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Amorphous and crystal inulin behavior in a water environment

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ABSTRACT

The aim of present study was rheological analysis of gels obtained from different kinds of high performance inulins and analysis of an effect of crystal seeds addition on inulin gel formation. Inulin gels (20%) were produced at different temperatures. Wide-angle X-ray scattering allowed for monitoring the crystallographic changes in used inulins. A light microscopy enabled to register the inulin particles reaction in water during the first seconds. Amorphous inulin forms, in contrast to crystal one, stable crystal structure in water at 20 °C. The presence of crystal seeds is essential to form gel structure at temperatures above 70 °C. These crystal seeds occur naturally as not dissolved inulin particles (at 72 °C) or must be added when the inulin solution is heated at 100 °C. Five per cent of a crystal or amorphous inulin added to a cooled preheated inulin solution allows forming a stable gel structure.

1. Introduction

Inulin is often used as a texturizing agent and fat replacer in low-fat food systems (Tungland & Meyer, 2002). From nutritional point of view inulin is a dietary fiber (Hond, Geypens, & Goos, 2000) and exhibits a prebiotic function of stimulating the growth of *Bifidobacterium* sp. in large intestine (Roberfroid, van Loo, & Gibson, 1998). Inulin also enhances calcium absorption (Griffin, Hicks, Heaney, & Abrams, 2003) and reduces the level of cholesterol (Liong & Shah, 2005).

Commercially available inulin is produced from chicory roots in the powder form (Franck, 2002). Native chicory inulin chains vary from 2 to 60 fructose molecules connected by $\beta(2 \rightarrow 1)$ glycoside bonds. Most of inulin chains have a glucose molecule at one end of the chain (Ronkart, Blecker, et al., 2006). An average DP (degree of polymerization) of a native inulin is 10–12 however during manufacturing process involving purification and removing low DP's fractions a high performance (HP) inulin with DP 23–25 can be produced (Franck, 2002).

HP inulin is hardly soluble in water at 20–25 °C. Temperature increase of a water solvent causes the increase of HP inulin solubility up to 34% at 90 °C (Kim, Faqih, & Wang, 2001). Inulin dissolution is not necessary to obtain a gel. Fifteen per cent of inulin suspension is able to form three-dimensional particulate gel network that extend through out the volume of the container (Roberfroid, 2005). Inulin suspensions have milky color. Temperature increase turns the suspension into a clear yellowish solution. Depending on the heating time and temperature the inulin solution can form a strong, weak or a partial gel, or does not form gel at all (Glibowski & Wasko, 2008; Kim et al., 2001).

Gelation mechanism is induced by the crystal seeds presence. Heating inulin solution at high temperatures can cause a complete dissolution of crystal seeds and as a consequence, stopping the gelation process (Bot, Erle, Vreeker, & Agterof, 2004). These observations have been confirmed in other studies (Glibowski & Wasko, 2008; Kim et al., 2001). However, to the best of our knowledge there is a lack of information available on the effect of crystal seeds addition on the inulin gelation.

The process of commercially available inulin powders production involves extracting inulin from chicory roots by diffusion in hot water and after many purification steps spray drying (Franck, 2002; Roberfroid, 2005). When the inlet temperature of air during spray drying reaches 230 °C, inulin is completely amorphous. Storing amorphous inulin at 75% relative humidity causes formation of crystals (Ronkart et al., 2009) and caking phenomena. Probably the same phenomena can be observed during inulin addition to water. Fast addition of amorphous inulin powder to water causes clumps formations (Glibowski, 2009, 2010). These clumps are very hard to dissolve or disperse. Ronkart et al. (2009) when testing a low temperature of inulin suspension designed for drying and low inlet temperature of drying air, obtained inulin in a crystal form. To the best of our knowledge there is no study which compares rheological properties of gels achieved from amorphous and crystal HP inulins. Besides there is no study analyzing the effect of initiation

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the inulin gelation by crystal seeds addition thus, the aim of present study was the rheological analysis of gels produced from different kinds of HP inulins and the analysis of an effect of crystal seeds addition on inulin gel formation. This study also contains the first micrographs picturing of inulin particles reaction in water during the first seconds.

2. Experimental

2.1. Materials

Inulin Frutafit® TEX! was kindly delivered by Sensus Operations C.V. (Roosendaal, The Netherlands). Inulin Beneo™ HPX was purchased from Orafti (Oreyle, Belgium). Inulins were extracted from chicory root and its average degree of polymerization is ≥23 (producer's data).

