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Title: Storage stability of starch-based dairy desserts containing long-chain inulin: Rheology and particle size distribution.

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Storage stability of starch-based dairy desserts containing long-chain inulin: Rheology and particle size distribution.

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Abstract

Variations in the rheological properties and particle size distribution in dairy desserts containing long-chain inulin during storage were studied. While control samples without inulin proved stable, rheological properties of desserts containing 7.5% inulin changed gradually during storage time. There was a progressive aggregation of inulin crystals in the continuous phase, thereby increasing the effective fraction volume and leading to a more thixotropic, consistent, pseudoplastic and elastic system. These time-course effects were greater in the case of skimmed-milk sample than for the whole-milk sample. Depending on the type of milk, different sized inulin aggregates were formed during storage time, and indicated that the presence of fat hindered the formation of large inulin aggregates.

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1. Introduction

Inulin is a soluble dietary fibre forming a subset of nutraceutical ingredients that is increasingly used in food products (Fagan, O'Donnell, Cullen, & Brennan, 2006). Inulin is a natural non-digestible storage polysaccharide comprising a chain of fructose molecules with a terminal glucose molecule. It is found in many vegetables, amongst which chicory roots are considered most suitable for industrial applications (Flamm, Glinsmann, Kritchevsky, Prosky, & Roberfroid, 2001; Robinson, 1995). Native chicory inulin contains molecules of different degrees of polymerization (from 2 to 60) and commercial products containing only short-chain inulin (DP 2-7) or long-chain inulin (22-25) are obtained from native inulin industrially. Inulin and oligofructose are of interest in human nutrition due to their prebiotic effect, i.e., specific stimulation of growth and/or activity of colonic bacteria that benefit the host, as well as inhibiting the growth of pathogens and harmful microorganisms (Roberfroid, 2007). The addition of inulin to different foods has aimed to supplement them to increase fibre ingestion, in amounts that oscillate between 3-6 g per portion, or to assure its bifidogenic nature, adding 3-8 g per portion (Coussement, 1999).

In addition to its beneficial health effects, inulin also has interesting technological properties and has been proposed as a low-calorie sweetener or fat substitute.

There are a number of recent studies in the literature investigating the effect inulin addition has on both rheological and sensory properties of dairy products. In the case of long-chain inulin, most studies focus on its use as a fat substitute in low-fat or reduced-fat products. Adding 4–6% inulin to skim milk beverages increased viscosity, approximating it to that of 3.1% fat beverages (Villegas & Costell, 2007). The same effect was observed on adding 6 % inulin to low-fat custard systems with low starch concentration (2.5 and 3.25%) (Tárrega & Costell, 2006). In reduced fat ice-cream, the addition of long-chain inulin increased both hardness and viscosity, and also lowered the freezing point (Schaller-Polovny & Smith, 2001) and improved sensory properties (Schaller-Povolny & Smith, 1999). Long-chain inulin improved the creamy mouthfeel of low-fat stirred yoghurt by enhancing the airy, thickness and stickiness attributes (Kip, Meyer, & Jellema, 2005).

Guggisberg, Cuthbert-Steven, Piccinali, Bütikofer, and Eberhard (2009) showed that yield stress, firmness and creaminess of low-fat set yoghurt increased with both inulin and fat content; however, the highest inulin level considered (4%) was not enough to imitate whole-milk yoghurt. In contrast, Paseephol, Small, and Sherkat (2008) found that adding 4% inulin to non-fat set yoghurt gave lower yield stress and gel stiffness that were similar to those of the full-fat yoghurt. The addition of 5 % inulin to low-fat fresh Kashar cheese also improved its textural, melting and sensory properties (Koca & Metin, 2004). In this case, the low-fat cheese with inulin and full-fat cheese were not as hard, elastic or gummy as the low-fat cheese and had greater meltability.

According to these studies, the inclusion of long-chain inulin in a formulation have different effects on the rheological properties depending on product type and composition and it seems inulin can act as a filler or as breaker of structure in the same way as fat globules do. González-Tomás, Coll-Marqués, and Costell, (2008) studied the effect of long-chain inulin on viscoelastic properties of both low and full-fat desserts, with results indicating that although the effect of inulin concentration (from 2.5 to 7.5%) had low impact on the viscoelasticity of whole-milk desserts, they were key factors affecting skimmed-milk desserts.

Besides these studies on inulin applications, several fundamental studies on inulin rheology and gelling mechanisms can be found in the literature. Native and long-chain inulin in concentrate aqueous solutions develop a gel structure formed by a network of crystalline particles (Hébette et al., 1998; Chiavaro, Vittadini, & Corradini, 2007). The characteristics of these gels have been shown to depend on multiple factors. In general, both crystallization rate and gel firmness increased with inulin concentration, shear treatment, and with the presence of seeding crystals after preparation, which depends on the thermal treatment (Bot, Erle, Vreeker, & Agterof, 2004; Duynhoven, Kulik, Jonker, & Haverkamp, 1999; Sensus Operations CV, 2002). The kinetics of ageing in long-chain inulin gels was deduced from the amount of solid-like component through NMR cross-relaxation experiments (Duynhoven et al., 1999), and gel firming was observed to take longer when starting from totally dissolved inulin, for instance inulin prepared at temperatures exceeding 82 ºC. Bot et al. (2004) also studied how the crystallisation process affected rheological properties of inulin gels during ageing and observed that shear-stress values at deformation increased progressively during ageing and the effect endured for at least 64h.

