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Factors affecting inulin crystallization after its complete dissolution

Pawel Glibowski[a,*], Stanislaw Pikus[b], Justyna Jurek[a], Marcin Kotowoda[a]

[a] Department of Milk Technology and Hydrocolloids, University of Life Science in Lublin, Skromna 8, 20-704 Lublin, Poland
[b] Faculty of Chemistry, The Maria Curie-Skłodowska University, Plac M.C. Skłodowskiej 3, 20-031 Lublin, Poland

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ABSTRACT

In this study, we analyzed inulin crystallization during one year after its complete dissolution and an effect of inulin crystal seeds concentration on rheological and textural properties of inulin gels. 20% and 25% solutions of three different inulins, one native and two high performance (crystal and amorphous), were prepared by heating at 100 °C for 5 min. During one year of storage at 20 °C, inulin did not form a gel structure, but only precipitates and a crystal layer on the walls of the containers. Addition of crystal seeds (0.02–2%) caused formation of gel structure. Minimal concentration of the crystal seeds necessary to form a strong inulin gel was 0.4%. Crystallographic structure of inulin powder did not have an influence on the formed gels. The obtained results allow inulin gelation to control which can be crucial in novel foods, the structure of which is based on inulin.

1. Introduction

Inulin is a polyfructan consisting of fructose (F) residues usually terminated by a glucose (G) moiety. Its simplified formula can be described as GF*n* and F*m*, where *n* or *m* is between 2 and 60, and represents the number of fructose units (Ronkart et al., 2006). Industrially inulin is mainly produced from chicory (Franck, 2002). Global production of inulin is estimated at 100 000 tons per year. Inulin is applied in food industry as sugar and fat substitute as well as gelling substance or bulking agent. Substitution of fat by inulin was successfully applied in milk drinks, cheese and meat products (Hennelly, Dunne, Sullivan, & Riordan, 2006; Mendoza, Garcia, Casas, & Selgas., 2001; Mittal & Bajwa, 2012). Such substitution caused significant decrease in caloric value of the final product (Glibowski & Kowalska, 2012; Mittal & Bajwa, 2012). Due to β (2–1) glycosidic bonds, inulin is not digestible by human enzymes and is classified as a soluble dietary fiber (EFSA, 2010). Moreover, inulin has prebiotic properties stimulating growth of pro-healthy bacteria in the colon (Gibson & Roberfroid, 1995).

Usually, inulin is purchased as a white powder, however sometimes it can be sold in a syrup form. The most important parameter describing inulin properties is average degree of polymerization (DP). Oligofructose (OF), native inulin and high polymerized (high performance) inulin has DP about 4, 10 and 23 respectively. Native inulin and OF are slightly sweet (up to 30% of sucrose) thus HP inulin is not sweet at all (Franck, 2002).

15–20% solution of HP inulin is able to form particulate gel mimicking fat (Kim, Faqih, & Wang, 2001). Besides DP and concentration, formation of inulin gel depends on pH, heating temperature and time, the presence of crystal seeds and crystallographic form of inulin powder (Glibowski & Pikus, 2011; Glibowski & Wasko, 2008). Inulin is hydrolyzed at pH below 4. The longer time, the higher temperature and the lower pH the faster hydrolysis takes place. Above pH 5, inulin is not hydrolyzed even after one hour of heating at 100 °C (Glibowski & Bukowska, 2011).

Recent studies showed that crystallographic form of inulin powder is quite important functional property (Glibowski & Pikus, 2011). Nowadays, inulin can be purchased in two forms – amorphous and semi-crystalline. Semi-crystalline form is easily dispersed. Fast addition of amorphous inulin powder to water causes very hardly dissolving clumps. This phenomenon results from a rapid bounding of water moieties by inulin to form crystal structure which energetically is more favorable than amorphous one (Glibowski & Pikus, 2011; Ronkart et al., 2009). As a consequence, dispersion of amorphous inulin powder in water at ambient temperature can cause a formation of strong inulin gel. To form similar gel, crystal inulin powder solution needs to be heated up to 72 °C (Glibowski & Pikus, 2011).