2.2. Preparation of samples

2.2.1. Effect of filtration on inulin gels texture

Inulin (20%) was suspended in distilled water (20 °C) using a magnetic stirrer. This step took approximately 2 min. To avoid clump formation inulin was sprinkled to the beaker through a sieve. The suspensions were stirred for 5 min. Subsequently inulin suspensions were heated in conical flasks in a water bath up to 72 °C at heating rate 25 °C min^{-1}. Temperature was controlled by a laboratory thermometer. Immediately after heating the evaporated water was filled up and the inulin solutions were filtered through filter membranes with a pore size 0.2, 0.45 and 1.0 μm (Carl Roth GmbH, Karlsruhe, Germany). 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 for 21 h at 20 °C in a thermostatic cabinet.

2.2.2. Effect of preparation temperature on viscoelastic properties of the inulin gels

Inulin (20%) was suspended in distilled water and as it was described in Section 2.2.1. Some suspensions were heated up to 72 °C. The heated samples were cooled in a tap water to 20 °C. Afterwards the solution/suspension was poured into a rheometer cup.

2.2.3. Effect of crystal seeds addition on inulin gel formation

Inulin (19%) was suspended in distilled water and 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 20 °C. Afterwards further inulin was added to reach 20% concentration in the final samples. The additional inulin, called crystal seeds, was added 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 in Section 2.2.1.

2.3. Light microscopy

Changes of inulin particles after contact with water were observed with a light microscope Studar Z1 (PZO, Warsaw, Poland) equipped with Escar camera (Elemis, Warsaw, Poland) at resolution 480 × 640 pixels. The micrographs were registered by Winam Suite software (ELSA AG, Aachen, Germany).

2.4. 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). Dynamic oscillatory rheological measurements were conducted using a concentric-cylinder-fixed cup (43 mm diameter) and 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 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 (Zimeri & Kokini, 2003).

2.5. Texture 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.

2.6. Wide-angle X-ray scattering

The wide-angle X-ray scattering (WAXS) investigations involved the use of Seifert URD-6 diffraction instrument. The radiation of Cu Kα and the nickel filter were employed. The operating conditions for the instrument were as follows: accelerating voltage −40 kV, anode current −30 mA. X-ray diffraction patterns were taken within the range of 2θ from 4° to 50°, at the increments of 0.02°, and at counting intervals of 6 s.

2.7. Statistical analysis

The data were analyzed by 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 filtration on inulin gels texture

Inulin gelation/crystallisation depends on the presence off crystal seeds (Bot et al., 2004). For this reason filtration was applied to examine the effect of pore size on inulin gels hardness (Table 1). Theoretically the filter membranes let a passage of particles with dimensions up to 0.2, 0.45 and 1.0 μm. Statistical analysis showed that filtration through the membranes with pore size 0.45 μm and smaller significantly ($p \leq 0.05$) affected the hardness of the obtained gels. Hardness of the gels achieved after filtration through the membranes with pore size 1 μm or without filtration was significantly higher. The presence of bigger and/or greater amount of crystal seeds probably allowed obtaining more stable gel structures. Studying the phenomena of inulin gelation Bot et al. (2004) estimated the size of not dissolved crystalline inulin particles which formed a haze in an inulin solution, in the range of 50–100 nm. In our study we did not see any haze at 72 °C. In the quoted study, an average inulin DP was about 10 and the authors estimated the size after filtration. The size of the inulin particles in commercial powders is about two-, three-fold higher, usually in the range of 30–200 μm (Glibowski & Wasko, 2008).

Filtration was employed to solutions obtained from inulin TEX with an amorphous structure (Fig. 1). Inulin HPX with crystal struc-

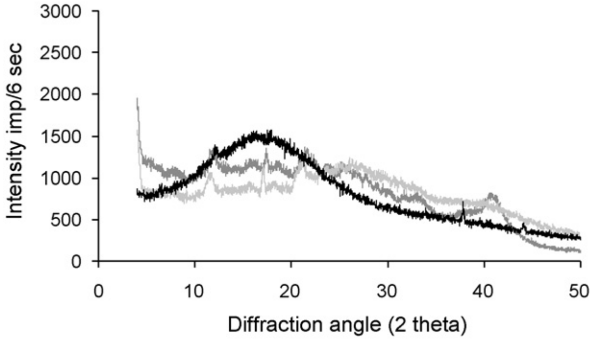

Fig. 1. Wide-angle X-ray scattering diffractograms of inulin TEX: powder (black line), gel obtained at 20 °C (dark grey line) and 72 °C (light grey line).

