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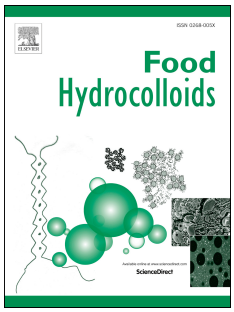

Gelation properties of calcium-inulin gels

Carlos Bengoechea, María Luisa lópez-castejón, Sandra Márquez, Victoria Salinas, Cecilia Puppo, Antonio Guerrero

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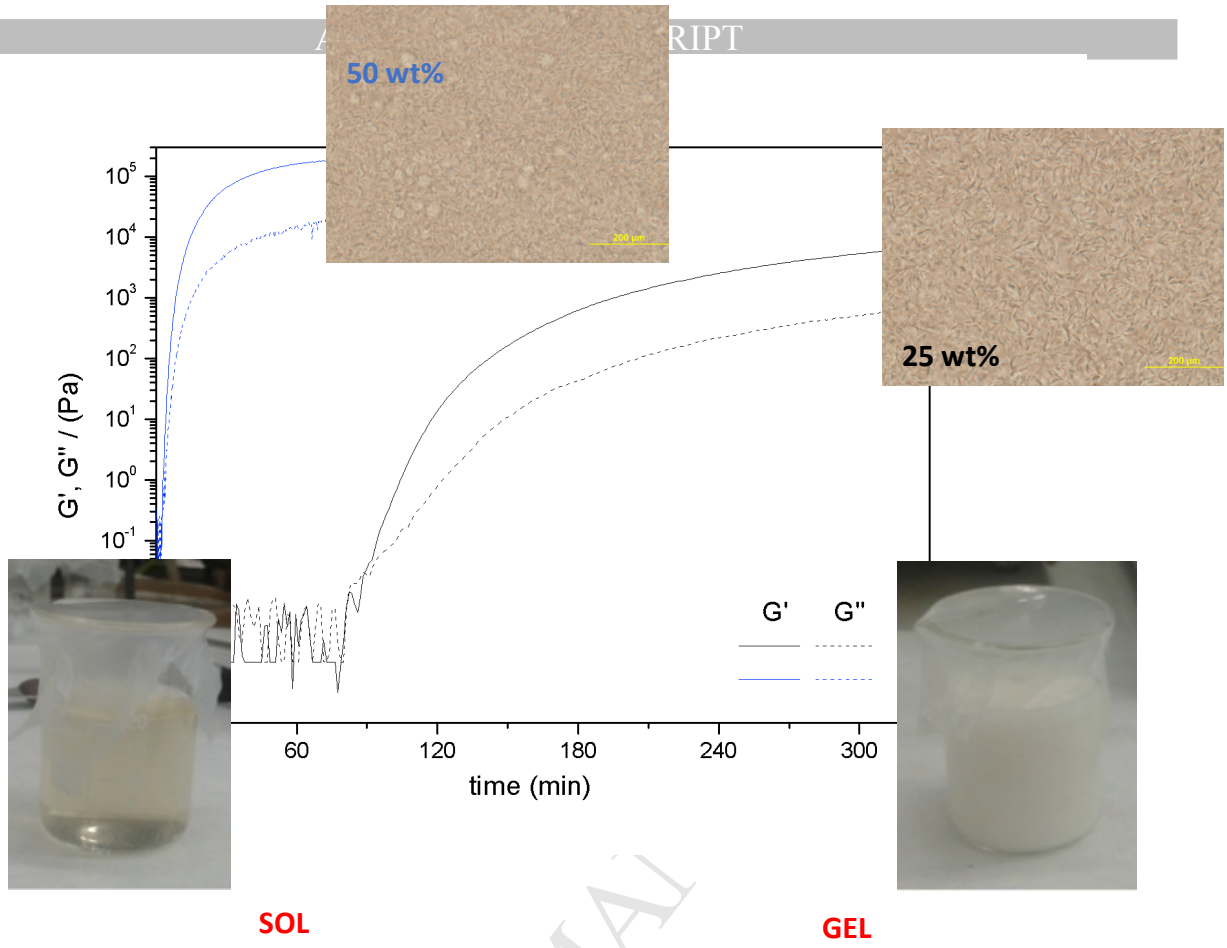
50 wt%
25 wt%
G', G'' / (Pa)
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
G'
G''
60
120
180
240
300
time (min)
SOL
GEL

GELATION PROPERTIES OF CALCIUM-INULIN GELS

CARLOS BENGOECHEA[1], MARÍA LUISA LÓPEZ-CASTEJÓN[1] (✉), SANDRA MÁRQUEZ[1], VICTORIA SALINAS[2], CECILIA PUPPO[2], & ANTONIO GUERRERO[1]

1 Departamento de Ingeniería Química, Universidad de Sevilla, Facultad de Química. Calle Profesor García González 1, 41012 Sevilla Spain

2 Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA, CICPBA-CONICET Facultad de Ciencias Exactas UNLP). Calle 47 y 116, 1900 La Plata Argentina.

Abstract

Inulin is a prebiotic ingredient that is being increasingly used in food formulations as fat replacer. The present manuscript focuses on the formation of gels formed from inulin aqueous dispersions, observing the effect of inulin content, ranging from 25 to 50 wt%, and of the presence of calcium salts (chloride, lactate) at different concentrations up to 5 wt% Gels are observed to be stronger and formed in a shorter time as the polysaccharide content is higher. Both backscattering and rheological techniques can be used to follow the gelation, being able to detect a significant initial setting stage at 25 wt% inulin content. When calcium salts are added, weakening of gels takes place, resulting in lower values of the storage and loss moduli, G' and G'', respectively, as well as in a reduction of the linear viscoelastic range. Moreover, gelation seems to be retarded on a large scale of time due to the presence of salt. Prebiotic gels containing calcium are of special interest for the development of functional foods, always considering the effect they exert on the rheology when formulating these products.