As occurs in aqueous systems, when inulin is included in a product formulation the crystallization process could also be expected to occur, thereby affecting time-course rheological properties. However, to the best of our knowledge, very little information has been reported on the inulin structuring process and its stability in dairy products during the initial storage period. The aim of this work was to study the variations in rheological properties and particle size distribution in custard desserts with different fat content containing long-chain inulin during refrigerated storage.

2. Materials and methods

2.1. Samples composition and preparation

The following ingredients were used: hydroxypropylated tapioca di-starch phosphate (C* Creamtex 75720; Cerestar Ibérica, Barcelona, Spain); inulin of long chain length (≥ 23 monomers, Frutafit TEX! from Sensus, Brenntag Química, Barcelona, Spain); colouring (Vegex NC 2c; CHR Hansen S.A., Barcelona, Spain), vanilla aroma (37548A; Lucta S.A., Barcelona, Spain); skim and whole-milk powders (Central Lechera Asturiana, Valladolid, Spain); mineral water (Font Vella, Girona, Spain) and commercial sucrose.

Custard desserts with either whole milk (3.5% fat) or skimmed milk (0.14% fat) were prepared containing 7.5% of long-chain inulin while control samples were made without inulin. All samples included fixed amounts of starch (3.75%, w/w), milk (80%, w/w), sucrose (6%, w/w), colouring (0.052%, w/w) and vanilla aroma (0.016, w/w). Milk was prepared 24 h in advance by dissolving 13.5% (w/w) milk powder in deionised water and stored under refrigeration (4±1 ºC).

Samples were made in batches of 800 g. All ingredients were weighed in a flask and mixed by magnetic stirring for 10 min. The flask was placed in a water bath at 97 ± 1 ºC and stirred constantly with a propeller stirrer. After 10 min the product temperature reached 85 ± 1 ºC and heating was maintained at this temperature for 15 min. After the heating process, the evaporated water was replaced gravimetrically. The sample was cooled in a water bath at 20 ºC until reaching temperatures of about 40 ºC and then the aroma was added. Samples were homogenised, transferred to closed flasks and stored under refrigeration (4 ± 1 ºC). Particle size distribution and rheology were measured after 1, 2, 3, 6 and 7 days of storage.

2.2. *Rheological measurements*

Both flow behaviour and viscoelastic properties were measured in a controlled stress rheometer RS1 (Thermo Haake, Karlsruhe, Germany), with parallel plates geometry of 6 cm diameter and 1 mm gap. During the measurement a temperature of 10 ±1 ºC was maintained using a Phoenix P1 Circulator device (Thermo Haake, Karlsruhe, Germany). Samples were allowed to rest for 10 min before measurement. Two batches of each sample were prepared and two measurements were run per batch. A fresh sample was loaded for each run.

2.2.1. *Flow behaviour*.

Flow curves were obtained by shearing up from 1 to 200 s^{-1} in 60 s and down (Tarrega, Durán, & Costell, 2004). Values of the area under the upstream data points (A_{up}) and under the downstream data points (A_{down}) as well as the hysteresis area (A_{up} - A_{down}) were obtained. Data from the ascending flow curve were fitted to the Ostwald de Waele model

$$\sigma = K \dot{\gamma}^{n}$$

that relates the variation in shear stress values (σ, Pa) with shear rate ($\dot{\gamma}$, s^{-1}) and where K (Pa s^n) is the consistency index and n is the flow index. These calculations were made using the Rheowin Pro software (version 2.93, Haake).

2.2.2. *Viscoelastic properties*.

To determine the linear viscoelastic region, stress sweeps were run at 1 Hz. The frequency sweeps were performed over the range f = 0.01-10 Hz and the values of the storage modulus (G'), the loss modulus (G''), the loss tangent angle (tan δ), as a function of frequency, were calculated using the Rheowin Pro software (version 2.93, Haake).

2.3. Particle size distribution

Particle size distribution analysis was determined using a Laser Diffraction Particle Size Analyzer (MasterSizer 2000, Malvern Instrument Ltd., Worcestershire, England). A refractive Index value of 1.53 was selected for measurements considering the starch refractive index as a reference. About 0.2 g of custard was dispersed in distilled water at room temperature (20 ± 2 °C) until an obscuration of 0.2 was obtained. The sample was placed under ultrasonic dispersion for 2 min to ensure particles were independently dispersed and thereafter maintained by stirring during measurement. Distributions were made in triplicate for each sample. Particle size calculations were based on the Mie-Scattering theory. Volume mean diameter values (D[4 ,3]) and the percentage of volume corresponding to each observed population were obtained using the software provided with the equipment (Mastersizer 2000 V. 5.40).

2.4. Statistical analysis

The effects of storage time, type of milk and addition of inulin on the rheological properties were studied through an analysis of variance (ANOVA) of three factors with interactions. The Fisher test (α=0.05) was used to calculate the minimum significant difference. Calculations were carried out with XLSTAT-Pro Version 2007 (Addinsoft, Paris, France)

3. Results and discussion

3.1. Rheological properties

3.1.1. Flow behaviour

Different curves were observed depending on storage time and sample composition (Fig. 1). A hysteresis loop (different ascending and descending curves) was observed for all of them, indicating flow-time dependency. To quantify this phenomenon, the thixotropic area values were calculated corresponding to the area encircled between the two curves. According to Halmos and Tiu (1981), this area is an index of the energy per unit time and unit volume needed to eliminate the influence of time on flow behaviour. Subjecting the thixotropic area values to an ANOVA showed a significant ternary interaction among the effects of storage time, inulin addition and type of milk. This indicates that variations in the values over time depended on both the presence of inulin and type of milk. Fig. 2 gives the thixotropic area values and their variation throughout the storage time for all evaluated samples. For samples without inulin the thixotropic area values were higher for whole milk than for skimmed milk and in both cases values did not change significantly during storage time. However, for samples containing inulin the values of the area increased notably over time indicating a change in the system structure during storage. This inulin-induced increase in the area over time was higher in skimmed-milk samples. The skimmed-milk sample displayed a lower thixotropic area than the whole-milk sample only on day one, while after day two the highest thixotropic area was always observed for the skimmed-milk sample.