Table 1
Hardness (*N*) of the samples contained 20% inulin as affected by filtration[a].

Pore size (μm)	TEX	HPX
Not filtrated	$0.863^{abA} \pm 0.041$	$0.816^{A} \pm 0.044$
1.0	$0.963^{a} \pm 0.093$	–
0.45	$0.794^{b} \pm 0.035$	–
0.2	$0.748^{b} \pm 0.156$	–

[a] Data are presented as means ± standard deviation. Means in the same column with different lowercase superscript letters are significantly different ($p < 0.05$). Means in the same row with different uppercase superscript letters are significantly different ($p < 0.05$). A dash shows not analyzed sample.

ture did not form clear solutions but milky suspensions at 72 °C. Viscosity of these suspensions increased significantly during filtration which made the process impossible. The differences in crystallographic structure did not influence significantly ($p \leq 0.05$) on the hardness of the gels obtained without filtration (Table 1).

3.2. *Effect of crystal seeds addition on inulin gel formation*

Heating 20% inulin solution for 5 min at 100 °C caused completely lack of gel structure (Table 2) or even inulin in a sediment form. These results are in agreement with other studies (Bot et al., 2004; Kim et al., 2001). The gel structure did not form regardless of the amorphous or crystal structure of the inulin powder used. However, a small addition of inulin (5% of the total inulin amount present in the sample) to the cooled preheated inulin solution caused formation of a stable gel structure. This addition was called in this study crystal seeds because it initiated crystallisation/gelation of inulin solutions. The hardness of gels produced after crystal seeds addition did not significantly ($p \leq 0.05$) differ regardless their crystallographic structure (amorphous or crystal). The structure of the inulin which was used to prepare the heated solutions (95% of the total inulin amount present in the sample) did not significantly influenced on the hardness either. On the other hand, a considerable ($p \leq 0.05$) higher and lower value of hardness for the gels obtained at 20 °C from TEX and HPX inulin respectively is seen.

Table 2
Hardness (*N*) of the samples contained 20% inulin as affected by crystal seeds addition[a].

Preparation temperature °C	Crystal seeds addition	TEX	HPX
20	None	$3.529^{a} \pm 0.171$	$0.113^{c} \pm 0.005$
	None	–	–
100	Amorphous	$1.990^{b} \pm 0.104$	$1.885^{b} \pm 0.256$
	Crystal	$1.990^{b} \pm 0.151$	$1.983^{b} \pm 0.188$

[a] Data are presented as means ± standard deviation. Means with different superscript letters are significantly different ($p < 0.05$). A dash shows non-gelled sample.

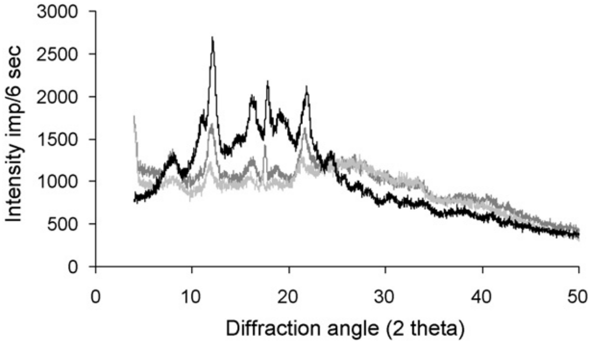

Fig. 2. Wide-angle X-ray scattering diffractograms of inulin HPX: powder (black line), gel obtained at 20 °C (dark grey line) and 72 °C (light grey line).

3.3. *Effect of preparation temperature on viscoelastic properties of the inulin gels*

The differences observed in the HPX and TEX inulin gels hardness are very prominent during monitoring of the gelation process (Fig. 3). HPX inulin forms much weaker gel at 20 °C than a gel obtained from inulin with an amorphous structure. The storage modulus values for a TEX sample during the last seconds of the measurements are about million Pascals, thus for HPX sample — about 26 thousands. The gel preparation temperature increase to 72 °C caused about 10-fold increase of the G' value for HPX and significant decrease for TEX inulin (227,000 and 31,000 Pa in the last seconds of the measurements respectively). Quite fast formation of a gel structure after heating HPX inulin solution at 72 °C deserves a special notification. A clear distinction of loss and storage moduli in the case of inulin TEX, which tells about domination of the elastic over viscous features, can be seen only after about 3 h of a measurement.