In the case of amorphous inulin, gel formation results from swelling inulin powder particles due to water binding. Crystal

* Corresponding author. Tel.: +48 81 462 3349; fax: +48 81 462 3354.
E-mail addresses: glibowskipawel@up.lublin.pl, glibowskipawel@wp.pl (P. Glibowski), stanislaw.pikus@poczta.umcs.lublin.pl (S. Pikus), justyna.jurek@gmail.com (J. Jurek), marcin.kotowoda@gmail.com (M. Kotowoda).

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inulin powder forms a weak gel when dispersed at ambient temperature, probably because the crystals already exist in the powder and the formed gel structure is based on much less connection points between the present crystals than the primary structure formed by amorphous inulin. To form gel, crystal inulin has to be dissolved nearly completely (Glibowski & Pikus, 2011). When inulin solution reaches sufficient concentration (about 20%), gelation is possible as long as crystal seeds are present (Bot, Erle, Vreeker, & Agterof, 2004; Glibowski & Wasko, 2008). HP inulin dissolves poorly. At 50 °C, solubility of HP inulin is 1.2%. Further temperature increase causes significant increase in solubility (Kim et al., 2001). At 70–80 °C, 20% inulin solution is clear thus some microscopic inulin particles are still not dissolved. These particles, called crystal seeds, are necessary to form white creamy inulin gel after cooling. However, excessively long heating at 80 °C and above, can cause some problems with forming the compact gel structure (Glibowski & Wasko, 2008; Kim et al., 2001). Heating of 20% inulin solution at 100 °C for a few minutes makes formation of a gel structure impossible because of complete dissolution of inulin including most resistant particles. As Glibowski and Pikus (2011) mentioned in their studies, when such a solution is stored for several weeks at room temperature, gradual inulin precipitation occurs, but the gel structure is not formed even after a year of storage. However, to the best of our knowledge, there are no studies analyzing this phenomenon more deeply.

In spite of complete dissolution of native crystal seeds, inulin gelation can be initiated by addition of a small amount of inulin. Glibowski and Pikus (2011) showed that 5% addition of inulin (percentage calculated as a share in the total amount of inulin present in the sample) allowed the solution to turn into gel. These results were confirmed by Arcia, Navarro, Costell, and Tárrega (2012) in their studies concerning model desserts. They proved that seeding technique favored a faster formation of a greater amount and more regular sized inulin particles.

Because the knowledge about inulin crystallization in water environment is still lacking, the aim of this study was the estimation of inulin crystallization dynamic during one year after its complete dissolution and analysis of the impact of inulin crystal seeds concentration on rheological and textural properties of inulin gels.

2. Experimental

2.1. Materials

Inulin Frutafit® IQ (IQ) and Frutafit® TEX! (TEX) were kindly delivered by Sensus Operations C.V. (Roosendaal, The Netherlands). Inulin Beneo™ HP (HP) and Beneo™ HPX (HPX) were purchased from Orafti (Oreyle, Belgium). Inulins were extracted from chicory root and their average degree of polymerization is ≥23 with the exception of native inulin Frutafit® IQ with DP 9-12 (producer's data).

2.2. Preparation of samples

2.2.1. Long term observation

Inulin IQ, TEX and HPX (20% and 25%) was suspended in distilled water (20 °C) using a magnetic stirrer. To avoid clump formation inulin was gradually added to the beaker, which took approximately 2 min. The suspensions were stirred for 5 min. Subsequently, inulin suspensions were heated in conical flasks on an electric cooker up to boiling and boiled 5 min. After heating, the evaporated water was filled up with hot distilled water. Afterwards, the solutions were poured into plastic cylindrical containers of 35 mm inner diameter and the lids were twisted on to prevent evaporation. The containers were stored at 20 °C in a thermostatic cabinet. The whole procedure was repeated three times for each inulin and concentration.

2.2.2. Effect of crystal seeds addition on inulin gel formation

Inulin HP and HPX (18–20%) was suspended in distilled water as it was described in Section 2.2.1. The suspensions were heated up to boiling point and boiled for 5 min. Subsequently, the evaporated water was filled up with hot water and the solutions were cooled in a tap water to 40 °C. Afterwards, further inulin was added to reach 20% concentration in the final samples. The additional inulin, called crystal seeds, was added in the same way as it was described earlier. After 5 min of stirring, the solutions were poured into containers and stored as it was described above. The samples were analyzed after one week of storage. Some of the solutions were poured into rheometer cup for monitoring of gelation process.

