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ORIGINAL ARTICLE

Effect of Inulin Seeding on Rheology and Microstructure of Prebiotic Dairy Desserts

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Abstract Long-chain inulin in the presence of water forms a particulate gel of inulin crystals that can not only improve the consistency of low-fat products, but can also be responsible for a rough sensation. The objective of this work was to study the rheological properties and microstructure of inulin-enriched desserts when using seeding to control inulin particle size. Dairy desserts were prepared with 2.5%, 5% and 7.5% of long-chain inulin, and during cooling, they were seeded with a small amount of powdered inulin. After 1, 4 and 7 days of refrigerated storage, the rheological properties and microstructure of samples were studied and compared with control (unseeded) samples. Results indicated that seeding had a significant effect on both rheological properties and microstructure of desserts. For all inulin concentrations, the seeding technique favoured a faster formation of a greater amount and more regular sized inulin particles.

Keywords Inulin · Seeding · Rheological properties · Microstructure

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Introduction

Inulin is a prebiotic dietary fibre that is included in the formulation of food products due to its nutritional or technological properties.[1] Inulin with a high polymerisation degree can be used as a thickener and a fat substitute in low-fat foods because in the presence of water inulin forms microcrystals which interact with each other, forming small aggregates that can occlude a great amount of water and create a particulate gel.[2, 3] Gel characteristics depend on factors such as inulin concentration and processing conditions that affect nucleation, crystallisation and the arrangement of inulin crystals. Duynhoven et al.[4] studied the kinetics of ageing in long-chain inulin gels through NMR cross-relaxation experiments and showed that gel firming takes longer when starting from a totally dissolved inulin solution (heated at temperatures exceeding 82 °C). Bot et al.[5] showed that the inulin crystallisation rate decreased greatly when inulin solution had been heated at temperatures near the dissolution point, and Glibowski and Wasko[6] showed that gelation was inhibited after heating inulin solutions above 80 °C while at lower temperatures solutions formed firm inulin gels. According to these authors, when an inulin solution is heated and inulin dissolves completely, a longer time must elapse before crystallisation begins than for lower preparation temperatures where the presence of crystals acts as a nucleus, facilitating faster overall growth of smaller inulin crystallites. The importance of the crystal seeds' presence on inulin gel formation was confirmed recently by Glibowski and Pikus[7] on adding inulin (amorphous or crystalline) to the preheated inulin solution as an alternative to have inulin seeds in products that have to be processed at temperatures above