On observing shear stress variation with shear rate, samples showed a non-Newtonian shear-thinning flow behaviour, in accordance with previous observations of commercial samples of semi-solid dairy desserts (Tárrega & Costell, 2007; Doublier & Durand, 2008; González-Tomás & Costell., 2006). Sample flow behaviour was characterized using the experimental data obtained in the upward rheogram. Flow data were well fitted to the Ostwald de Waele model ($0.951 \leq R^2 \geq 0.990$) and variations in consistency coefficient (K) and flow index (n) values were studied (Figs. 2b and 2c). For both parameters, analysis of variance showed a significant interaction between the three effects (Table 1). While the consistency coefficient of samples without inulin did not change over time, this parameter underwent a notable time-course increase in samples containing inulin, the magnitude of this effect being greater for the skimmed-milk sample. Regarding the flow index, skimmed-milk samples with inulin displayed lower values than the control skimmed-milk sample, the value decreasing with storage time. Inulin addition to whole-milk samples did not modify the flow index value initially; however, it decreased with time. During storage the presence of inulin induced a change in the matrix structure that gave a more consistent product with a more pseudoplastic flow. Therefore, the effect of inulin during storage increased both the time dependency and consistency of the system, indicating structural changes at two different levels. Part of the newly formed structure proved more labile and responsible for the time dependency while another part of the structure, responsible for the increase in consistency, proved independent of the shearing time but varied with shear rate.

3.1.2. Viscoelastic properties

For the analysis of viscoelastic properties, mechanical spectra of samples were obtained at a range of frequency values between 0.01 and 10 Hz. Mechanical spectra for samples made with skimmed milk are shown in Fig. 3. For all samples the response was typical of weak gels, with storage modulus (G’) higher than loss modulus (G’’) and relatively low values for both moduli. Differences in mechanical spectra were observed depending on composition and storage time. Similar spectra were obtained for the different samples at the beginning of storage; however, after seven days mechanical spectra obtained for samples containing long-chain inulin gave higher viscoelastic moduli values, thus indicating more structured systems.

For comparative purposes, G' and tan δ values were considered at a frequency of 1 Hz (Table 2). Analysis of variance showed a significant ternary interaction effect between inulin addition, type of milk and storage time (Table 3).

For samples without inulin, G’ values did not display significant differences during storage. However, tan δ values slightly increased during storage time in skimmed-milk samples indicating that the relative contribution of the viscous component to the viscoelasticity of the system increased. This more fluid-like behaviour may be attributed to a possible release of water from starch granules in the continuous phase during storage. This phenomenon did not seem to affect the whole-milk sample structure as tanδ values showed very slight variations. Samples containing inulin gradually increased in the storage modulus and decreased in tan δ values during storage time. The increase in G’ was more pronounced for the skimmed-milk sample. In this case the initial value of G’ was 23.70 Pa and reached 214.80 Pa after seven days of storage, while for the whole-milk sample the G’ value varied from 21.03 Pa to 152.95 Pa. The decrease in tan δ values with time was similar for both types of system. In agreement with that observed in flow behaviour, in custard with inulin the strength of the structure increased during storage time, exhibiting a more elastic response under non-destructive small deformation tests.

3.2. Particle size distribution

Particle size distribution for samples without inulin is shown in Figs. 4a and 4b. The skimmed-milk sample showed a mono-modal distribution (P1) ranging from 10.0 μm to 91.2 μm with 34.46 μm mean diameter ($D_{4,3}$). This distribution represents the particles to be found in a basic custard system and should correspond to the dispersed swollen starch granules. A bimodal distribution was obtained for the whole-milk sample. The major population coincided with that observed for the skimmed-milk sample and a small population (P2; 1.87 % of total volume) of lower particle sizes (1.91 μm and 7.58 μm) with 4.64 μm of mean diameter, which can be ascribed to the presence of fat globules. Regarding possible variations during storage, no time-course variation in particle size distribution was found for these two samples.

However, more complex variations in particle size distribution were observed for samples containing inulin depending on storage time and type of milk (Figs. 4c and 4d). Different populations below 10 μm were recorded and the relative percentage of volume was determined for each population at different storage times (Figs. 5 and 6). For skimmed-milk samples with inulin, besides the typical peak corresponding to the basic custard system (P1), a new small population (P2), located at lower particle size (1.9-10 μm), appeared after two days of storage (Fig. 5). As storage time increased, so too did the relative percentage of volume corresponding to this population until reaching 7.47% of the volume. This new population can be attributed to an inulin crystallization process. Furthermore, after three days of storage, this distribution showed a shoulder from 0.48 to 1.45 μm conformed by a group of smaller particles that could indicate the formation of smaller inulin crystals (Fig. 4c).