2.3. Rheological measurements

Rheological measurements were conducted using a Haake RS 300 rheometer (Haake, Karlsruhe, Germany). Temperature control was maintained by a Haake DC30 circulator water bath (Haake, Karlsruhe, Germany). All rheological data were collected and calculated by Haake Rheowin software version 3.61.0004 (Haake, Karlsruhe, Germany). All measurements were done at 20 °C. The apparent viscosity was measured at 20 s^{-1} shear rate for 120 s. For analytical purposes the average value was calculated from the 90th, 105th and 120th second of measurement (Glibowski, Zarzycki & Krzepkowska, 2008). Dynamic oscillatory rheological measurements were conducted using parallel plate geometry (both 35 mm diameter and serrated). These measurements were carried out at 1 mm gap. Dynamics of the gelation process were conducted using rotating vane (22 mm diameter, 112 mm height). Measurement began when the sample was poured into the cup, 7 ml of oil was put on the surface of the sample to prevent evaporation, the lift moved and the vane took the measuring position (8 mm clearance to bottom). The oscillatory measurements were conducted at a frequency of 1.0 Hz and a strain of 0.001. Used strain corresponded to the maximum found within the linear viscoelastic region of the studied material (Glibowski & Pikus, 2011).

2.4. Hardness analysis

Hardness analyses were performed according to the method previously described by Glibowski (2009). Briefly, samples were punched by a cylindrical stainless steel probe (1 cm diameter) with the crosshead speed 1 mm s^{-1} at 15 mm depth, using a TA-XT2i texture analyzer (Stable Microsystems, Goaldming, England). The maximal peak value after punching the sample 15 mm down was considered as gel hardness. The analysis was performed without removing the samples from the containers.

2.5. Wide angle X-ray scattering

The wide angle X-ray scattering (WAXS) investigations were performed according to the method previously described by Glibowski and Pikus (2011). Briefly, we used Seifert URD-6 diffraction instrument. X-ray diffraction patterns were taken within the range of 2θ from 6° to 35°, at the increments of 0.02°, and at counting intervals of 6 s. Crystallinity indexes were calculated according to Ronkart et al. (2007) from the ratio of the integrated intensity of the crystalline peaks to the total integrated intensity of coherent scattering after appropriate baseline subtraction.

2.6. Dry matter

Dry matter content in inulin solutions was analyzed by oven drying for 15 h at 60 °C. For this purpose 5 ml of clear solution was taken with a pipet from above the precipitated inulin and poured into glass dried, previously weighted containers. Subsequently, containers with inulin solutions were weighted and put into oven.

2.7. Photographs

Photographs were taken using Coolpix L27 camera (Nikon, Tokyo, Japan).

2.8. Statistical analysis

The data were analyzed by means of the Statistical Analysis System (SAS Enterprise Guide 3.0.3.414) using the ANOVA procedure for analysis of variance and Student–Newman–Keuls *t*-test for ranking the means.

3. Results and discussion

3.1. Effect of storage time on inulin gelation after its complete dissolution

Boiling of inulin solutions for 5 min affected on the lack of compact gel structure throughout a year of storage (Fig. 1). Such a structure was not formed both for 20% and 25% inulin solutions. Although these concentrations can be not sufficient for inulin with low DP (IQ) because solutions containing native inulin do not form compact gels up to 30% (Chiavaro, Vittadini, & Corradini, 2007), however even samples containing HP inulin did not form any gel. Crystallographic structure of HP inulin did not matter as well, both solutions prepared from amorphous (TEX) and semi-crystal (HPX) inulin powder did not form gel structure. Storage at room temperature for one day did not cause even any sediment precipitation in most samples except TEX sample containing 25% inulin. Further storage affected the precipitation, though no noticeable differences were seen between the samples stored 4 and 12 months. Concentration of stored solutions seemed to be more important. After visual assessment, it can be said that the level of the sediment in samples containing 25% inulin was higher which was confirmed by further analysis.