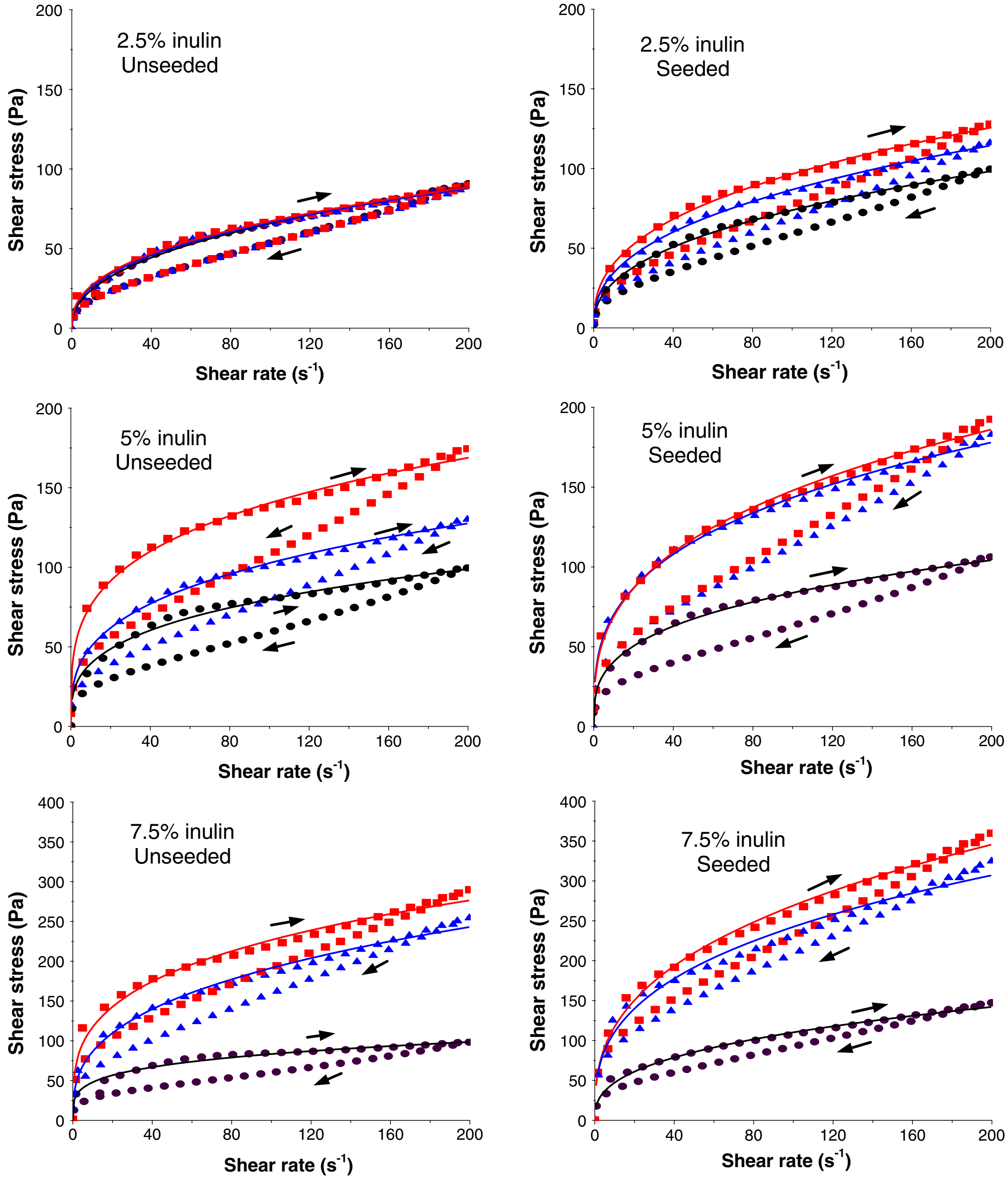


Fig. 1 Flow behaviour (*upward* and *downward curves*) of inulin-enriched dairy desserts at 10 °C. Fits to Ostwald–de Waele model (*line*) of the *upward curve*. Measurements were carried out at different storage time: 1st (*filled circle*), 4th (*filled triangle*) and 7th day (*filled square*)

80 °C. The solution inoculated with inulin showed a fast crystallisation and the formation of a stable gel, while in the non-inoculated solution, only precipitation of inulin particles was observed. All these studies have been carried out in water solutions. In dairy desserts enriched with inulin (7.5%) and prepared with thermal treatment, Torres et al.[8] showed the formation of inulin particles during a 7-day-storage period that produced an increase in both viscosity and elasticity of products. By varying inulin chain-length distribution, the characteristics of inulin particles and thus the rheological properties of products can be varied.[9] Seeding has previously been used by producers of condensed milk and “dulce de leche” to control particle size and to avoid the formation of big lactose crystal agglomerates,

Table 1 Characterisation of the flow behaviour of desserts enriched with inulin. Average values and standard deviation (n=2) for the consistency index (K) and flow index (n) obtained from Ostwald–de Waele model

Procedure	Storage time (days)	2.5% inulin		5% inulin		7.5% inulin	
		K (Pa s^n)	n	K (Pa s^n)	n	K (Pa s^n)	n
No seeding	1	9.1 (0.3)	0.44 (0.01)	20.9 (2.0)	0.31 (<0.01)	26.5 (1.6)	0.35 (0.02)
	4	9.6 (0.8)	0.40 (0.01)	25.8 (2.2)	0.31 (<0.01)	29.4 (1.1)	0.30 (0.03)
	7	11.0 (0.3)	0.40 (<0.01)	35.3 (3.5)	0.27 (0.01)	40.1 (0.6)	0.30 (<0.01)
Seeding	1	10.5 (0.9)	0.40 (0.01)	23.4 (0.3)	0.32 (0.01)	35.2 (0.5)	0.30 (<0.01)
	4	14.4 (0.9)	0.40 (<0.01)	32.2 (2.7)	0.33 (0.01)	49.5 (1.6)	0.29 (0.01)
	7	15.8 (1.4)	0.38 (<0.01)	34.0 (2.8)	0.34 (0.01)	51.1 (1.2)	0.29 (0.02)

which are partly responsible for product sandiness.[10] The aim of the present work was to study the effect of inulin seeding on the rheological properties and microstructure of low-fat desserts enriched with different concentrations of long-chain inulin (2.5%, 5% and 7.5%).

Materials and Methods

Sample Composition and Preparation

Samples were prepared using the following ingredients: long-chain inulin (Frutafit ® TEX; Sensus, Brenntag Química, Spain), skimmed milk powder (Central Lechera Asturiana, Spain), modified tapioca starch (C * CreamTex 75,720, Cargill, Spain), commercial sucrose, mineral water (Font Vella, Spain) and the following preservatives: potassium sorbate and potassium benzoate (Panreac, Quimica SA, Spain). Inulin content was varied from 2.5%, 5% and 7.5%, while contents of starch (3.75%), sucrose (6%), milk (80%) and preservatives (potassium sorbate 500 ppm; potassium benzoate 500 ppm) were constant. Skimmed milk was prepared 24 h in advance by dissolving the milk powder (13.5%) in mineral water and storing under refrigeration (4± 1 °C). Samples were prepared in batches of 800 g. Starch, sucrose, inulin and milk were weighed in a flask and mixed under magnetic stirring for 10 min. The flask was placed in a water bath at 97±1 °C and stirred constantly with a propeller stirrer for 25 min (paddle stirrer, Heidolph RZR 1, Germany). Then the sample was cooled in a water bath at 20 °C with stirring for 10 min. When samples reached a temperature of 40 °C, 1.6 g of inulin (0.2% of total sample weight) was added as inoculum. Finally, the preservatives and the water evaporated in the process, were added, and then samples were transferred to closed flasks and stored under refrigeration (4±1 °C). Desserts with the same total amounts of inulin but without inoculums were also prepared as control samples. Rheological, particle size and microscopy analyses were carried out after 1, 4, and 7 days of storage. Two replicates of each sample were prepared and measured.

Rheological Measurements

All rheological measurements were carried out at 10±1 °C with a controlled stress rheometer (RheoStress 1, Karslruhe, Germany), using a parallel-plate sensor system (6 cm diameter and 1 mm gap). Samples were left to relax for 5 min before shearing.