Distributions with three modes were observed for whole-milk samples containing inulin, with the main population corresponding to the basic custard system (P1), and two smaller populations that appeared below 10 μm. In this case and according to the results observed before, the particles ranging from 1.9 to 10 mm (P2) could correspond to both fat globules and inulin aggregates. On the first day, the volume percentage represented by this population was similar to the control sample without inulin, indicating that the most part corresponds to fat globules. The population of particles ranging from 0.41-1.90 mm (P3) could correspond to those observed as a small shoulder in the skimmed-milk sample with inulin stored for three days. In the case of the whole-milk sample, these particles were distributed in a well conformed population, which represented 1.29 % of the volume on storage day one. On storage day two, the percentage of volume corresponding to P2 particles increased, indicating an inulin aggregating process. However, subsequently the volume percentage corresponding to P2 decreased while that corresponding to P3 increased, indicating no further large size aggregate formations but the emergence of smaller aggregates. Comparison of these results with those obtained for skimmed milk indicates that the presence of fat hindered the formation of large sized aggregates. The larger the aggregate size, the more water they held. The lower number of big inulin aggregates would explain the weaker structure formed during storage in whole-milk samples as compared with skimmed-milk samples.

4. Conclusions

The presence of 7.5 % long-chain inulin induced important rheological changes in custards during storage time. A progressive aggregation of inulin crystals took place in the continuous phase, thereby increasing the effective fraction volume and leading to a more thixotropic, consistent, pseudoplastic and elastic system. The magnitude of this time-course changes was greater in the skimmed-milk sample than for the whole-milk sample because the presence of fat hindered the formation of large sized aggregates.

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Figure legends

Fig. 1. Flow behaviour for custards. Control samples (without inulin) made with skimmed milk (a) and whole milk (b), and samples containing 7.5% long chain inulin made with skimmed milk (c) and whole milk (d). Measurements were carried out at different times of storage: day 1 (☐), day 2 (◇), day 3 (△), day 6 (×) and day 7 (○).

Fig. 2. Variation of thixotropic area, consistency (K), and flow index (n) values for control samples (open symbols) and samples with 7.5% long chain inulin (filled symbols) containing skimmed milk (- - -) and whole milk **(** —) during storage time. Error bars correspond to the Least Significant Difference interval as obtained from Fisher test (α=0.05).

Fig. 3. Mechanical spectra for custards. Control samples (without inulin) made with skimmed milk (a) and whole milk (b), and samples containing 7.5% long chain inulin made with skimmed milk (c) and whole milk (d). Measurements of G' (filled symbols) and G" (open symbols) were carried out at different time of storage: day 1 (☐**,** ■), day 3 (△, ▲) and day 7 (○**,** ●).

Fig. 4. Particle size distribution for custards. Control samples (without inulin) made with skimmed milk (a) and whole milk (b), and samples containing 7.5% long chain inulin made with skimmed milk (c) and whole milk (d). Measurements were carried out at different time of storage: day 1 (—), day 3 (− − −) and day 7 (- - -).

Fig. 5. Variation during storage time of the percentage of volume occupied by particles of population P2 (1.90 μm – 10.00 μm) in the skimmed milk sample (white bars) and in the whole milk sample (dark bars) containing 7.5% long chain inulin. Average values (n=2) and standard deviation.

Fig. 6. Variation during storage time of the percentage of volume occupied by particles of population P3 (0.41 μm – 1.90 μm) in the whole milk sample containing 7.5% long chain inulin. Average values (n=2) and standard deviation.

Table 1.
Effects of type of milk, storage time and presence of inulin on consistency (K) and flow index (n) values, F ratio (F) and probability (*p*) values.

	K (Pa s^{-1})		n	
Effects	F	*p*	F	*p*
Type of milk	383.98	< 0.0001	3031.34	< 0.0001
Time	2741.54	< 0.0001	365.72	< 0.0001
Inulin	23292.52	< 0.0001	7426.85	< 0.0001
Type of milk * Time	141.88	< 0.0001	80.76	< 0.0001
Type of milk * Inulin	2989.17	< 0.0001	3607.24	< 0.0001
Time * Inulin	2732.78	< 0.0001	327.42	< 0.0001
Type of milk * Time * Inulin	158.64	< 0.0001	83.97	< 0.0001

Table 2.

Storage modulus (G'), loss modulus (G") and loss angle tangent (tan δ) average values (n=2) at 1 Hz for dairy dessert samples with and without inulin, during storage time[a].

	Time (days)	Without inulin			With long chain inulin		
		G' (Pa)	G" (Pa)	tan δ	G' (Pa)	G" (Pa)	tan δ
Skimmed milk	1	15.25^{ab}	11.05^{bc}	0.72^{f}	23.70^{bc}	10.08^{abc}	0.43^{d}
	2	19.32^{abc}	12.31^{c}	0.64^{e}	59.53^{g}	18.50^{d}	0.31^{ab}
	3	17.73^{abc}	12.72^{c}	0.72^{f}	149.05^{i}	48.31^{g}	0.32^{b}
	6	11.56^{a}	11.20^{c}	0.97^{h}	242.65^{l}	75.33^{i}	0.31^{ab}
	7	11.88^{a}	10.60^{abc}	0.89^{g}	214.80^{k}	61.85^{h}	0.29^{ab}
Whole milk	1	26.61c^{de}	11.28^{c}	0.42^{d}	21.03^{bc}	7.94^{a}	0.38^{c}
	2	20.46a^{bc}	8.15^{ab}	0.40^{cd}	38.28^{f}	11.91^{c}	0.31^{ab}
	3	32.81^{def}	12.91^{c}	0.39^{cd}	87.36^{h}	25.44^{e}	0.29^{ab}
	6	25.79^{cd}	10.48^{abc}	0.41^{cd}	163.30^{j}	45.67^{fg}	0.28^{a}
	7	34.94^{ef}	12.86^{c}	0.37^{c}	152.95^{i}	43.53^{f}	0.28^{a}
Standard Error Values		3.04	0.99	0.02	3.04	0.99	0.02

[a] Values are averages of two sample replicates. Standard error values were obtained from ANOVA. For each parameter, values with different superscript letters are significantly (α=0.05) different according to Fisher test.