Inulin in water environment is able to form gel structure, however under several conditions. Heating of 20% inulin solutions at 70–80 °C causes its almost complete dissolution. Only small amount of inulin remains not dissolved (Bot et al., 2004; Glibowski & Wasko, 2008) and able to cause complete or partial gelation (Kim et al., 2001). But when inulin solution is heated at 100 °C for 5 min, at concentrations up to 25%, inulin is completely dissolved. For this reason, formation of gel structure is not possible, which was proved previously with 20% solutions (Glibowski & Pikus, 2011).

Another factor which could have an influence on gel formation was the storage temperature. Glibowski and Wasko (2008) studied gelation of inulin solutions stored at 5 and 20 °C and proved that the storage temperature did not significantly affect the formed gels.

3.2. Dry mass content

Inulin in the stored containers did not form only sediments seen on Fig. 1. A part of inulin formed a transparent layer on the walls of the containers. In order to analyze which part of inulin was precipitated, the content of inulin in the solution above the sediments was measured (Table 1). Statistical analysis showed significant ($p \leq 0.05$) differences between the content of dry mass between samples stored one day (storage time 0 month) and those stored 4,

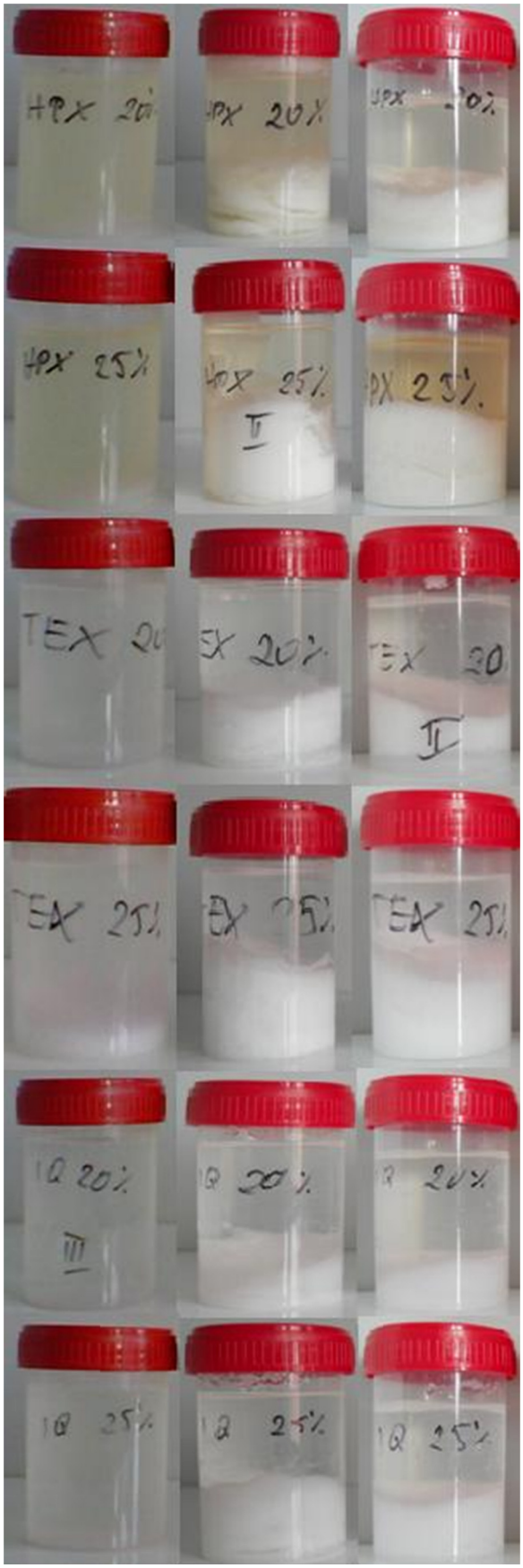

Fig. 1. Samples of HPX, TEX and IQ inulin after 1 day (left column), 4 months (middle column) and 12 months (right column) of storage at 21 °C.