Keywords: *Inulin; calcium, gelation; rheology; backscattering*

1. Introduction

Inulin is a prebiotic β-fructan that is present in many plant species such as Jerusalem artichoke, dahlia and chicory root, or synthesized synthetically from sucrose (Esmaeilnejad Moghadam, Keivaninahr, Fouladi, Rezaei Mokarram, & Nazemi, 2019; Nguyen, Mattes, Hoschke, Rezessy-szabó, & Bhat, 1999; Santos et al., 2018). The degree of polymerization (DP) of inulin, as well as the presence of branches, is an important property since they influence its functionality to a striking extent. Inulin possesses a neutral bland taste, is moderately soluble in water and confers body and palatability. In addition, from the techno-functional point of view, inulin is considered a texturizing agent and/or a stabilizer for emulsions and foams (López-Castejón, Bengoechea, Espinosa, & Carrera, 2019). Even if the health claim associated with prebiotic foods can no longer be used freely by the industry (Salminen & van Loveren, 2012), soluble fiber products, such as inulin, still remain important for the health nutrition (Slavin, 2013). For all these reasons, it has various applications in the food industry, being frequently added to milk, fermented products, jellies, aerated desserts, mousses, ice cream and bakery products (Balthazar et al., 2018; Guimarães et al., 2018). In these food formulations, inulin is used as a substitute for sugar or fat replacement obtaining calorie-reduced food that additionally provide added health benefits. Inulin and fructo-oligosaccharides (FOS) are non-digestible food ingredients that selectively stimulate growth and/or activity of a number of potentially health-stimulating intestinal bacteria (Balthazar et al., 2017). This ability to modify the intestinal flora is called prebiotic effect and the prebiotics are defined as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited group of bacteria in the colon” (Gibson & Roberfroid, 1995). Inulin does not release fructose in the gastro intestinal tract and is thus classified as a low-calorie food ingredient (Roberfroid & Delzenne, 1998).

Studies show evidence that inulin and FOS contribute to increasing the absorption of calcium, magnesium and iron in humans (Coudray et al., 1997; Coudray, Tressol, Gueux, & Rayssiguier, 2003). In addition, an increase of calcium absorption, bone mineral density of proximal tibia, total bone mineral content and bone volume was observed in growing male rats fed with breads fortified with calcium and inulin/FOS together with an increase of anaerobic bacteria, probably bifidobacteria and *Lactobacillus*, correlated with a decrease in the pH due to the rapid fermentation of inulin and FOS by the colonic flora resistant to acid medium (Salinas et al., 2017). In order to face health issues (osteopenia, osteoporosis) derived from a poor absorption of calcium (Berdanier & Berdanier, 2015), different calcium salts (e.g. lactate, chloride) have been introduced in food systems.

Gels are solid tridimensional networks that trap and immobilize the solvent (e.g. water), exhibiting a wide range of microstructural and mechanical properties (Clark, 1996). A gelation mechanism taking place as the result of the precipitation of molecules present in an initial aqueous solution has been proposed for inulin (Kim, Faqih, & Wang, 2001). The main factors affecting the formation of the gel are inulin concentration, temperature/heating time, the solvent used and the pH. A comprehensive rheological characterization considering the effect of these factors of gel systems is very important for developing new products, estimating their shelf-life, sensory assessment, as well as stability evaluation (Abu□Jdayil, Shaker, & Jumah, 2000; Barnes, Hutton, & Walters, 1991; Xu, Zhang, Liu, Sun, & Wang, 2016). Additionally, some authors (Arango, Trujillo, & Castillo, 2015, 2018) have used a light backscattering technique to predict the rheological gelation and curing times, as well as coagulation and syneresis parameters, in milk gels at different inulin, protein and calcium concentrations.

Although there are several studies on the effect of calcium and inulin on gels including proteins such as whey (Glibowski & Glibowska, 2009), studies on gels based solely on

calcium and inulin have not been documented so far. Thus, the objective of the present work was the development and characterization of inulin gels containing calcium as potential health-promoting food systems. These systems were characterized through rheological dynamic tests and backscattering measurements in order to evaluate the gelation kinetics and the effect of the presence of calcium salts on inulin gelation properties.

2. Experimental

2.1 Materials and sample preparation

The oligofructose-enriched inulin used was Orafti® Synergy1, a combination of longer and shorter chain inulin with specific physiological effects (~92wt% inulin content) and was provided by BENEO (Germany). Calcium chloride (100 %) and calcium lactate (min. 98 %) were supplied by Sigma-Aldrich (United States).

Different inulin suspensions in distilled water (25, 35, 45, 50 wt%) were prepared, and the effect of calcium salt (chloride or lactate) at different concentrations (0, 0.1, 1, 5 wt%) was evaluated on aqueous suspension of 25% wt of inulin. The 25% wt system was selected due to its poorer rheological behaviour, which may be affected in a larger extent by the presence of salts. Solids (inulin or inulin-calcium salt) and water were mixed for 1 hour at room temperature using a magnetic stirrer in order to ensure an adequate homogenization of the system. After stirring, the mixture solutions were stored at 25 ºC for 24 hours, observing a transition from a yellowish solution into a white gel system.

2.2. Methods

2.2.1. Rheological properties

Shear rheology tests for inulin or calcium-inulin gels were performed using a MARS II rheometer (Haake, Germany). A stainless-steel parallel plate geometry with a rough surface with a diameter of 35 mm and a gap of 1 mm was used for all the shear rheological tests.

Small Amplitude Oscillatory Shear (SAOS) measurements were conducted in the same rheometer in order to obtain the linear viscoelastic properties for all the samples studied as a function of frequency (between 0.01 to 10 Hz) at different storage times. Shear stress sweep tests were previously performed to determine the linear viscoelastic range (LVR). The critical stress (τ_c) and critical strain (γ_c) were determined from the strain graph as a function of the stress, being the values of stress and strain, respectively, at which the linear viscoelastic region ends (Bengoechea, Romero, Aguilar, Cordobés, & Guerrero, 2010). It must be taken into account that those critical parameters depend not only on the material properties, but also on the specimen geometry and possibly on strain rate and inertia (Tanna, Wetzel, & Winter, 2017).