Table 3.
Effects of type of milk, storage time and presence of inulin on values of storage modulus (G'), loss modulus (G'') and loss tangent (tan δ).

	G' (Pa)		G'' (Pa)		tan δ	
Effects	F	*p*	F	*p*	F	*p*
Type of milk	142.07	< 0.0001	342.92	< 0.0001	973.32	< 0.0001
Time	692.64	< 0.0001	534.63	< 0.0001	20.62	< 0.0001
Inulin	4747.70	< 0.0001	2832.21	< 0.0001	1696.40	< 0.0001
Type of milk * Time	21.34	< 0.0001	30.59	< 0.0001	23.33	< 0.0001
Type of milk * Inulin	460.71	< 0.0001	307.21	< 0.0001	767.13	< 0.0001
Time * Inulin	686.78	< 0.0001	510.61	< 0.0001	39.04	< 0.0001
Type of milk * Time * Inulin	37.53	< 0.0001	38.87	< 0.0001	24.55	< 0.0001

Figure revisions required

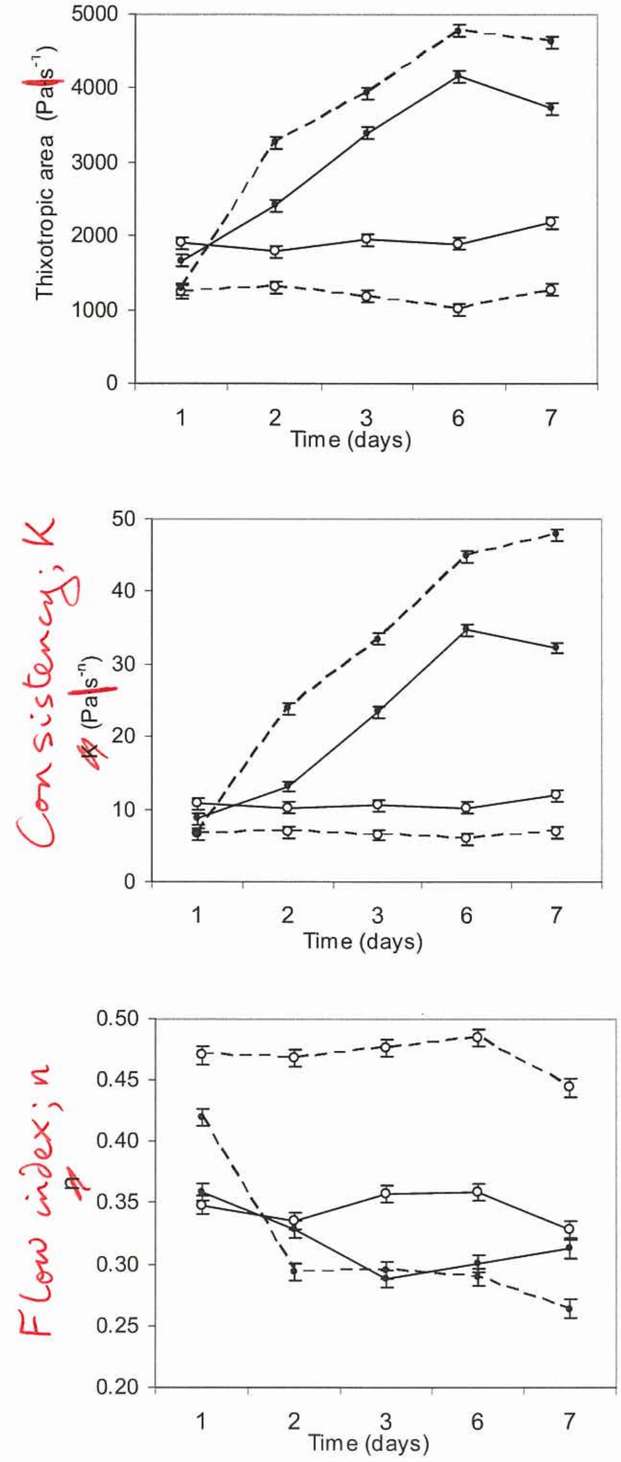

Figure 2. Torres et al.