Table 1
Dry mass content in the inulin solution above sediment.[A]

Inulin concentration (%)	Storage time (month)	Dry mass (%)		
		IQ	TEX	HPX
20	0	19.58[a]	18.71[b]	16.07[c]
	4	12.18[d]	4.12[e]	3.56[e]
	8	12.13[d]	3.67[e]	3.49[e]
	12	12.80[d]	3.80[e]	3.78[e]
25	0	23.72[a]	15.56[c]	17.73[b]
	4	15.05[c]	4.50[d]	4.50[d]
	8	15.10[c]	4.50[d]	3.77[d]
	12	15.90[c]	4.80[d]	4.17[d]

[A] Values are means. Means with different superscript letters for the same inulin concentration are significantly different, $p \leq 0.05$.

8 and 12 months. The differences were seen both for 20% and 25% solutions. Dry mass content was not affected by crystallographic structure of HP inulin. An average DP had significant importance. Native inulin solutions contained higher ($p \leq 0.05$) dry mass level. It was caused probably by the higher content of well dissolved sugars like fructose, sucrose and glucose, the concentration of which can reach even 8%, in the contrary to HP inulin where it is up to 0.5% of dry mass (Franck, 2002).

3.3. Crystallographic changes

The recent studies demonstrate that amorphous inulin changes its structure to semi-crystalline in a water environment (Glibowski & Pikus, 2011; Ronkart et al., 2009). In the case of inulin with semi-crystalline structure, decrease in the share of the crystal structures was demonstrated (Glibowski & Pikus, 2011). The results of crystallinity index (CI) of inulin powders and transparent layers deposited on the walls of containers containing inulin solutions stored for different periods are given in Table 2. The CI values exceeded 70% for most samples. Twelve months storage caused a little increase in CI values in comparison to four months of sample storage. Since energetically, crystalline structure is more stable than amorphous one (Ronkart et al., 2009) a noteworthy change in CI value can be noticed in the case of amorphous inulin powders. A little decrease in CI value of HPX inulin powder probably resulted from high water content. Inulin powder was completely dissolved, and the semi-crystalline structure was built anew in the samples containing 80% water.

Analysis of CI was carried out to show changes in a deposited transparent layer during storage. The obtained results suggest that the biggest change takes place during first four months. However, earlier analysis could not be done because the layer had been forming for several weeks.

The main reason of a lack of a compact gel structure in the case of HP inulin was the absence of crystal seeds, while in the case of native inulin, too low inulin concentration was the additional factor enhancing gelation. Inulin precipitation on the bottom and deposition on the walls of containers was a consequence of its solubility. Depending on a source of information, solubility of native and HP inulin at ambient temperature is not higher than 2.5% and 12% respectively (Franck, 2002; Kim et al., 2001). Storage of oversaturated solution for a long time caused precipitation of inulin excess.

Table 2
Crystallinity index changes of inulin sediments during storage.

Inulin concentration (%)	Storage time (month)	Crystallinity index (%)		
		IQ	TEX	HPX
Powder	–	0.0	0.0	86.0
20	4	66.8	73.3	74.6
	12	84.2	74.6	79.0
25	4	72.3	78.9	73.4
	12	76.1	84.4	78.9

3.4. Hardness analysis

In the previous studies, it was concluded that gel structure can be formed by inulin when crystal seed are added (Glibowski & Pikus, 2011). But a question about minimal concentration necessary to form inulin gel was open. Results of hardness analysis of 20% inulin gels formed after inulin seeds addition are given in Table 3. We analyzed effect of concentration and crystalline structure on the final gel's hardness. It is necessary to mention that the collected data present in Table 3 is a final effect of the two-step experiment. In the first step, we analyzed the addition of crystal seeds in the range 0.1–2.0%. When it turned out that even 0.1% addition (0.5% of the total inulin amount present in the sample) initiate forming compact gel structure, in the second step, we analyzed 0.08–0.02% addition (0.4–0.1% of the total inulin). Hardness of the inulin gels significantly ($p \leq 0.05$) depended on concentration of crystal seeds addition. An increase in crystal seeds concentration caused formation of harder inulin gel for each analyzed set – HP/HP, HP/HPX, HPX/HP, HPX/HPX (inulin used for preparation of basic solution/inulin used as crystal seeds). In most cases, 0.4% and higher crystal seeds addition did not significantly influence ($p \leq 0.05$) on inulin gels hardness. Analysis of the effect of crystallographic form at the same crystal seeds addition did not reveal unambiguous tendencies. What was noteworthy, in most analyzed sets, crystallographic form did not affect hardness significantly ($p \leq 0.05$) at crystal seeds addition above 0.2%.