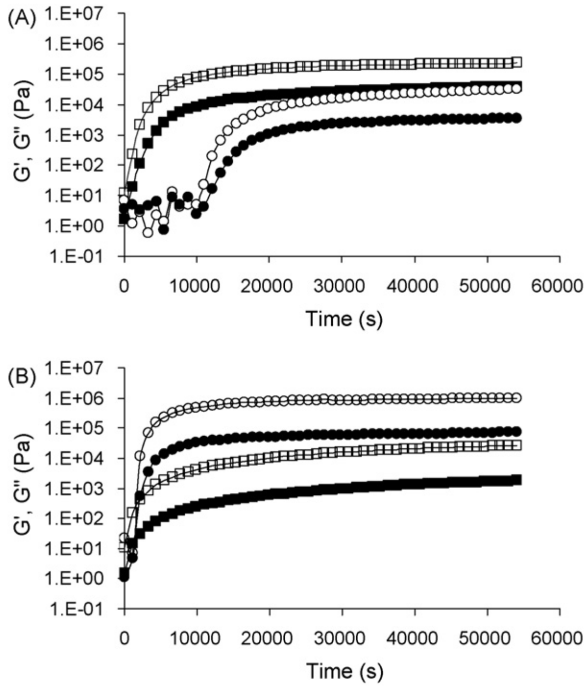

Fig. 3. Changes in loss (G'') and storage moduli (G') for 20% HPX (□■) and TEX (○●) suspensions/solutions obtained at (A) 72 °C and (B) 20 °C. Open symbols represent storage modulus (G'), closed symbols represent loss modulus (G''). Three replicate measurements of each curve were performed with little variation, only one example is displayed.

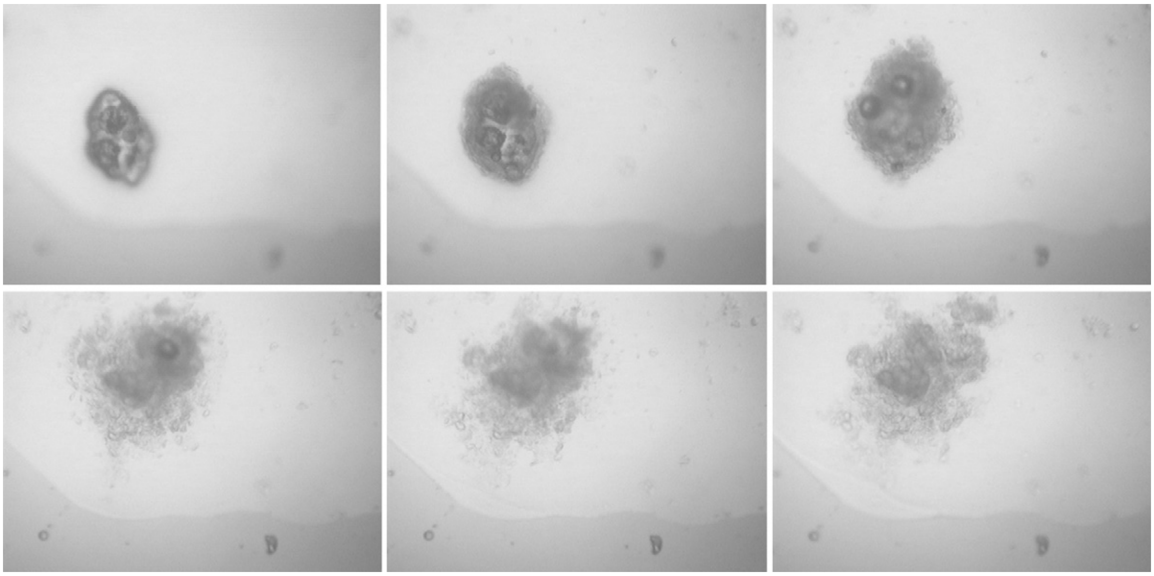

Fig. 4. Micrographs of HPX inulin particle during the very first seconds after contact with water. Top row from the left 1, 2 and 10 s after contact with water, bottom row from the left 18, 30 and 43 s after contact with water. Image width 50 μm.