Figure 1

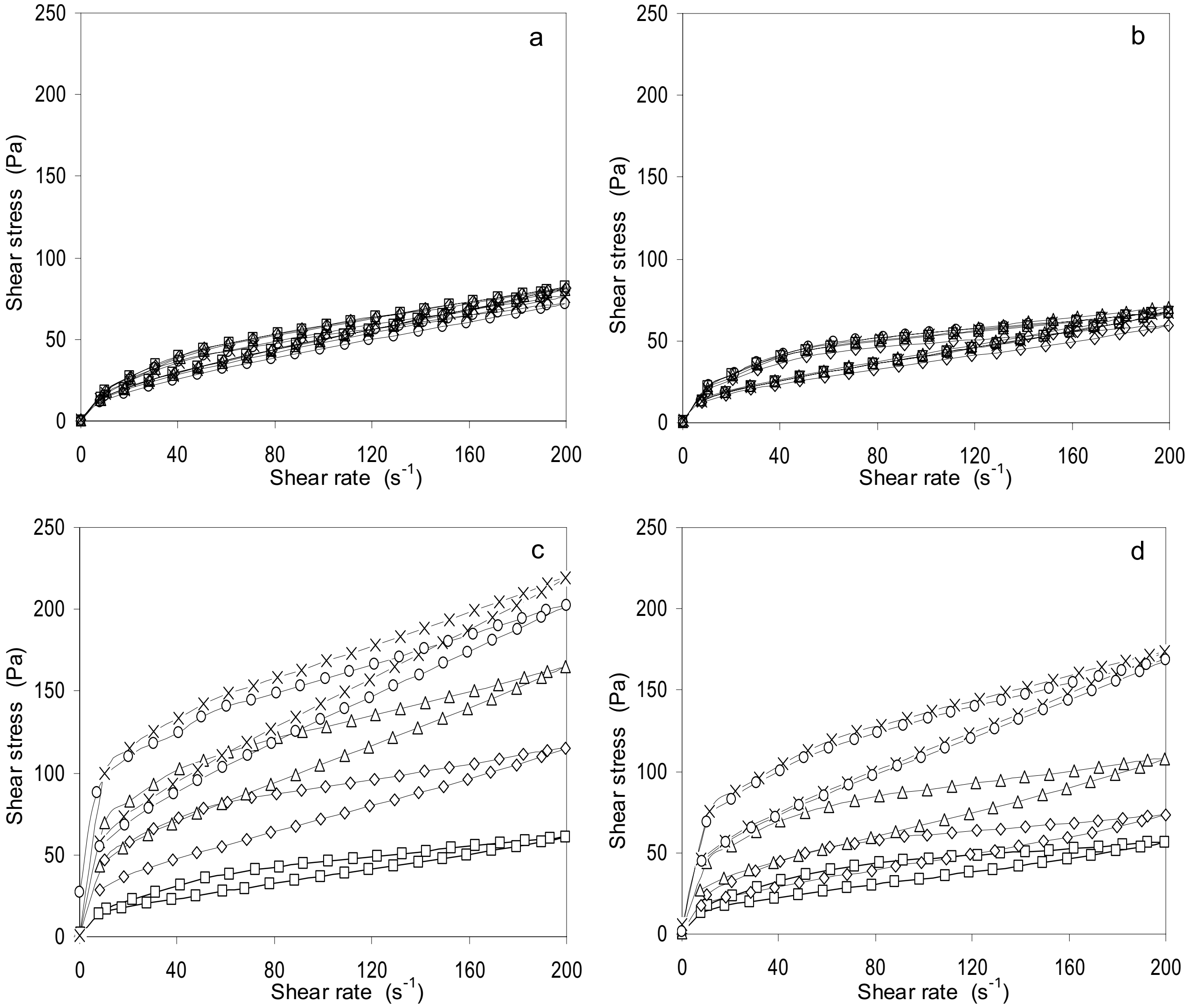

Figure 1. Torres et al.