Gelation kinetics of the different systems were studied *in-situ* through dynamic time sweep tests at 0.1 Hz in an AR2000 rheometer at 25 ºC (TA Instruments, USA). This frequency was selected in order to keep the sample within the LVR during the whole test. In order to study the *in-situ* gelation, inulin solutions were mixed for 1 hour at 25 ºC using a magnetic stirrer, and then were subsequently placed on an aluminium low-inertia parallel plate geometry with a diameter of 60 mm and a gap of 1 mm.

2.2.2. Backscattering

A vertical scan analyzer Turbiscan MA 2000 (Formulaction, France) was used in order to study the evolution of the gelation of the suspension along time, by measuring the backscattering of a pulsed near infrared light source (λ=850 nm) as a function of the suspension height (Mengual, Meunier, Cayré, Puech, & Snabre, 1999). Freshly prepared

suspensions were placed into cylindrical glass tubes and stored at 5 °C. The backscattering of light along the entire height of each suspension was then measured at room temperature (20–23 °C) as a function of time. The results are presented as the backscattering signal (ΔBS/100%) at a certain height (4 cm) as a function of time.

2.2.3. Statistical analysis

The data were presented as mean ± standard deviation (SD) of three determinations. Variable results were subjected to one-way ANOVA according to the general linear model procedure with least-square mean effects. Significantly different means ($p < 0.05$) were determined according to Fisher's least significant differences (LSD) test.

3. Results and Discussion

3.1. Influence of inulin concentration

Inulin gels were prepared at different polysaccharide contents (25, 35, 45, 50 wt%) in order to study the effect of inulin content on viscoelastic properties and backscattering profiles of the gels. Kim et al. (Kim et al., 2001) already reported the existence of a critical concentration of inulin to be overpassed in order to produce gels (10-15 wt.%), either thermally or shear induced. It must be highlighted that the inulin concentrations studied in the present manuscript are well above of those reported for critical concentrations.

Mechanical spectra

Before frequency sweep tests were carried out, the Linear Viscoelastic Range (LVR) was established at 1 Hz for every system considered. Thus, Table 1 displays the critical stress (τ_c) and the critical strain (γ_c) for the gels formed at different inulin contents above from which their structure starts to breakdown (Tadros, 2004). The parameter τ_c increased around ten times its original value when the polysaccharide content doubled from 25 to 50 wt%, which

would point out the strengthening of the structure as result of higher number of interactions (e.g. van der Waals, Hydrogen bonding). These interactions would contribute to the development of physical gels especially at higher inulin contents, when the polymeric chains are proximate with an increase in polymer-polymer interactions. Kim et al. (Kim et al., 2001) indicated that no gel was formed at lower inulin contents (10, 15 wt%) at room temperature, although they were able to obtain gels fully formed when heating the systems (70, 40 ºC, respectively). On the other hand, γ_c diminished with the inulin content, going from $8.0 \cdot 10^{-4}$ for the 25 wt% inulin system to 3.6 10^{-4} for the concentrated gels (50 wt%), which is associated to the formation of a more structured matrix that is susceptible to be deformed at lower values of strain.

Figure 1 shows the mechanical spectra obtained for the inulin systems at the different polysaccharide concentrations studied, observing that they all show the same qualitative response: the elastic modulus (G') is well above the viscous modulus (G''), denoting the expected predominantly elastic behaviour of a gel. In Figure 1A, it may be observed that an increase in the inulin content in the systems results in an increase of their viscoelastic moduli, G' and G''. A network composed of solid crystalline particles conferring a gel-like texture to the matrix has been reported for native inulin at high concentrations (>25 wt%) after shearing (Franck & De Leenheer, 2005). When inulin is thoroughly mixed with water or another aqueous liquid using a shearing device such as a rotor-stator mixer (e.g. Ultra-Turrax) or a homogenizer, a white creamy structure is formed which can easily be incorporated in food to replace fat (up to 100%) (Frank, 1993). Moreover, it is possible to establish an empirical superposition for all those spectra using concentration-dependent vertical shift factor (a_c) using the system containing 25 wt% of inulin as a reference (Figure 1B). The shifting factors a_c were 1.0, 3.1, 8.1 and 11.4 for 25, 35, 45, and 50 wt%. inulin concentration of gels, respectively. The linear viscoelastic behaviour shown by the gelled systems may be

reproduced to a generalized Maxwell (GM) model (Mohsenin & Mittal, 1977; Steffe, 1992). GM model consists of a superposition of n independent relaxation processes with their corresponding relaxation times, λ_k, and relaxation strengths, G_k. According to this model, the linear material functions may be defined as follows:

$$G'(f) = G_e + \sum_{k=1}^{n} G_k \frac{(2\pi f)^2 \lambda_k^2}{1+(2\pi f)^2 \lambda_k^2} \quad (1)$$

$$G''(f) = \sum_{k=1}^{n} G_k \frac{2\pi f \lambda_k}{1+(2\pi f)^2 \lambda_k^2} \quad (2)$$

$$G(t) = G_e + \sum_{k=1}^{n} G_k e^{-t/\lambda_k} \quad (3)$$

The discrete relaxation spectrum may be estimated through the former equations for G' and G'' as a function of the frequency (f) by selecting a set of relaxation times λ_k and calculating the values of G_k that minimize the sum of deviation squares between predicted and experimental moduli. Figure 1B shows the predicted mechanical spectra obtained for the reference system (25 wt% inulin) as a continuous line, showing a reasonably satisfactory fitting to the proposed GM model (n = 4), especially for G'. It is well known that lower relaxation times regulate the high frequency region, while higher relaxation times influence strongly the low frequency region of the spectra. It is expected that, conveniently adjusting G_k parameters with a_c, the GM model used would fit the rest of the experimental data. Some authors have already pointed out the ability of the GM model to describe relaxation processes in solid-like foods (Del Nobile, Chillo, Mentana, & Baiano, 2007).