Flow Behaviour

Sample flow was measured by recording shear stress values when shearing the samples at linear increasing shear rates from 1 to 200 s^{-1} through 60 s and down in reverse sequence during the same time[11] using the "controlled rate" mode of the rheometer. Data from the ascending segment of the shear cycle were fitted to the Ostwald–de Waele model (Eq. 1) using Rheowin Pro software (version 2.93, Haake).

$$\sigma = \mathrm{K}\dot{\gamma}^{\mathrm{n}} \tag{1}$$

where K (Pa s^n) is the consistency index and n is the flow index. Apparent viscosity values at 10 s^{-1} were calculated using Eq. 2.

$$\eta_{10} = \mathrm{K}\dot{\gamma}^{(n-1)} \quad \text{for } \dot{\gamma} = 10 \tag{2}$$

Viscoelastic Properties

Stress sweeps were run at 1 Hz to determine the linear viscoelastic region. The frequency sweeps were performed

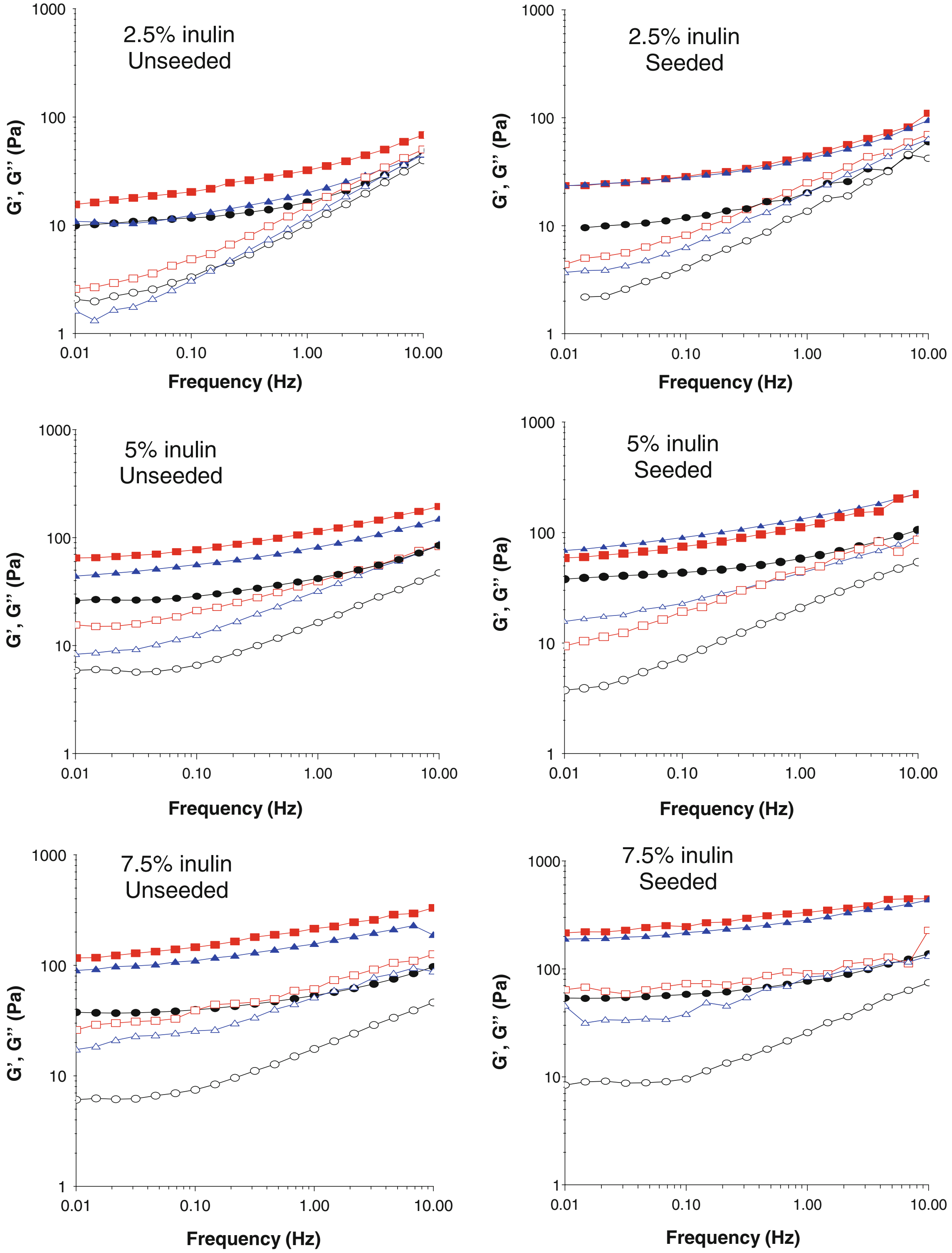


Fig. 2 Mechanical spectra of inulin-enriched dairy desserts at 10 °C. Measurements were carried out at different storage time: 1st (*filled circle*), 4th (*filled triangle*) and 7th day (*filled square*). Values of G′ (*filled symbols*) and G″ (*open symbols*)

over the range f=0.01–10 Hz, and the values of the storage modulus (G′) and the loss modulus (G″), as a function of frequency, were calculated using the Rheowin Pro software (version 2.93, Haake).