Figure 2

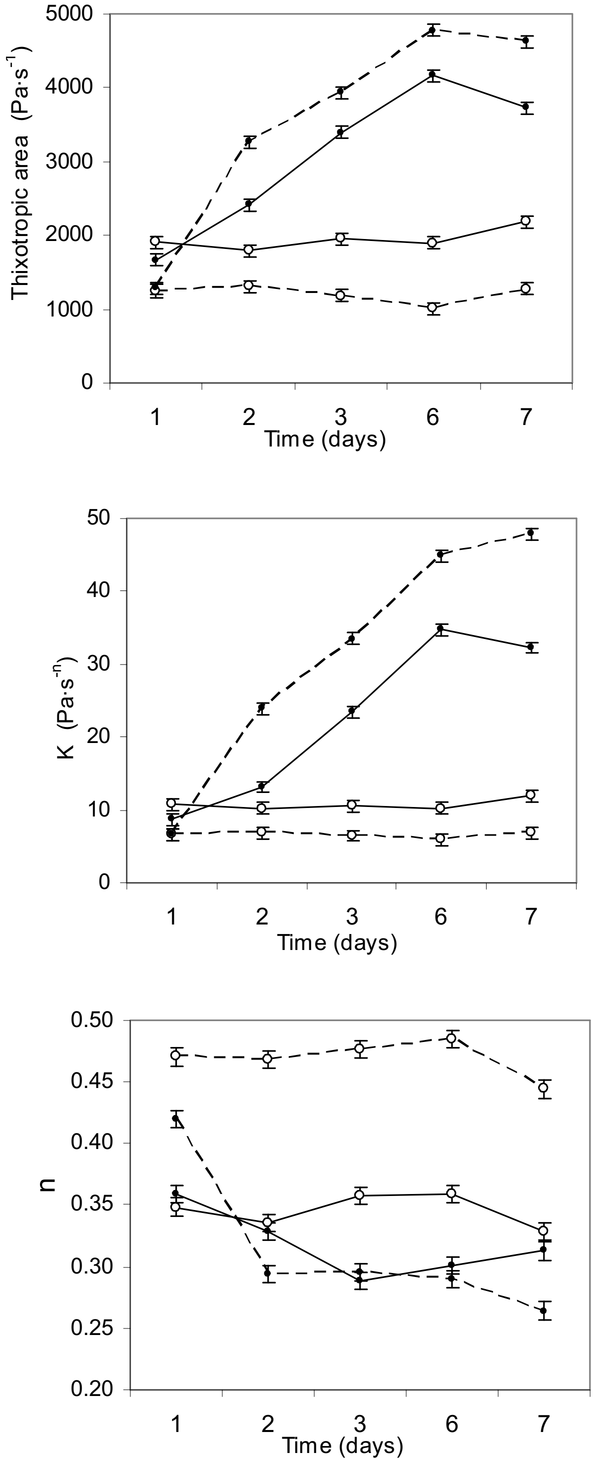

Figure 2. Torres et al.

Figure 3

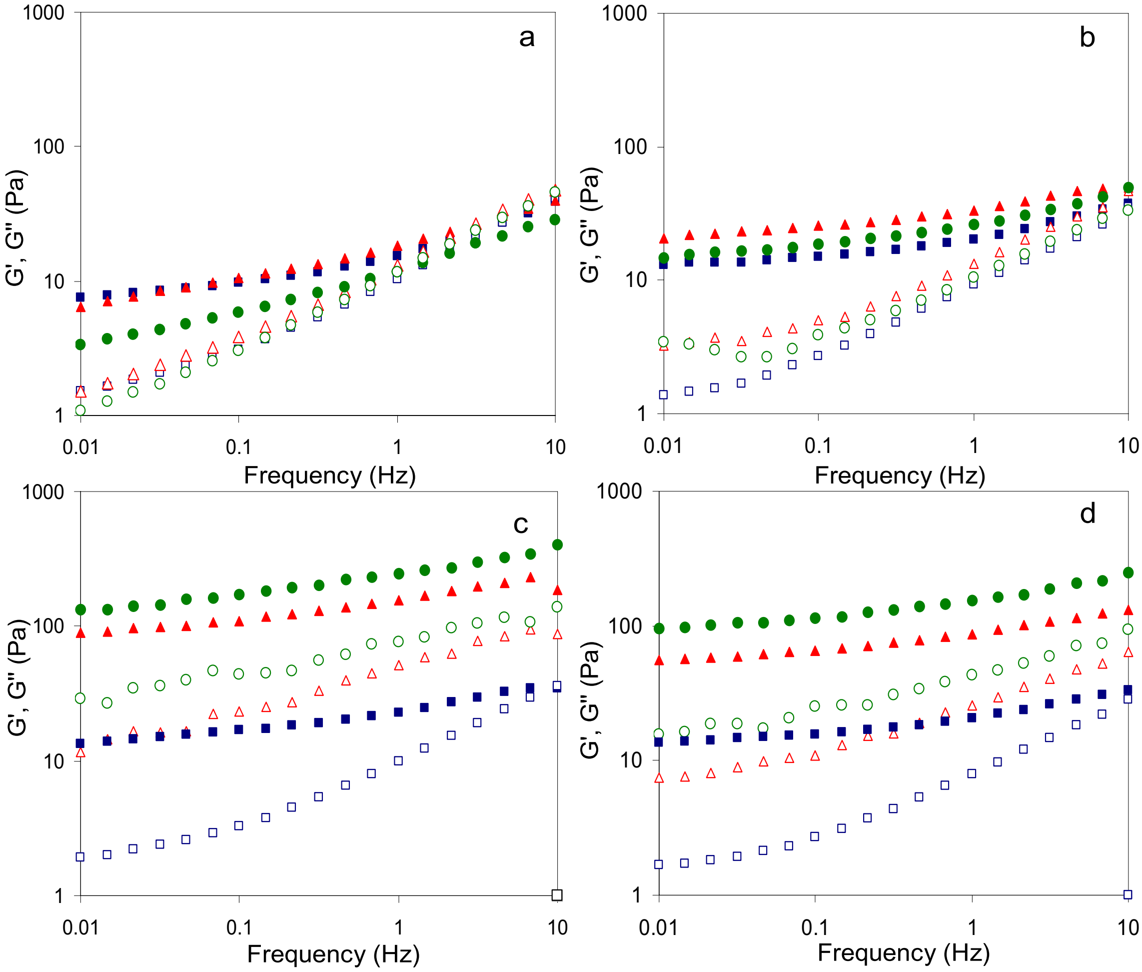

Figure 3. Torres et al.