Kinetic study

Previously, some authors have used turbidimetric methods to study gelation processes (Hermansson, 1982; Ozcan, Horne, & Lucey, 2015), reporting a dependence of the aggregation on the concentration of the biopolymer. In this sense, Figure 2A shows the evolution of the percentage of backscattering (BS) at a specific height (4 cm) along gelation

time for aqueous systems with different inulin contents (25 and 50 wt%). It may be observed how for the system with the lower inulin content there is a sudden decrease of BS in the first stages of gelation. Then, the BS value stabilize and subsequently starts increasing until reaching a BS plateau value around 60 % at much longer times (>2000 min). On the other hand, the system containing 50 wt% of inulin does not show any decay of the BS signal, but it increases from the first moment until it reaches a plateau around 65 % at 240 min approximately. These differences found between the two systems imply the commented effect of concentration on gelation kinetics: aggregation processes occur generally faster when higher biopolymer contents are present (Ross&Murphy, 2005).

For the same samples, Figure 2B shows the *in-situ* gelation taking place once fresh samples (25, 50 wt%) were put into the rheometer geometry, indicating the evolution of the viscoelastic moduli along gelation time obtained from SAOS tests. It may be observed that there is a characteristic setting time for an incubation period prior the increase of the elastic modulus over the viscous one take place, during which both moduli seem to be similar. It may be attributed to the signal noise during this lag phase that no clear difference is observed among the viscoelastic moduli, as a predominance of the viscous modulus over the elastic one may be expected in the solutions before a significant number of aggregates start to form (sol-gel transition). This setting time may be determined as the time at which tan δ starts to deviate from the unity onto lower values (Ross&Murphy, 2005), being 96.7 ± 10.3 min and 4.5 ± 2.7 min for the 25 and 50 wt% systems, respectively. When the setting time is surpassed, viscoelastic properties start to increase exponentially eventually reaching a plateau once the gelation has finished. Thus, it seems that higher inulin contents apparently promote faster gelation processes, with shorter setting times and a more rapid exponential growth. Previously, other authors have pointed out that an increase in biopolymer concentration results in a greater proximity between molecules, favouring their interaction and the gelation

kinetics (Fernández Farrés & Norton, 2014). Furthermore, Kim et al. (Kim et al., 2001) explained inulin gelation through a so-called crowding effect, in which molecules that are in solution precipitate into particles, forming a gel. On these terms, they established that inulin concentrations lower than 5 wt% did not result on gel formation, as molecular density of inulin chains would be below the critical concentration to promote the mentioned crowding effect.

Beccard et al. (Beccard et al., 2019) studied physical properties and the development of a consistent model, explaining the gel formation of inulin with a high DP as potential fat replacer in fat containing foods. These authors found that a 20 wt% gel prepared at 25°C had sufficient amount of inulin that remained undissolved during sample preparation and acted as crystallization nuclei, ensuring a seeded crystallization. After 24 h of crystallization, the resulting gel consisted of small primary particles and exhibited a hard texture. When this gel was prepared at higher temperatures (60 °C), the solubility of inulin in water increased and, therefore, the number of undissolved nuclei decreased, lowering the crystallization velocity but eventually producing gels formed by larger particles.

A comparison of the results achieved through backscattering measurements (Figure 2A) and rheological tests (Figure 2B) permits to obtain a similar conclusion, that is, that the higher the inulin content, the faster the gelation process. However, the similarity is only qualitative, as substantial quantitative differences are found for the characteristic times of the incubation period and subsequent gelation. Rheological measurements are commonly used, as they show the solution (G''>G') to gel (G"<G') transition as it takes place. However, when the kinetic is slow, this technique may be time-consuming and difficult to control (e.g. drying of the sample, modification of the linear viscoelastic range). On the other hand, BS tests allow the kinetic study through one-time measurements of variations of the particle size (e.g. aggregation) detected by the optical device. Even if results are not worthy for a quantitative

kinetic characterization through model fitting, the simplicity of the measurement along with its control easiness, make BS measurements a suitable alternative to rheological tests in certain cases. Other authors have previously used backscattering measurements as a tool to study gelation kinetics (Boul et al., 2015; Ozcan et al., 2015)

Figure 3 shows the effect of storage time on the rheological properties of the inulin systems at 25 (Figure 3A) and 50 wt% (Figure 3B). An evolution onto higher values is observed for both viscoelastic moduli, G' and G'', for the 25 wt% inulin system, which may imply that gelation is still in process. In contrast, the mechanical spectra obtained for the 50 wt% inulin system remained constant along time, which may support the fact that gelation was completed within 90 min, as observed in Figure 2B. If loss tangent (tan δ = G''/G') is estimated at 1 Hz for the 25 wt% inulin system, it may be observed that it decreased from 0.10 to 0.08 from 24 to 96 h, while the 50 wt% systems kept a constant tan δ value around 0.09. This would be also indicative of the on-going gelation process for the 25 wt% system. The absence of evolution for the 50 wt% system should be related to the well-known fact that higher polymer concentrations lead to a faster gelation process (Ross&Murphy, 2005).

3.2. Influence of the presence of Calcium salts

Hydrocolloids may form gel structures in the presence of positively charged ions (e.g. calcium), being the positive ion fitted into negatively charged areas within the hydrocolloid molecule.

Dynamic tests

Table 2 shows the values for both critical stress (τ_c) and strain (γ_c) for the 25 wt% gels formed in the presence of different contents of the considered salts. It can be observed that, although no clear evolution is found for chloride salt, τ_c remained practically unaffected by

the presence of salt. On the other hand, an increase in γ_c was observed for the maximum salt concentration used (5 wt%).