Particle Size Distribution

Particle size distribution analysis was performed with a Laser Diffraction Particle Size Analyzer (Mastersizer 2000

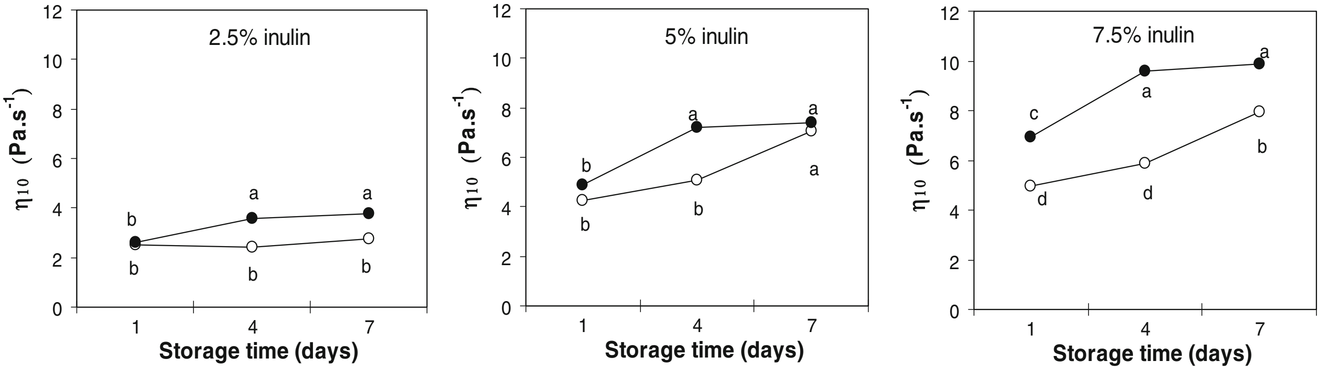


Fig. 3 Average values of apparent viscosity at 10 s^{-1} (η_{10}) for inulin-enriched dairy desserts. The significant differences among values are indicated by the *letters*: values not sharing letters differ significantly (p=0.05); seeded samples (*filled circle*) and unseeded samples (*circle*)

Malvern Instruments, Worcestershire, UK), connected to a cell for liquid measurements (Hydro 2000S mixing, Malvern Instruments, Worcestershire, UK) with distilled water as dispersant. The refractive index used was 1.53. Particle size calculations were based on the Mie scattering theory. The percentage of volume (% volume) and mean diameter ($D[_{4,3}]$) corresponding to each observed population were calculated. The calculations were done with the software provided with the equipment (Mastersizer 2000V. 5.40).

Light Microscopy

The samples were placed on slides with a cover slip and observed at a magnification of 20× under a light microscope

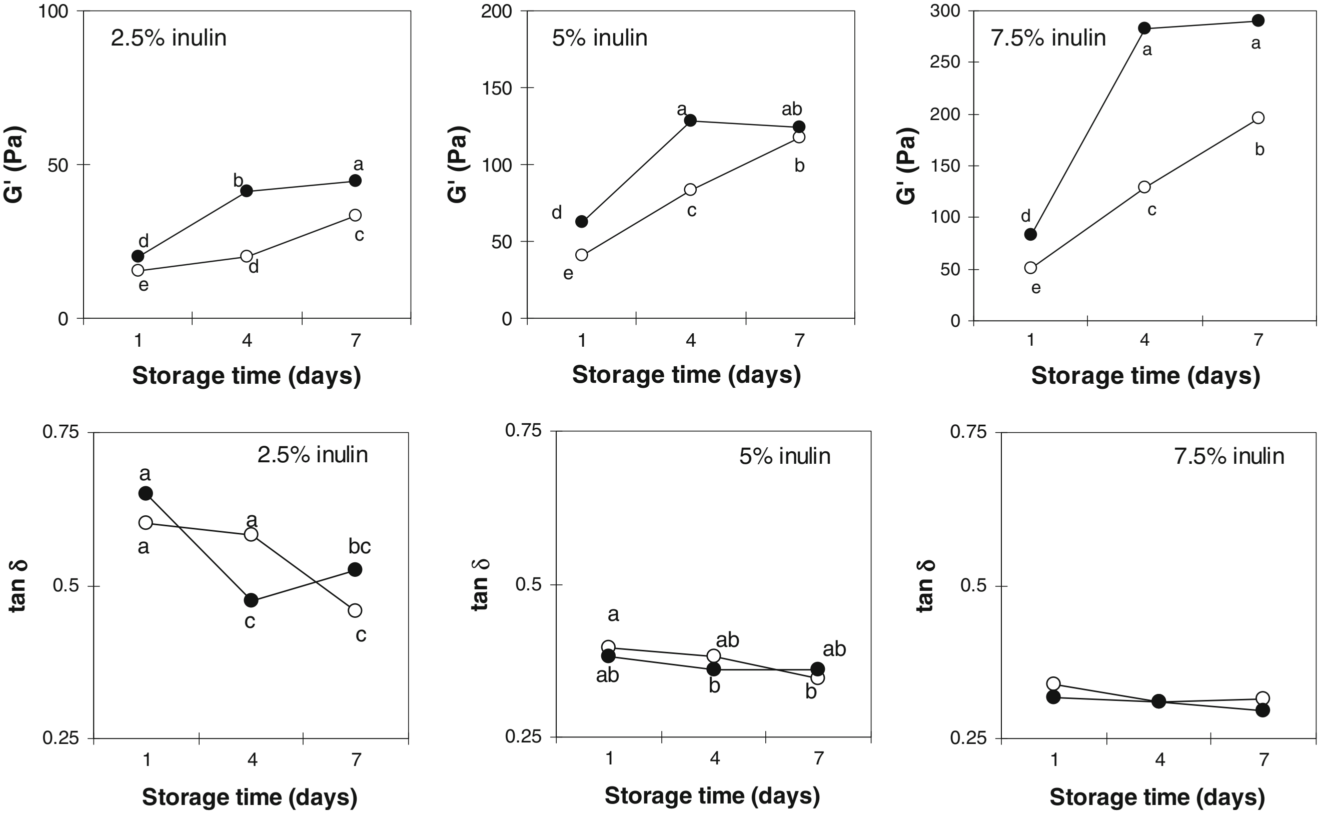


Fig. 4 Average values of storage modulus for inulin-enriched dairy desserts. Significant differences among values are indicated by the *letters*: values not sharing letters differ significantly (p=0.05); seeded samples (*filled circle*) and unseeded samples (*circle*)