3.4. *Wide-angle X-ray scattering*

For better understanding the mechanism, which inulin undergoes in a water environment, inulin powders and obtained gels were analyzed using a wide-angle X-ray scattering (WAXS) technique. Fig. 1 shows inulin TEX diffractograms. Within analyzed range inulin TEX powder has a broad halo pattern which is characteristic for amorphous substances. The WAXS pattern for the inulin present in gel obtained from TEX inulin at 20 °C is radically different and has diffraction peaks characteristic for semi-crystalline materials (Ronkart, Blecker, et al., 2006; Ronkart, Paquot, et al., 2006). This difference is caused by the presence of water, which enables inulin to change its structure from an amorphous to a semi-crystal one. Inulin stored at high relative humidity changed its structure in the same way (Ronkart et al., 2009). The decreasing intensities of the peaks in gel obtained at 72 °C prove a lesser share of the crystal structure. The drop in the share of crystal structures in the inulin samples heated at high temperatures was also noticed by Ronkart et al. (2007). Heating inulin solution at 90 °C and drying at 120 °C caused significant increase in the amorphous index as compared to dispersions heated at 40 °C.

Diffractograms representing the HPX inulin are shown in Fig. 2. The crystallinity degree of HPX inulin powder is in the high level. In the sample obtained at 20 °C, a decrease in the share of the crystal structures can be seen. An increase in the preparation temperature of the inulin gel to 72 °C deepened this tendency.

3.5. *Light microscopy*

The picture of inulin behavior in a water environment is completed by the micrographs of inulin during the first seconds after contact with water (Figs. 4 and 5). Inulin particle with a crystal structure (Fig. 4) swells and partly disintegrates into small particles with irregular shapes. In the case of inulin with an amorphous structure (Fig. 5), almost an explosion from the very first seconds can be seen. Small, regular in shape particles with 0.6–0.8 μm diameter spread into the surrounding environment. A noticeable difference in the spreading speed of the component inulin particles is caused probably by the difference in the structure construction. Amorphous particles try to build water moieties into their structures which results from more stable crystal structure (Ronkart et al., 2009). Inulin crystals contain water in the structure (Andre et al., 1996) thus the spreading speed for the inulin powder with crystal structure in a water environment is considerably lower.

3.6. *Understanding the mechanism of inulin gel formation*

Presented results allow better understanding the mechanism of inulin gel formation by inulin powders with different crystallographic structures. Inulin with a crystal structure undergoes dispersion and forms a suspension in a water environment at room temperature. Probably some of the smallest crystals dissolve. Most of the crystals do not change their structure. The crystals which did not dissolve form a weak three-dimensional network which is strengthened by dissolved inulin. These crystals have much less connection points than inulin which turns its amorphous structure into crystal one. As a consequence low values of hardness or storage modulus for crystal gels and significantly higher values for the gels formed from inulin which had an amorphous structure can be observed. Similar rheological behavior was observed in the earlier study (Glibowski, 2010). Inulin gel formed from amorphous inulin subjected to shear forces formed significant weaker gel structure after a day of storage. Rebuilding the destroyed gel structure by the semi-crystal inulin caused formation a weak gel as it is in the case of a crystal inulin. The previously formed crystals are not able to form as strong gel structure as the primary structure formed by amorphous inulin.

In the case of amorphous inulin a temperature increase makes its dissolution from a visual assessment point of view. However, some of the inulin particles do not dissolve completely and become a crystal seeds. Filtration through the membranes with pore size up to 0.45 μm partly confirms this supposition since the inulin solutions which were not filtrated or were filtrated through the membranes with pore size larger than 0.45 μm formed gels with higher hardness. A lesser amount and/or a size of the crystal seeds present in the filtrates caused formation of a weaker gel structure.