Figure 4 shows the mechanical spectra for 25 wt% inulin gels containing different amounts (0-5 wt%) of calcium salt: Calcium chloride (Figure 4A) and calcium lactate (Figure 4B). All frequency tests were always carried out within the linear viscoelastic range ($\tau<\tau_c$; $\gamma<\gamma_c$). A predominantly elastic behaviour was always found for the systems studied (G´>G´´), confirming the gel character of the samples. However, the presence of calcium salts resulted in a decrease in both viscoelastic moduli which takes place in a greater extent as the salt concentration is higher. This decrease may be explained on basis of the disruption of aggregates that interlink different chains present in the gel particles (Bozzi, Milas, & Rinaudo, 1996). Different authors have indicated that at low concentrations of Ca^{2+}, an increase of the viscoelastic properties up to a plateau is found, decreasing abruptly once a critical concentration is exceeded (0.015-0.02 wt%)(Bozzi et al., 1996; Meng, Hong, & Jin, 2013). Considering the mass percentage of calcium in a mol of lactate and chloride salt (18.3 and 36.0 wt%, respectively), it may be quantified that in the present study the reported critical concentration was surpassed in every case, as the minimum Ca^{2+} concentrations considered were 0.018 and 0.036 wt% in the lactate and chloride calcium systems, respectively. This fact may explain the slighter decrease found for the lactate system at 0.1 wt% compared to the chloride system. However, at higher concentrations, it may be observed how, in spite of containing a lower content of Ca^{2+}, viscoelastic properties decreased in a greater extent for systems containing lactate than the corresponding chloride system. Similar results were obtained for gellan gum systems (Meng et al., 2013).

Gelation Kinetics

The influence of the presence of Calcium salts on the gelation kinetics of 25 and 50 wt% inulin systems containing a 5 wt% salt content is displayed in Figure 5.

Systems with lower inulin contents (25 wt%) are still evolving 96 h after their preparation, suggesting that their kinetic is much slower than that observed at higher contents (50 wt%), as it shown in Figure 3. Figure 5A shows the evolution of the percentage of backscattering (BS) at a specific height (4 cm) along gelation time for 25 wt% aqueous systems with or without Calcium salts (5wt% of chloride or lactate). All systems studied show an initial decrease for BS, which has already been related to particles rearrangement. Subsequently, a BS increase takes place for the three systems, although it is observed how systems containing calcium salts display higher backscattering values, at shorter times than the reference system. This effect might imply that the presence of salts initially promotes the aggregation of particles, being especially remarkable for the chloride system. However, all systems studied eventually reached similar BS values around 70 hours (4200 min) after their preparation. When studying the *in-situ* gelation of 50 wt% system of inulin (Figure 5B), all samples display a similar rheological response along time, with the viscoelastic moduli increasing till they stabilize around 50 min after their placing onto the rheometer plate and reaching similar values, independently of the presence of salt. However, it is observed that the presence of salt slightly increased the gelation rate during the first stages, as it both G' and G'' are higher for chloride and lactate systems compared to the system without salt. These rheological results may be consistent with the BS results described above for the 25 wt.% systems, as the presence of salt leads to a faster evolution of the studied parameter (e.g. G', BS (%)) during time.

The evolution of both the elastic and viscous moduli with storage time is shown in Figure 6, for the 25 wt% inulin systems containing 5wt% of calcium chloride (Figure 6A) or calcium lactate (Figure 6B). As observed in Figure 5, in Figure 6 it can be observed that the gelation seems to last longer times for the systems containing the chloride salt, as mechanical spectra kept evolving 96 hours after its preparation, while gels containing calcium lactate were

formed within 48 hours, not showing remarkable differences since then. This difference might be related to the different interactions established between anions (lactate, chloride) with inulin and water during gelation. Thus, calcium lactate gels were just formed at 48 hours, although values of G' and G'' were significant lower (Figure 6 B) in comparison with those found for calcium chloride-inulin gels. Previous results obtained on pasting properties of calcium salts-inulin-wheat flour systems, also confirmed that the calcium lactate salt developed a paste of low viscosity, in comparison to calcium carbonate and citrate salts (Salinas & Puppo, 2013).

These results are different from those found for alginate gels in the presence of calcium salts, as a faster gelation was reported in the presence of chloride compared to lactate (Lee & Rogers, 2012). However, just like happened with inulin gels, lactate gave poorer viscoelastic parameters (Lee & Rogers, 2012; Meng et al., 2013). This difference could be attributed to the distinct form that calcium salts interact with a polymer as alginate or with an oligosaccharide as inulin. According to Hofmeister series of stabilizing effect of anions, chloride is a strongest hydrated anion than lactate (Salinas & Puppo, 2013), favouring a great interaction inulin-water via hydrogen bonds, leading to more elastic gels (> G', Figure 6 A).

The proven formation of inulin gel containing calcium is strongly related with the development of innovative health-promoting applications of prebiotics through their applications to food gels that may enlarge the offer options of food industry to consumers. These products can replace fat and sugars and, at the same time, enhance the mouthfeel by providing better tongue lubrication (Singla & Chakkaravarthi, 2017). In addition, these gels will be suitable for elderly people that usually need consuming healthy-soft foods.

4. Conclusions

Inulin gel systems were successfully prepared through water dispersion of the polysaccharide and subsequent storage at room temperature. As the inulin content increased from 25 to 50 wt%, gels became thicker, as denoted by a broader linear viscoelastic range and higher storage and loss moduli. Therefore, the more compact structure that results from a higher presence of inulin would contribute to its potential use as fat replacer in food systems. The shape of the mechanical spectra was independent of the inulin content, being possible to shift all results onto a general master curve. Additionally, those results could be fitted to a generalized Maxwell model of four elements. The kinetics of the initial stages of gelation was studied through different methods, like backscattering or rheological dynamic tests. Both techniques confirmed that gelation took place at a slower pace for the system with lower inulin content (25 wt%), being even possible to distinguish a setting time region. Moreover, when observing the effect of storage on the viscoelastic moduli, it was clear that gelation was still happening 96 hours after the preparation of gels with lower inulin content, while the 50 wt% inulin gel displayed constant rheological properties much sooner (48 hours).

The presence of ionic calcium from a chloride or lactate salt in the gel formulation resulted in poorer rheological properties, probably due to the rupture of aggregates within the gel. Gelation was affected by the nature of the salt used, as more time was needed to complete the gelation process when using chloride. In spite of the weakening observed, the rheological gel behaviour persisted after the addition of salt. The obtaining of a prebiotic gel including calcium in its formulation may be of great interest for the food industry aiming to promote the health of specific sectors of the population through the development of functional foods.