Figure 4

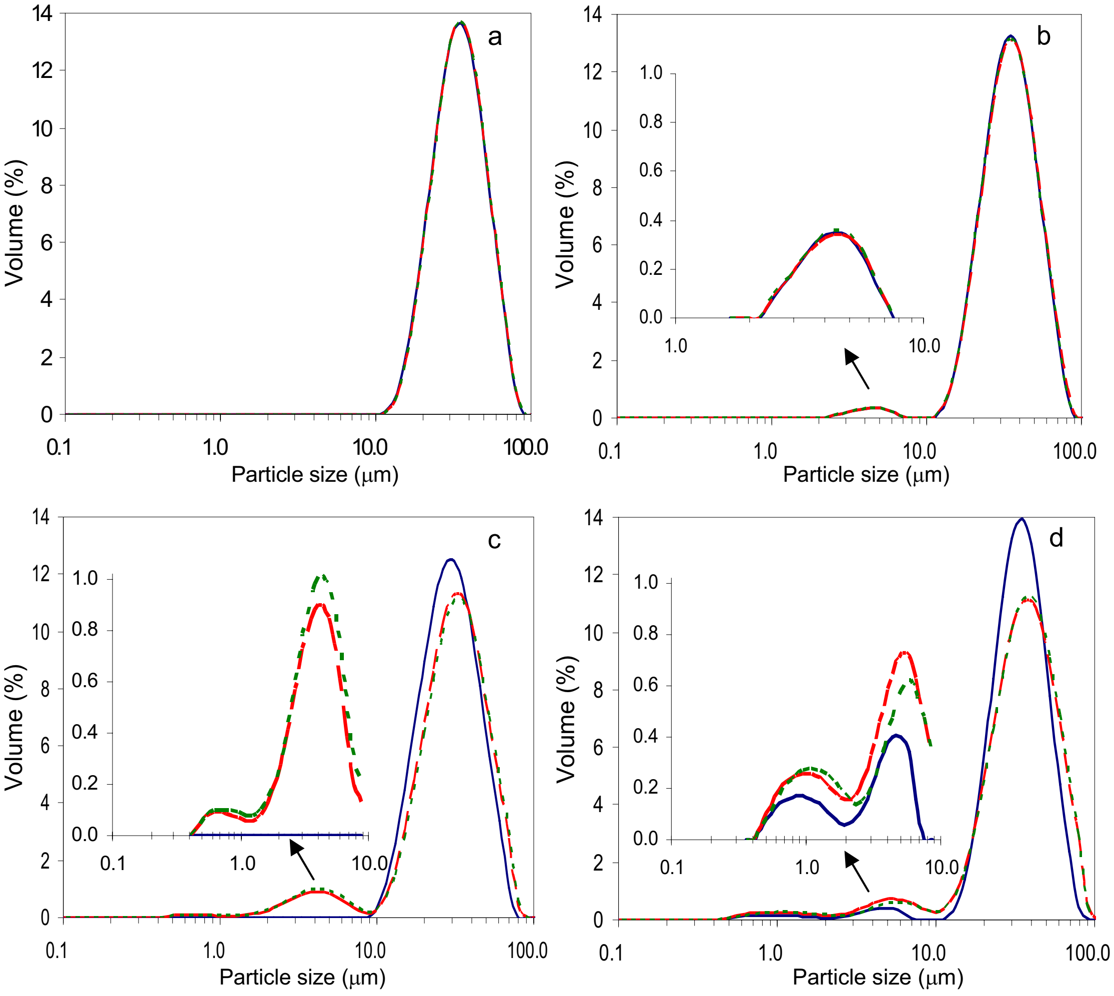

Figure 4. Torres et al.

Figure 5

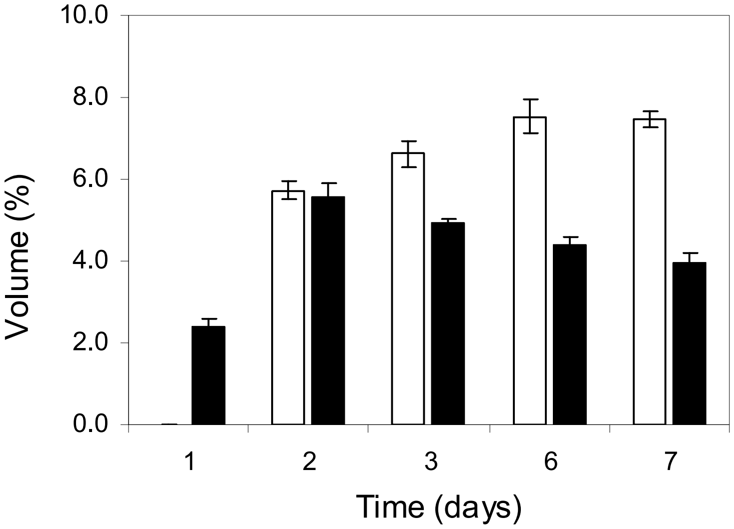

Figure 5. Torres et al.

Figure 6

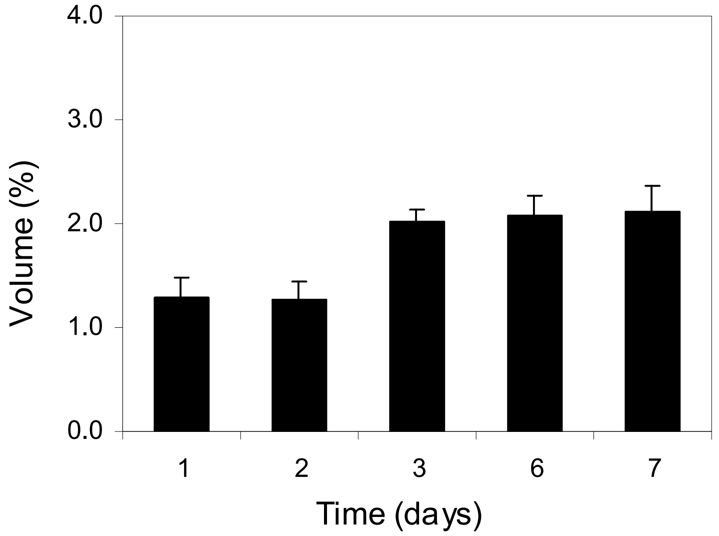

Figure 6. Torres et al.