Crystallographic structure is important during dissolving or rather addition inulin powder to water. Since crystalline inulin has water molecules built in the structure (Ronkart et al., 2009), such inulin powders can easily form suspensions in water at ambient temperature. Different behavior can be observed in case of amorphous inulin that has a tendency to form clumps at high humidity. However, after dissolving at high temperature (above 80 °C), crystallographic structure of the inulin powder is not important at all (Glibowski & Pikus, 2011).

Although inulin was completely dissolved in the basic solution, crystal seeds were added after cooling the basic inulin solution to 40 °C. We can assume that the added inulin was not dissolved completely, especially at 1–2% addition. Kim et al. (2001) showed that solubility of HP inulin at 50 °C is 1.25%. This is in agreement with visual assessment of the analyzed solution in our study. Inulin solution after addition of 1–2% crystal seeds was a little hazy. When the concentration was lower than 1%, this haziness was barely seen or the solutions were completely clear.

Statistical analysis indicates that crystallographic structure of the crystal seeds does not influence on gel hardness ($p \leq 0.05$). Gel formation is possible because inulin powder after addition as a seeding material suspends in the solution, which is clearly seen when it forms a haze. In this way dissolved inulin has a lot of contact points to built three dimensional gel structure.

3.5. Rheological analysis

Rheological analysis of inulin gels induced by 0.2%, 0.6% and 1% crystal seeds addition showed that both apparent viscosity as well as loss and storage moduli did not significantly change ($p \leq 0.05$) (Table 4). As it was in the case of hardness analysis, crystallographic structure of inulin was not important. Analysis of loss and storage moduli shows characteristic difference between their values

Table 3
Hardness (N) of the samples containing 20% inulin as affected by concentration and type of crystal seeds.[A]

Concentration of crystal seeds (%)	Type of inulin used for preparation of basic solution			
	HP		HPX	
	Type of inulin used as crystal seeds			
	HP	HPX	HP	HPX
0.02	$0.886^{c,BC}$	$1.157^{b,A}$	$0.761^{b,C}$	$1.025^{d,AB}$
0.04	$0.854^{c,B}$	$1.064^{b,A}$	$1.052^{b,A}$	$1.100^{cd,A}$
0.06	$1.032^{c,C}$	$1.196^{b,B}$	$1.291^{b,A}$	$1.200^{cd,B}$
0.08	$1.204^{c,A}$	$1.085^{b,A}$	$1.179^{b,A}$	$1.418^{cd,A}$
0.1	$1.248^{c,B}$	$1.525^{b,AB}$	$1.475^{b,AB}$	$1.688^{bcd,A}$
0.2	$1.169^{c,A}$	$1.788^{a,A}$	$1.520^{b,A}$	$1.800^{bcd,A}$
0.4	$2.716^{b,A}$	$3.797^{a,A}$	$2.922^{a,A}$	$4.288^{a,A}$
0.6	$3.042^{b,A}$	$3.769^{a,A}$	$3.758^{a,A}$	$3.315^{ac,A}$
0.8	$3.222^{b,A}$	$4.020^{a,A}$	$3.334^{a,A}$	$3.808^{a,A}$
1	$3.462^{b,A}$	$3.284^{a,A}$	$3.825^{a,A}$	$3.790^{a,A}$
2	$5.323^{a,A}$	$2.481^{ab,B}$	$4.146^{a,AB}$	$2.713^{abcd,B}$

[A] Data are presented as means. Means in the same column with different superscript small letters and in the same row with different superscript capital letters are significantly different, $p \leq 0.05$.