5. Acknowledgements

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Figure Captions

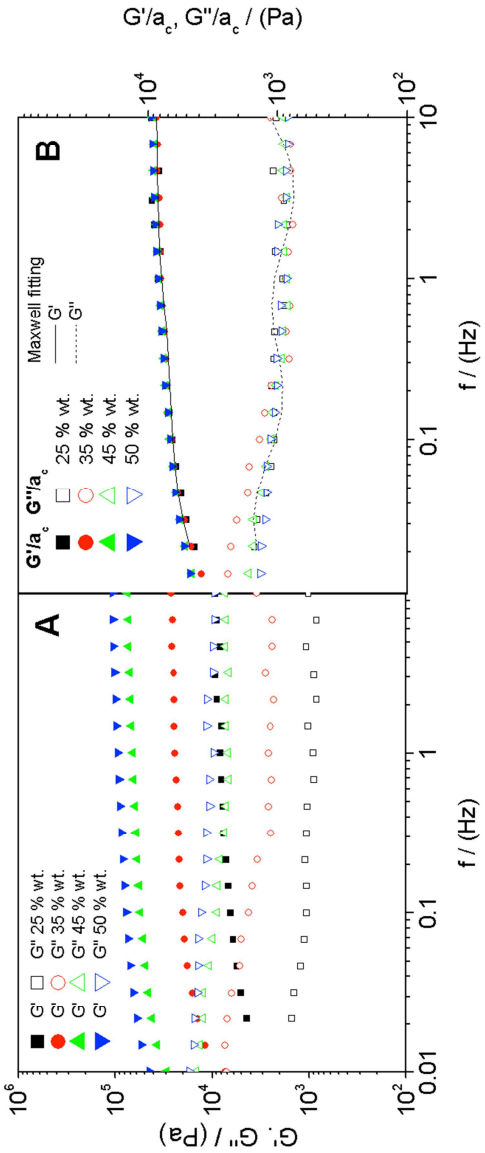
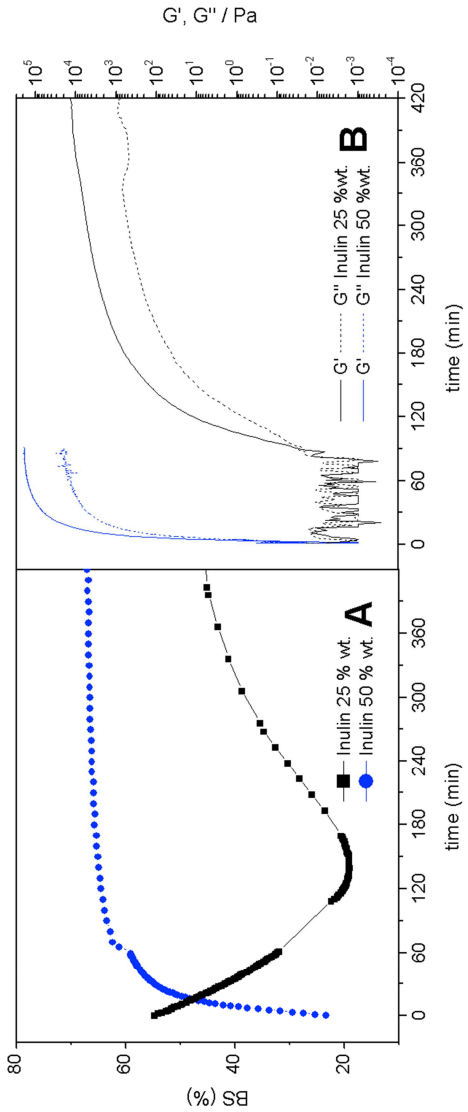


Figure 1. Mechanical spectra for inulin gels at different polysaccharide concentrations (25, 35, 45, 50 wt%) (A); Concentration master curve for mechanical spectra with a shifting factor, a_c, using the 25 wt% system as reference. Lines represent the fitting to a Generalized Maxwell model of four elements (G', continuous line; G'', dashed line) (B).


G', G'' / Pa
B
G' Inulin 25 %wt.
G'' Inulin 25 %wt.
G' Inulin 50 %wt.
G'' Inulin 50 %wt.
time (min)
A
Inulin 25 % wt.
Inulin 50 % wt.
BS (%)
time (min)

543

544 **Figure 2.** Evolution of backscattering signal (BS%) (A) and of elastic and viscous moduli
545 (G', G'') at 0.1 Hz (B) with time for inulin systems with different inulin contents (25, 50
546 wt%).

547

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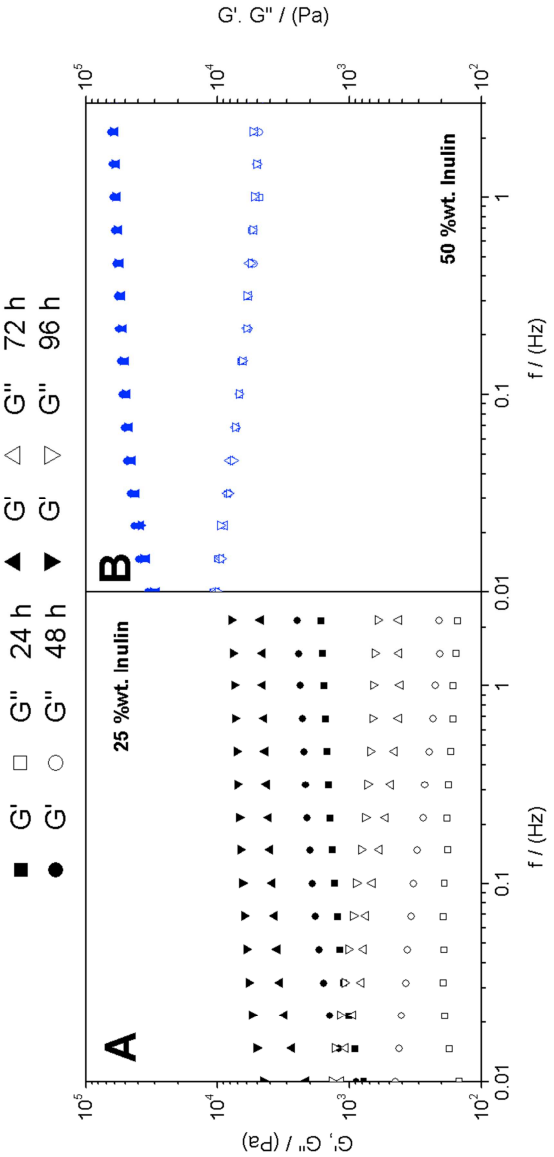
G'. G" / (Pa)
■ G' ● G" 24 h
□ G' ○ G" 48 h
◀ G' ▶ G" 72 h
◁ G' ▷ G" 96 h
A
25 %wt. Inulin
B
50 %wt. Inulin
G', G" / (Pa)
f / (Hz)
f / (Hz)