The crystal inulin in a water environment at 72 °C is partly dissolved to form a gel structure after cooling. Since we did not observe clearing the solution during temperature increase, as it was seen in case of amorphous inulin, it was obvious that only part of the inulin crystals was dissolved. The suspensions with crystal inulin cleared only at 82 °C (data not shown). The lack of a solution clearness that

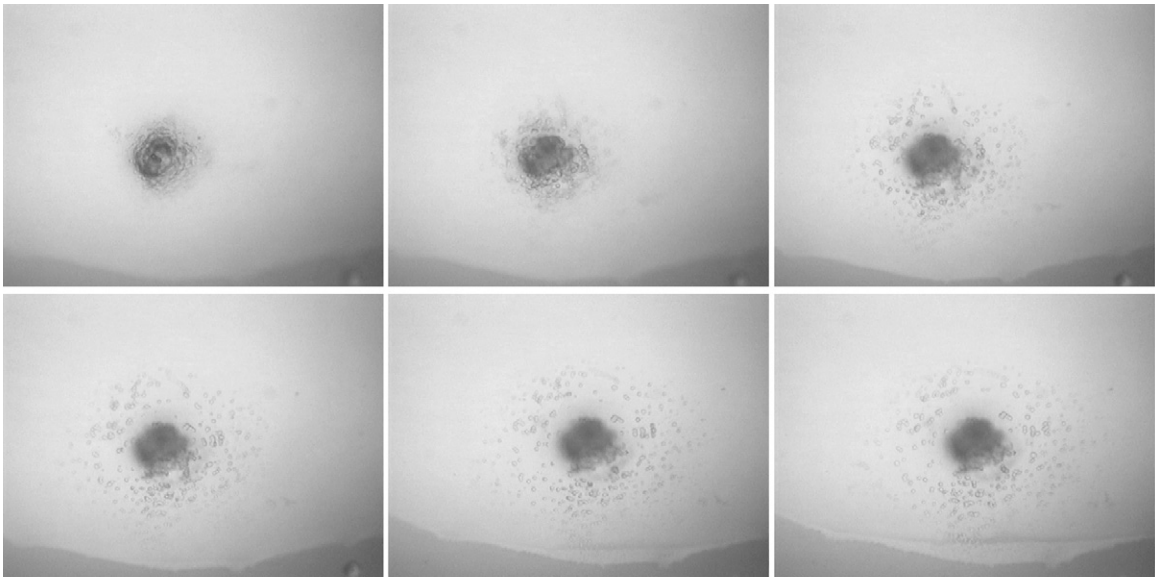

Fig. 5. Micrographs of TEX inulin particle during the very first seconds after contact with water. Top row from the left 1, 5, 8 s after contact with water, bottom row from the left 11, 15, 20 s after contact with water. Image width 50 μm.

is the presence of a part of inulin in a not dissolved form makes the formation of a gel easier which explains a greater dynamic in this process (Fig. 3).

Further temperature increase up to 100 °C causes completely inulin dissolution including crystal seeds. When heated for 5 min at 100 °C, inulin solution is not able to form gel structure regardless of the degree of structure ordering of inulin powder. Storage of such solution for several weeks at room temperature causes gradually inulin precipitation on the walls of the container in which the solution was stored however, the gel structure is not formed even after a year of storage (data not shown). Inulin addition in a crystal or an amorphous form just after heating for 5 min at 100 °C followed by cooling to 20 °C makes the dissolved moieties of inulin find points to bound and construct a gel network. A crystallographic structure of added crystal seeds is not apparently significant. Probably low, 5% share in the total amount of inulin present in the sample is the reason. Only the addition of the crystal seeds is significant.

4. Conclusions

Inulin gelation/crystallisation and rheological properties of inulin gels depends on the preparation temperature, presence of crystal seeds and the degree of structure ordering of an inulin powder. Inulin behavior in a water environment is different for crystal and amorphous inulin. Amorphous particles of inulin form stable crystal structure in water at 20 °C. Crystal inulin forms much weaker gels at this temperature, probably because the crystals already exist and the formed gel structure is based on much less connection points between the present crystals than the primary structure formed by amorphous inulin, which builds water moieties into its structures. Further temperature increase up to 72 °C causes almost complete amorphous inulin dissolution. Only the small crystallites remain not dissolved. The presence of a bigger and/or a greater amount of these crystal seeds causes an increase of the gels hardness. Crystal inulin in a water environment at 72 °C, in contrast to amorphous one, does not form clear solutions however, the formed gel is quite stable. Heating 20% inulin solution for 5 min at 100 °C regardless of the degree of structure ordering of the inulin powder causes complete lack of a gel structure. Only a small addition of crystal or amorphous inulin to a cooled, preheated inulin solution allows forming a stable gel structure.

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