Table 2 Analysis of variance of two factors (seeding and storage time) with interaction on values of apparent viscosity at 10 s^{-1} and storage modulus and loss tangent at 1 Hz

Factors	Apparent viscosity at 10 s^{-1}						Storage modulus at 1 Hz						Loss tangente at 1 Hz					
	2.5% Inulin		5% Inulin		7.5% Inulin		5% Inulin		5% Inulin		7.5% Inulin		2.5% Inulin		5% Inulin		7.5% Inulin	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
A: seeding	32.3	0.001	25.3	0.002	101.4	<0.001	53.4	0	85.8	<0.001	431.0	<0.001	0.02	0.889	0.7	0.441	2.5	0.163
B: storage time	8.9	0.016	57.3	<0.001	42..0	<0.001	17.8	0.003	248.0	<0.001	568.1	<0.001	24.5	0.001	5.9	0.038	2.7	0.15
A × B	6.0	0.037	7.1	0.026	11.8	0.008	5..3	0.047	17.4	0.003	60.7	<0.001	11.8	0.008	1.6	0.288	0.6	0.603

F and *p* values

Nikon Eclipse 90i (Tokyo, Japan). Photomicrographs were acquired with a digital camera (Nikon DS-5Mc).

Statistical Analysis

The effects of storage time and seeding on the apparent viscosity at 10 s^{-1} and the values of G′ and tan δ at 1 Hz were studied for each inulin level by analysis of variance (ANOVA) of two factors with interaction. The Fisher test (α=0.05) was used to calculate the minimum significant difference. The calculations were carried out with XLSTAT-Pro Version 2007 (Addinsoft, Paris, France).

Results

Rheological Behaviour

Rheological behaviour of seeded and unseeded desserts containing different inulin concentrations was studied during the storage time. The flow curves are shown in Figure 1, and in all the cases, they exhibited a shear-thinning flow behaviour with apparent thixotropy. The flow curves of samples changed during the storage differently depending on the concentration of inulin and on seeding (seeded or unseeded sample). In order to characterise the flow behaviour of each sample, data of the ascending curve were fitted to the Ostwald–de Waele model ($0.98 \leq R \leq 0.99$), and the values of consistency index (K) and the flow index (n) obtained are shown in Table 1. In general, the consistency index values increased during storage, while the flow index slightly decreased. On the seventh day of storage, the samples showed consistency index values that varied from 11.1 to 51.1 Pa s^n and flow index values that were low (from 0.27 to 0.40) indicating a high shear-thinning behaviour. Consistency values increased with inulin concentration, and in general, were higher for seeded samples than for unseeded samples. Flow index values decreased with inulin concentration, but slightly varied among seeded and unseeded samples.

To study viscoelastic properties, mechanical spectra of all samples were obtained (Figure 2). On the first day, the mechanical spectra of all samples presented values of storage modulus (G′) higher than loss modulus (G″), and both parameters were quite dependent on frequency, indicating a response typical of a weak gel. During storage time, the values of G′ and G″ increased and, in the case of 5% and 7.5% inulin samples, became less dependent on frequency indicating an increase in gel strength.

In order to quantify the changes in rheological properties during storage time and to compare among different desserts, the values of apparent viscosity at 10 s^{-1} and the values of G′ and tan δ at 1 Hz were taken (Figures 3 and 4,