549

550 **Figure 3.** Effect of storage time on viscous moduli (G', G'') of inulin systems at 25 (A) and
551 50 wt% (B).
552
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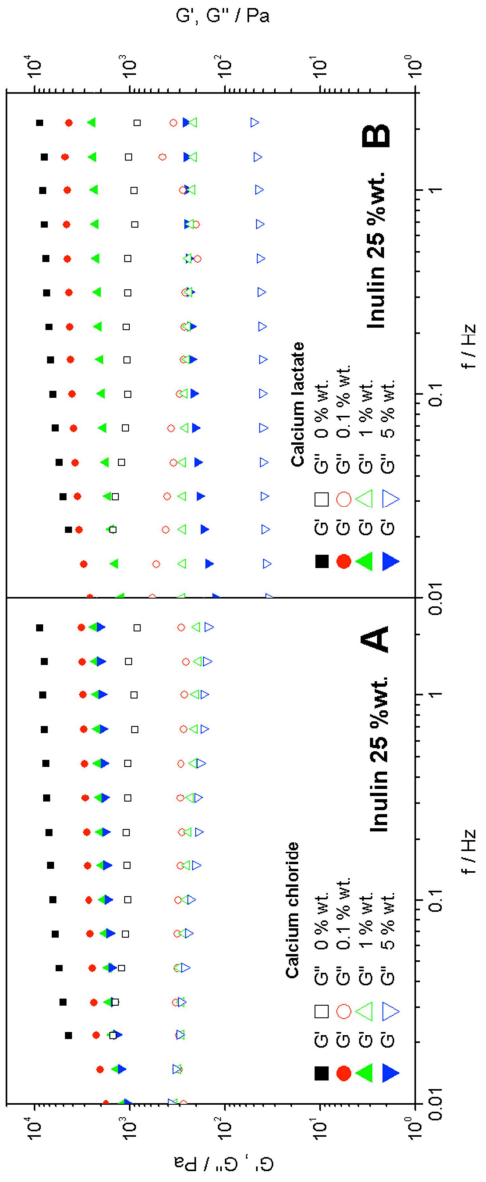
G', G" / Pa
A
Inulin 25 %wt.
Calcium chloride
G' 0 % wt.
G" 0 % wt.
G' 0.1 % wt.
G" 0.1 % wt.
G' 1 % wt.
G" 1 % wt.
G' 5 % wt.
G" 5 % wt.
f / Hz
B
Inulin 25 %wt.
Calcium lactate
f / Hz

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558

559 **Figure 4.** Mechanical spectra for 25 wt% inulin gels containing different amounts (0-5 wt%)

560 of Calcium chloride (A) and Calcium lactate (B).

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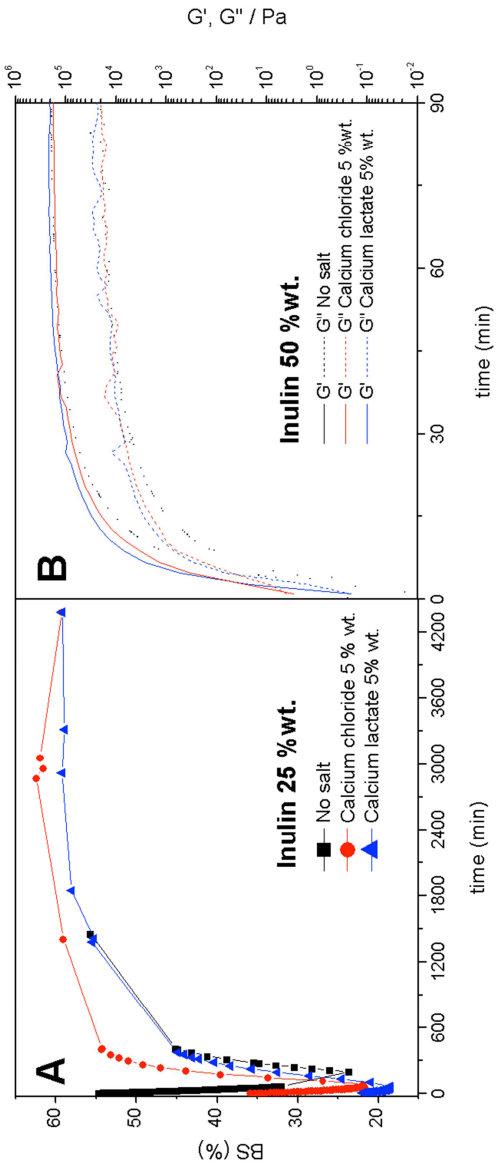
G', G" / Pa
A
B
Inulin 25 %wt.
No salt
Calcium chloride 5 % wt.
Calcium lactate 5% wt.
Inulin 50 %wt.
G' No salt
G" No salt
G' Calcium chloride 5 %wt.
G" Calcium chloride 5 %wt.
G' Calcium lactate 5% wt.
G" Calcium lactate 5% wt.
BS (%)
time (min)
time (min)

564

565

566 **Figure 5.** Evolution of backscattering signal (ΔBS/100%) at a certain height (4 cm) for 25
567 wt% inulin system (A); and of elastic and viscous moduli (G', G'') at 0.1 Hz with time for 50
568 wt% inulin system (B) as a function of time. Both systems are studied in the presence of
569 chloride or lactate salt (5 wt%) or in the absence of them