Table 4
Rheological parameters of the samples contained 20% inulin as affected by concentration and type of crystal seeds.[A]

Concentration of crystal seeds (%)	Type of inulin used for preparation of basic solution	Type of inulin used as crystal seeds	Apparent viscosity (Pa s)	G' (Pa)	G'' (Pa)
0.2	HP	HP	2138[a]	13,275[a]	1552[a]
		HPX	3886[a]	24,170[a]	3469[a]
	HPX	HP	2335[a]	14,545[a]	1920[a]
		HPX	4009[a]	24,940[a]	3489[a]
0.6	HP	HP	4962[a]	30,865[a]	4385[a]
		HPX	5552[a]	34,275[a]	6494[a]
	HPX	HP	4407[a]	27,395[a]	4004[a]
		HPX	4417[a]	27,435[a]	4174[a]
1	HP	HP	6075[a]	37,555[a]	6807[a]
		HPX	4370[a]	27,070[a]	4595[a]
	HPX	HP	5774[a]	35,765[a]	6054[a]
		HPX	4699[a]	29,160[a]	4579[a]

[A] Values are means. Means in the same column with different superscript letters are significantly different, $p \leq 0.05$.

($G' > G''$), which is typical for more elastic than viscous material (Glibowski, 2009, 2010; Zimeri & Kokini, 2003).

To illustrate the dynamic of inulin gel structure formation, we registered changes in G' and G'' moduli of 20% solution with or without crystal seeds addition (Fig. 2). The samples containing crystal seeds form gel structures (G' values are higher than G''). As we expected, formation of the gel structure was faster at 2% crystal seeds addition (crossover in 81st minute) than at 0.02% (crossover in 140th minute). Control sample revealed more viscous than elastic behavior ($G' < G''$), which was earlier demonstrated (Fig. 1). Faster gel formation for the samples containing more seeding material probably is caused by larger number of contact points necessary to form three dimensional gel structure (Glibowski & Pikus, 2011).

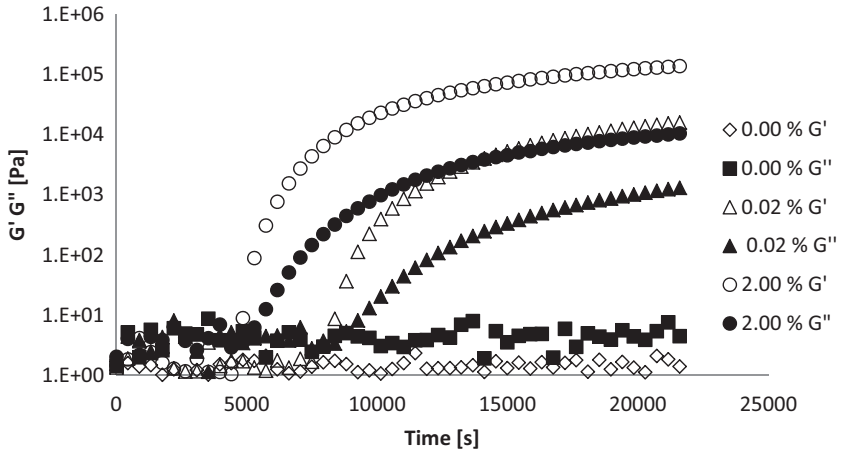

Fig. 2. Storage (G') and loss (G'') modulus changes for 20% inulin solutions with different seeding addition.

4. Conclusions

Gel formation by inulin in water solution depends on the presence of crystal seeds. It was important to establish minimal crystal seeds concentration necessary to form stable gel structure after previous complete inulin dissolution. Although a weak gel structure can be formed at 0.02% addition of crystal seed, strong stable gels are formed at 0.4% addition at least; however, the hardness of gels did not significantly change at higher addition. Gel formation is not influenced by crystallographic structure of the inulin with high DP when it is completely dissolved. Both amorphous and crystal inulin can form gels when there is enough seeding material in the solution. Crystallographic structure of crystal seeds is also unimportant. In order to maintain microbiological stability of the food product heating at 80–100 °C is a common practice in food industry. In the case of inulin, application of such temperatures could deteriorate its gelation properties or make it impossible. For this reason, estimation of the type and minimal concentration of the crystal seeds was important; especially in products which structure is based on inulin like desserts or margarines.

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