Fig. 5 Microscopy images for samples with 2.5% inulin at different storage time

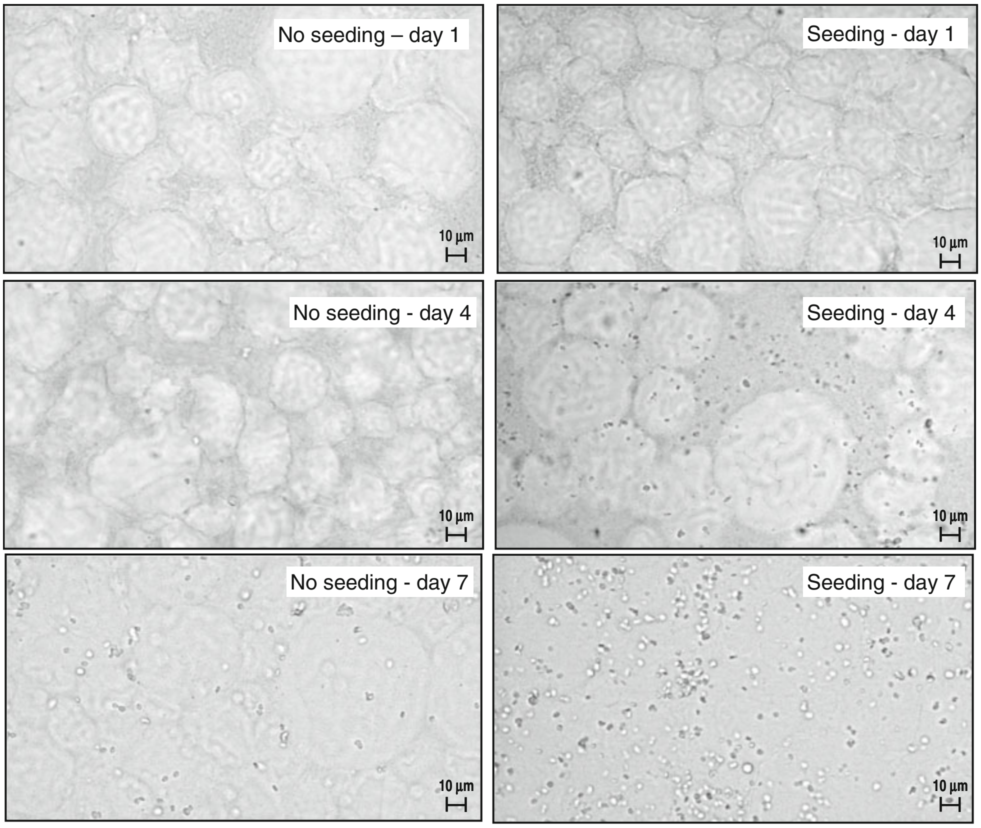


Fig. 6 Microscopy images for samples with 5% inulin at different storage time

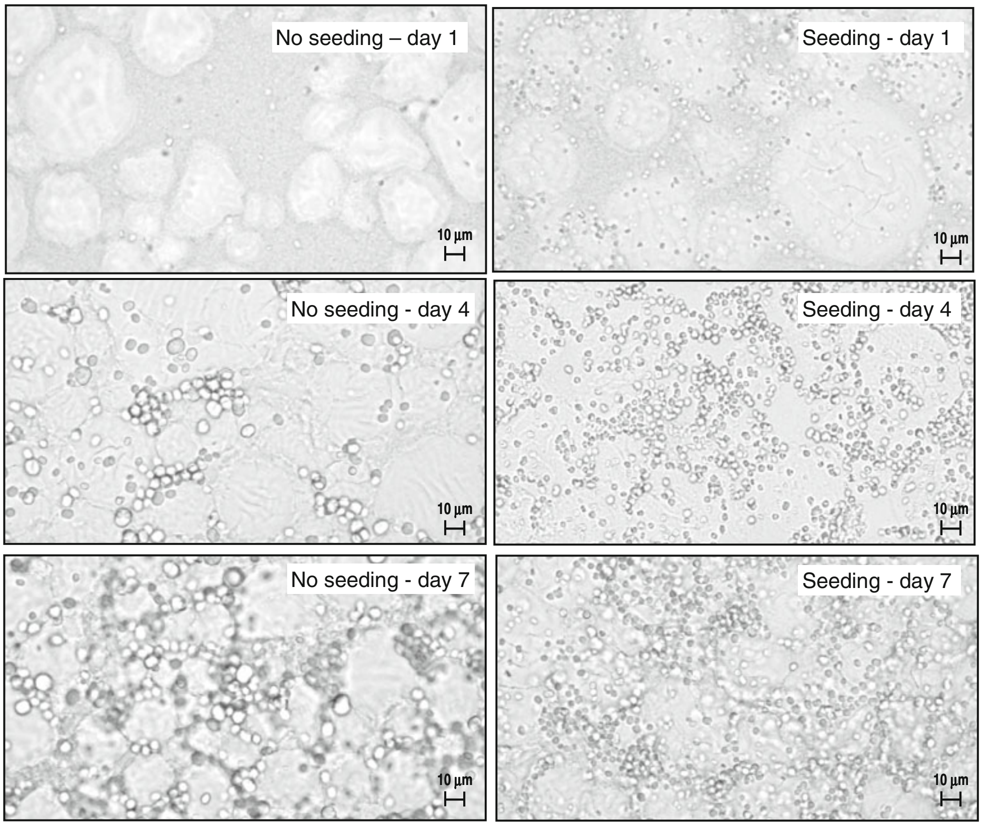
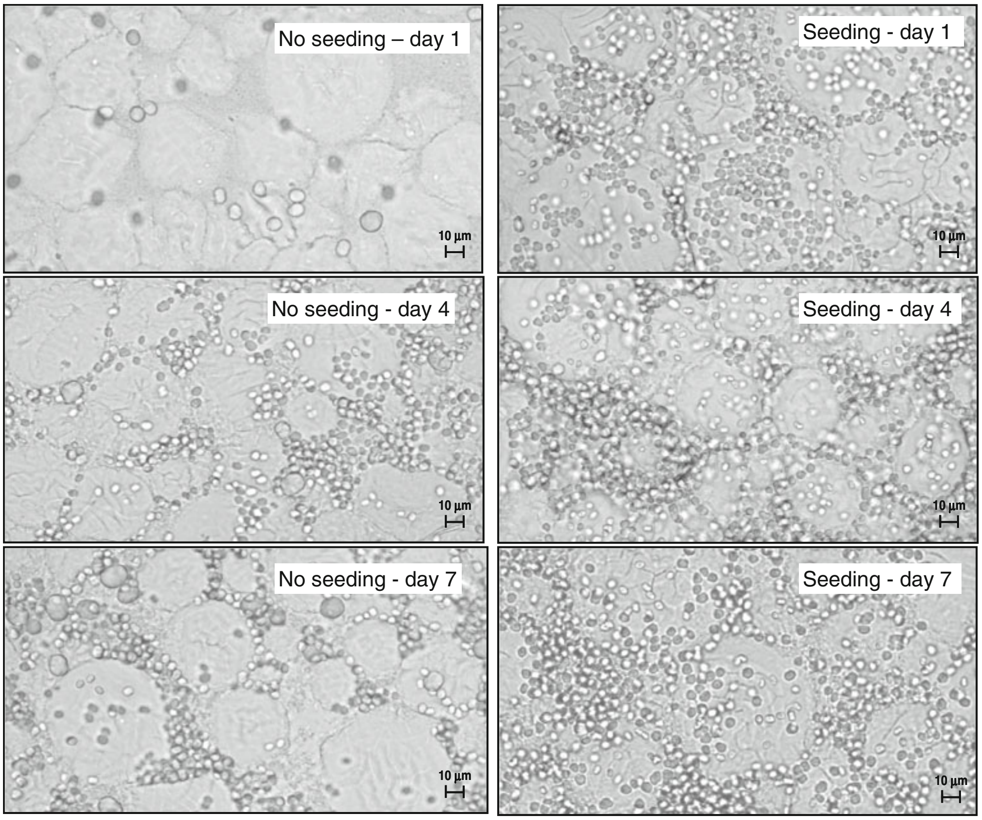