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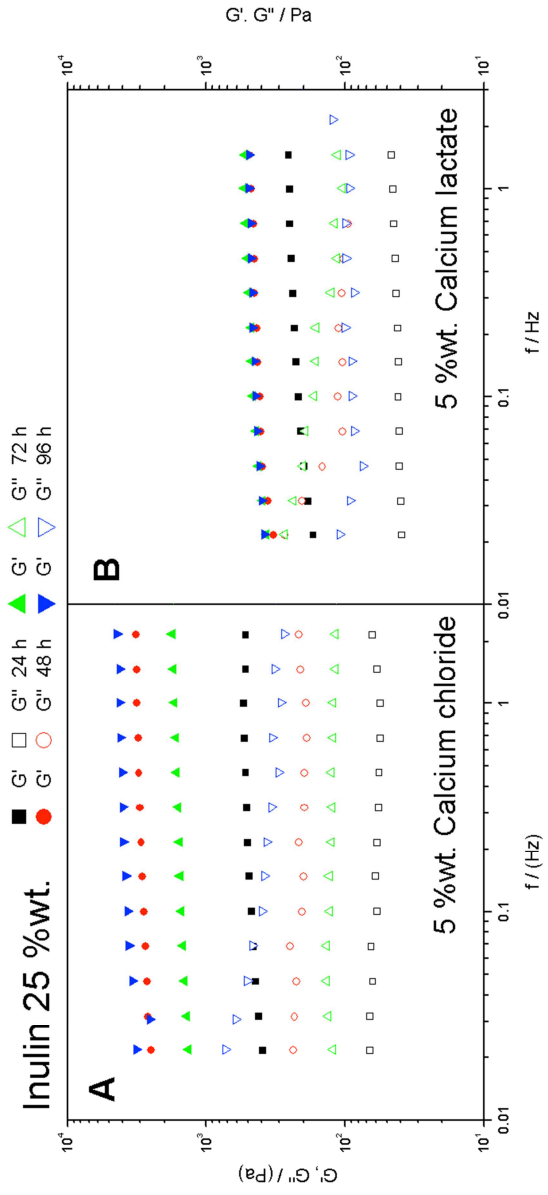
G', G" / Pa
Inulin 25 %wt.
G' 24 h
G" 48 h
G' 72 h
G" 96 h
A
5 %wt. Calcium chloride
B
5 %wt. Calcium lactate
G', G" / (Pa)
f / (Hz)
f / Hz

573

574

575

576

577 **Figure 6.** Effect of storage time on viscous moduli (G', G'') of 25 wt% inulin systems
578 containing 5 wt% of Calcium chloride (A) or Calcium lactate (B).

579

580

581 # Tables

582 Table 1. Critical stress (τ_c) and strain (γ_c) for inulin gels with different inulin contents

583

584 **Inulin (wt%)**	**τ_c (Pa)**	**γ_c (mm/mm)**
585 **25**	2.9 ± 1.4 **a**	$8.0 \cdot 10^{-4} \pm 3.0 \cdot 10^{-4}$ **b**
586 **35**	9.2 ± 0.0 **b**	$4.3 \cdot 10^{-4} \pm 0.8 \cdot 10^{-4}$ **a**
587 **45**	16.3 ± 0.6 **c**	$5.2 \cdot 10^{-4} \pm 0.3 \cdot 10^{-4}$ **ab**
588 **50**	30.3 ± 0.0 **d**	$3.6 \cdot 10^{-4} \pm 0.1 \cdot 10^{-4}$ **a**

589 Different letters in the same column indicate significant differences ($p < 0.05$).

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596

597 Table 2. Critical stress (τ_c) and strain (γ_c) for 25 wt% inulin gels with different calcium salt

598 contents.

Calcium Lactate (wt%)	τ_c (Pa)	γ_c (mm/mm)
0	2.9 ± 1.4 **a**	$8.0 \cdot 10^{-4} \pm 3.0 \cdot 10^{-4}$ **ab**
0.1	1.2 ± 0.5 **a**	$5.34\ 10^{-4} \pm 4.3 \cdot 10^{-4}$ **a**
1	1.5 ± 0.0 **a**	$6.0 \cdot 10^{-4} \pm 0.7 \cdot 10^{-4}$ **ab**
5	1.5 ± 0.0 **a**	$13.0 \cdot 10^{-4} \pm 0.6 \cdot 10^{-4}$ **b**
Calcium Chloride (wt%)	**τ_c (Pa)**	**γ_c (mm/mm)**
0	2.9 ± 1.4 **a**	$8.0 \cdot 10^{-4} \pm 3.0 \cdot 10^{-4}$ **a**
0.1	2.8 ± 0.0 **a**	$6.6 \cdot 10^{-4} \pm 1.2 \cdot 10^{-4}$ **a**
1	5.1 ± 0.0 **b**	$7.4 \cdot 10^{-4} \pm 0.8 \cdot 10^{-4}$ **a**
5	0.8 ± 0.0 **c**	$12.2 \cdot 10^{-4} \pm 0.2 \cdot 10^{-4}$ **b**

599

600 Different letters in the same column indicate significant differences ($p < 0.05$).

601

Highlights

- Higher inulin content results in a faster gelation.
- Gelation kinetics can be studied either by backscattering or rheological tests.
- Calcium salts resulted in inulin gels with lower viscoelastic moduli.
- The nature of the anion in calcium salts affect the rheology of inulin gels.

FACULTAD DE QUÍMICA
Departamento de Ingeniería Química

Sevilla, 21 June 2019

Dear Editor,

Enclosed please find a revised version of the manuscript entitled “Gelation properties of Calcium-Inulin Gels”.

We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere. We have no conflicts of interest to disclose.

Sincerely,

María Luisa López-Castejón

Departamento de Ingeniería Química
Escuela Politécnica Superior
C/Virgen de África, 7
41011
Sevilla
SPAIN

Tfno: +34 954557179
Fax: +34 954556447
Email: llcastejon@us.es