by small angle X-ray scattering (SAXS) that inulin crystallizes into stacks of lamellar crystals with a periodicity ranging between 9.0 and 11.0 nm [7]. As this distance represents the sum of the crystal core and amorphous length, the crystalline thickness alone is thus expected to be lower. By coupling the DSC results to time resolved SAXS experiments, these authors have related the complex melting behavior of inulin to the occurrence of two crystal populations with two distinct crystalline thicknesses, the thicker crystals having the higher melting point. This is in agreement with what is usually observed for lamellar crystals [17]. However their analysis is complicated by the presence of water and recrystallization phenomena. In the present study, dried powders of inulin are found to melt over a wide temperature range (>35°C). An important increase in the melting point is observed with increasing average *DP* of the sample. It seems very likely that increased crystal perfection accounts for this observation. Small molecules

bring a relatively high content in glucosyl end that may be considered as defects in the field of crystallization. During crystal growth, these defects are probably rejected from the crystal. This limits the thickening of the lamellae along the helix axis and thus induce a lower crystal perfection. On the other hand, as the average *DP* increases the number of defects decreases allowing a better packing of the chains and thus a larger crystal thickness.

Determination of the melting point of inulin samples by DSC might be a convenient way of assessment of the average molecular mass. However samples need to be of a comparable degree of crystallinity. This value is generally obtained via SAXS but melting enthalpy can also give this information (Table 2). Regarding this parameter, our samples can be divided into 2 groups, having a melting enthalpy comprised between 7 and 9 J g^{-1} for the first and 17 to 19 J g^{-1} for the second. For the 2 groups, linear relations can be drawn through the data of Fig. 4. These are all characterized by a determination coefficient higher than 0.978. Statistical analysis confirms that these correlations have a high signification for *DPn* as well as for *DPw* (Student *t* test, first relation: t_{10} obs=23.8 and 18.8, t_{th}=5.0 (P<0.001), second relation: t_4 obs=22.6 and 47.1, t_{th}=31.6 (P<0.001) or 9.9 (P<0.01)). Linear regression coefficients are mentioned in this Fig. 4. Note that the slope decreases strongly when the degree of crystallinity rises, that illustrates the importance of this parameter in the estimation of *DPn* and *DPw* by DSC analysis.

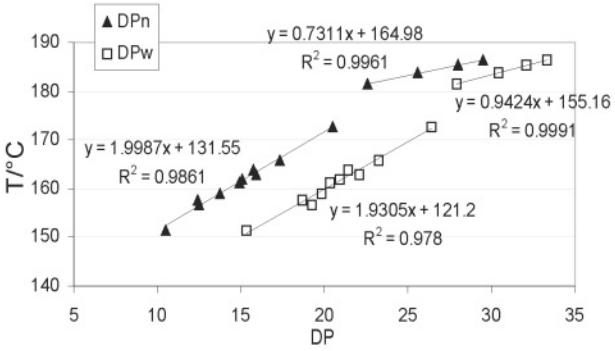

Fig. 4 Relations between ▲ – *DPn* or □ – *DPw* and inulin melting temperature

Finally, influence of a fast heating rate (25°C min^{-1}) has also been studied with success. With this new experimental condition, the major difference is a shift of 7–8°C of melting peak maximum towards higher temperatures. Similar linear regression has been observed and no loss of statistical signification has been observed.

Conclusions

These results show that DSC analysis can be very useful to estimate inulin mean polymerisation degree. For *DPn* as well as *DPw*, predictive measurement are effective for samples of similar crystallinity degree on a *DPn* range comprised between 10 and 30.

DSC could be considered as complementary to chromatographic methods and in some particular cases could replace them. Each method presents, however, its own advantages and limitations. DSC analysis does not give a complete determination of each degree of polymerisation and requires regular calibration but presents other interests such as short analysis time (reduction from more than 60 to 25 min), no solvent preparation, no risks of partial solubilisation or hydrolysis of sample, ... Chromatographic methods can provide quantification of each degree of polymerisation but need response factors determination beforehand.

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