Fig. 7 Microscopy images for samples with 7.5% inulin at different storage time



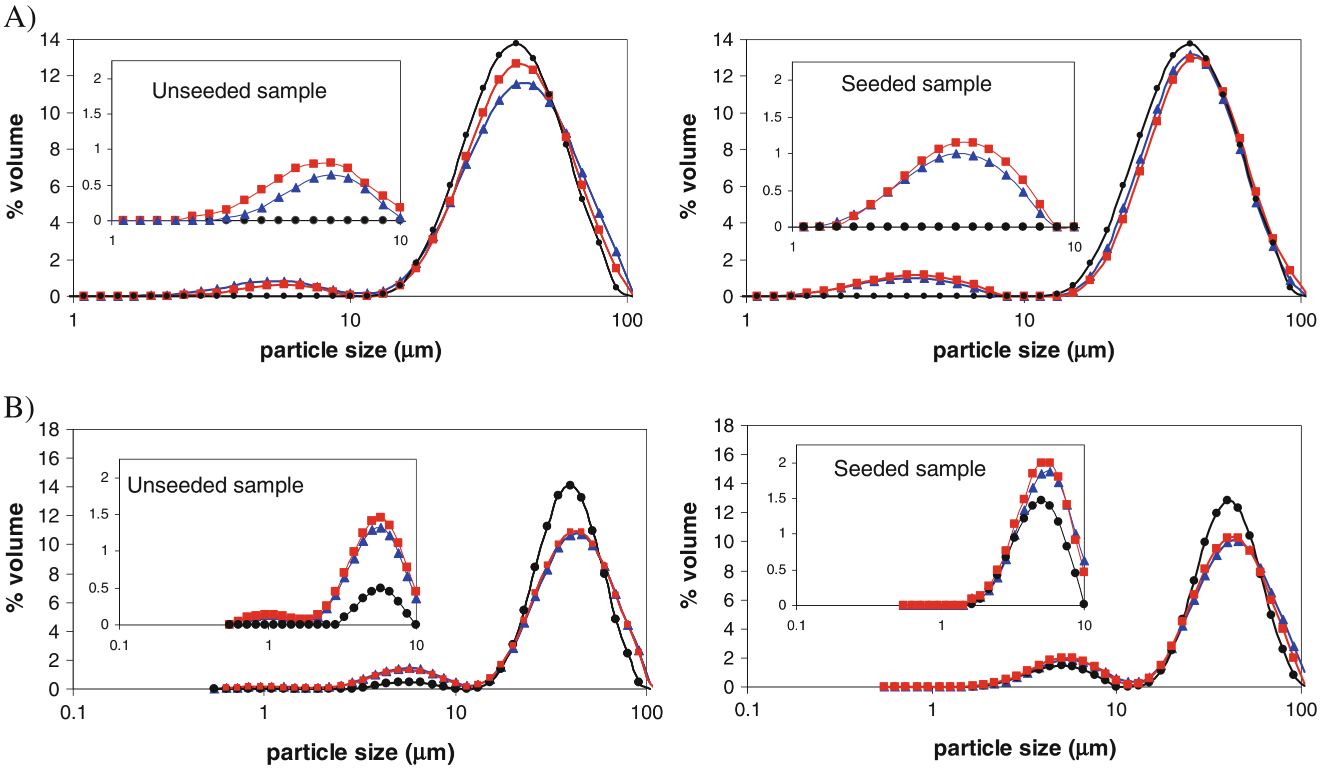


Fig. 8 Particle size distribution for samples with 5% inulin (**a**) and 7.5% inulin (**b**) at different storage time: 1st (*filled circle*), 4th (*filled triangle*), and 7th day (*filled square*)

Table 3 Characteristics of the population of particles with size <10 μm found in inulin-enriched desserts

Procedure	Storage time (days)	5% Inulin		7.5% Inulin	
		% volume	$D_{[4,3]}$ (μm)	% volume	$D_{[4,3]}$ (μm)
No seeding	1	–	–	2.41d	5.09a
	4	4.75c	5.12a	10.46c	4.07bc
	7	6.99b	4.82a	11.96b	4.76abc
Seeding	1	–	–	10.33c	4.49c
	4	7.83ab	3.89b	15.53a	4.77abc
	7	8.71a	4.00b	15.35a	4.91ab

For each column, values not sharing letters differ significantly ($p=0.05$)

respectively). Generally speaking, viscosity and elasticity of samples increased over time, indicating a reinforcement of the system structure. The magnitude of changes depended on inulin concentration. For samples containing 2.5% inulin, viscosity values slightly increased (seeded samples) or did not significantly vary (unseeded samples) during storage time, while a great variation was observed for samples with 5% and 7.5% inulin. The increase in storage modulus values during storage time was also low for 2.5% samples. However, tan δ values significantly decreased during storage for 2.5% inulin samples, while remained almost constant for 5% and 7.5% inulin samples (Figure 4). On the first day, samples with low amount of inulin showed high tan δ values due to the higher amount of water in the continuous phase resulting in a more fluid-like response. During storage, the water retention caused by this low amount of inulin not only slightly increased elasticity, but was also enough to significantly increase the relative contribution of the storage modulus with respect to the loss modulus. For the three concentrations of inulin, the ANOVA showed a significant interaction between the effects of seeding and storage time on apparent viscosity and storage modulus (Table 2) indicating that variations in both viscosity and elasticity of custards during storage depended on the presence of inulin seeds after thermal treatment. The increase in viscosity and elasticity was higher and faster when seeding procedure was used. On the fourth day of storage, seeded samples already reached the same values as those observed on the seventh day of storage time; while for unseeded samples, changes in flow properties continued to occur after the fourth day. This is in agreement with the results observed by Duynhoven et al.,[4] who showed gel firming to take longer in the absence of inulin crystal seeds.

Microstructure

Changes in sample microstructure during storage time were checked by observing samples under light microscopy and analysing particle size distribution. As stated previously,[8] in microscopic images (Figures 5, 6 and 7) big particles corresponded to swollen starch granules, which appeared as a population of particles ranging from 10 to 100 μm (Figure 8), while inulin particles can be observed at a smaller size range (<10 μm). The particle size distribution of 2.5% inulin samples (data not shown) revealed only the population corresponding to the dispersed swollen starch granules throughout the storage period. However, in microscopic images (Figure 5), small inulin particles can be observed. In seeded samples, inulin particles were observed sooner (on the fourth day) than for control samples (on the seventh day). Both the small size and the low abundance of these particles compared with those of starch granules would explain why they were not registered when particle size distribution was measured. Furthermore, that would also explain the small variation observed in the rheological properties of 2.5% inulin samples. For samples with 5% and 7.5% inulin, changes in particle size distribution were observed during storage time (Figure 8). Bimodal distributions were observed, with the population of inulin particles under 10 μm, for which the mean diameter and occupied volume percentage were calculated (Table 2). It should be noted that these values obtained for inulin particles are influenced by the significantly greater size of starch particles and are valid for comparison among samples since the percentage of the other ingredients in the formulation remained constant. According to the images of samples with 5% inulin (Figure 6), very few inulin particles appeared in control samples on the first day, while more appeared in the seeded sample. However, as what happened for 2.5% of inulin samples, these particles were not observed when particle size distribution was analysed (Figure 8). On the fourth day of storage, micrographs showed abundant inulin particles which, according to particle size distribution, were more abundant and smaller in size in the case of seeded sample than in control (Table 3). From the fourth to seventh day, the increase in particle number was only significant for the control sample. On the seventh day, the seeded sample still showed higher particle number and with lower size than the control. For samples with 7.5% inulin, on day 1, the seeded sample revealed a great amount of inulin aggregates (10.33% of volume) with regular size. The percentage of volume occupied by these particles also increased during storage time and its size increased slightly and uniformly. On day 1, control sample revealed few large-sized particles, and during storage time, small particles appeared, which lowered the mean diameter value. As a result, seeded and unseeded samples showed no differences in the mean diameter values ($D_{[4,3]}$); however, they were more regular in size in the seeded samples.

According to this result, changes in dessert microstructure during storage time were highly dependent on inulin concentration. Furthermore, the changes in inulin particle size during storage time differed for seeded and control samples, indicating that different phenomena occurred. In the case of unseeded samples, inulin particles appeared later because inulin was completely dissolved during heating (above 85 °C for 15 min), and in this situation, spontaneous nucleation takes longer. Furthermore, two different nucleation steps were observed in unseeded samples, the first leading to large particles and the second leading to smaller ones. The formation of different shaped and sized inulin particles has previously been observed under different conditions and described by different authors. Hebette et al.[2] showed that inulin solutions heated at 96 °C during cooling presented two nucleation steps, the first at high temperatures, yielding eight-like-shaped particles, and the second nucleation occurring at lower temperatures yielding smaller inulin particles. According to the authors, crystallites from the first nucleation had more time to grow at higher inulin concentrations and were growing at higher temperatures, where diffusion of molecules to the crystal surface was faster. Bot et al.[5] observed that, in inulin dispersions heated to high temperatures, slow crystallisation led to large primary particles and a very soft structure. According to the aforementioned authors, the large primary particles formed after removal of any seeding crystals had a low surface-to-mass ratio and, therefore, relatively few connection points, apparently leading to soft gels.

Regarding the seeded samples, inulin particles appeared sooner because inulin seeds initiated crystallisation.[7] Contrary to that observed for unseeded samples, only one nucleation of many small particles occurred; and during storage time, particle size grew slightly and homogeneously, reaching a size that depended on the amount of inulin present in the system. Similar differences among seeded and spontaneous crystallisation of lactose were observed in “dulce de leche” by Gimenez et al.[10] Big crystal agglomerates and small crystals were obtained when crystallised spontaneously, and only small crystals formed when lactose crystal seeds were added.

Finally, it should be indicated that inulin crystallisation is a complex phenomenon, depending on many factors, particularly the thermo-mechanical conditions used during manufacture.[5] In the present work, samples were prepared following a single method; however, during preliminary tests, the preparation procedure was varied and, although not quantified, cooling rate, shearing rate during cooling and storage flask volume were all found to affect crystallisation rate, inulin particle size and rheological properties, particularly in the case of seeded samples. The results of the present work showed that seeding can be used to control inulin particle size in real food, but the effects of these factors must be studied in each case to obtain the desirable amount and the size of inulin particles in the product.

Conclusion

The addition of inulin caused changes in rheological properties and microstructure of dairy desserts that highly depended on inulin concentration. For all inulin concentrations, the presence of inulin seeds favoured a faster crystallisation of a greater amount and more regular sized inulin particles, which generally resulted in more viscous and elastic desserts. Seeding proved effective for preparing low-fat custard desserts with regular sized inulin particles, whose abundance and size depend on inulin concentration.

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