



Shear and extensional properties of kefiran



Judith Piermaría^a, Carlos Bengochea^{b,*}, Analía Graciela Abraham^{a,c}, Antonio Guerrero^b

^a Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), CONICET, Facultad de Ciencias Exactas, UNLP, 47 y 116, La Plata, Argentina

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

^c Área Bioquímica y Control de Alimentos, Facultad de Ciencias Exactas, UNLP, 47 y 115, La Plata 1900, Argentina

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ABSTRACT

Kefiran is a neutral polysaccharide constituted by glucose and galactose produced by *Lactobacillus kefiranofaciens*. It is included into kefir grains and has several health promoting properties. In the present work, shear and extensional properties of different kefiran aqueous dispersions (0.5, 1 and 2% wt.) were assessed and compared to other neutral gums commonly used in food, cosmetic and pharmaceuticals industries (methylcellulose, locust bean gum and guar gum). Kefiran showed shear flow characteristics similar to that displayed by other representative neutral gums, although it always yielded lower viscosities at a given concentration. For each gum system it was possible to find a correlation between dynamic and steady shear properties by a master curve including both the apparent and complex viscosities. When studying extensional properties of selected gums at 2% wt. by means of a capillary break-up rheometer, kefiran solutions did not show important extensional properties, displaying a behaviour close the Newtonian.

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1. Introduction

Polysaccharides are employed in the food industry as thickeners, binders, stabilizers and gelling agents. In addition to the functional properties provided by these molecules, recently there has been an increasing attention to the potential beneficial health effects that these macromolecules may confer when they are included in foods.

Kefiran is a heteropolysaccharide constituted by D-glucose and D-galactose synthesized by *Lactobacillus kefiranofaciens*, a lactic acid bacterium present in kefir grains (Micheli, Uccelletti, Palleschi, & Crescenzi, 1999; Mukai, Toba, Itoh, & Adachi, 1988). The first study about kefiran structure was published by Kooiman (1968), who proposed a structure composed of two units: kefiran (polysaccharide) and kefirose (pentasaccharide). Kefiran is a water-soluble polysaccharide containing approximately equal amounts of glucose and galactose residues in a chain sequence (Micheli et al., 1999; Mukai et al., 1988). The structure of the repeating unit has been elucidated mainly by methylation analysis and NMR data (Maeda, Zhu, Suzuki, Suzuki, & Kitamura, 2004; Mukai, Toba, Itoh, & Adachi, 1990; Yokoi, Watanabe, Fujii, Toba, & Adachi, 1990). The branching structure is a hexasaccharide repeating unit with a single glucose residue attached to the branch point at the O-2 of Gal of the main chain. However, not all of the units contain a branched chain. The results

of structural elucidation also demonstrated that kefiran isolated from kefir grains is a heteropolysaccharide consisting of Glc and Gal with a molar ratio of 1.0:1.1, and has a backbone composed of (1 → 6)-linked Glc, (1 → 3)-linked Gal, (1 → 4)-linked Gal, (1 → 4)-linked Glc and (1 → 2, 6)-linked Gal, with branch attached to O-2 of Gal residues and terminated with Glc residues (Ghasemlou, Khodaiyan, Jahanbin, Gharibzahedi, & Taheri, 2012).

Kefiran, which has GRAS (generally recognized as safe) status, has been found to improve rheological properties of fermented milk (Rimada & Abraham, 2006), and to form cryogels (Piermaría, de la Canal, & Abraham, 2008; Zavala, Roberti, Piermaría, & Abraham, 2014) and edible films with good mechanical and barrier properties (Piermaría et al., 2011; Piermaría, Pinotti, García, & Abraham, 2009). Moreover, in relation to their biological activities, kefiran has been shown to exert immunomodulatory effect as well as the ability to protect epithelium against *Bacillus cereus in vitro* (Medrano, Perez, & Abraham, 2008; Medrano, Racedo, Rolny, Abraham, & Perez, 2011; Vinderola, Perdigon, Duarte, Farnworth, & Matar, 2006).

Most of the rheological studies in the literature were conducted under a pure shear flow. Nevertheless, many industrial applications often involve a component of extensional mode of deformation and not always shear flow results can be extrapolated directly to the extensional behaviour. In some cases, the extensional deformation could be dominating, as it is the case of a fluid flowing through a contraction pipe, or a fluid stretched by two rotating rollers. Even in dough baking process, the extensional deformation was also believed to play an important role (Dobraszczyk & Morgenstern,

* Corresponding author.

E-mail address: cbengochea@us.es (C. Bengochea).

2003). The extensional properties of the fluid can be quantified by monitoring the kinetics of filament thinning. This principle led to a development of a commercial extensometer, a Capillary Break-up Elongational Rheometer (CaBER) (Rodd, Scott, Cooper-White, & McKinley, 2005). Main advantages of this technique are that it can create a pure extensional flow and is applicable to viscous fluids over a wide viscosity range.

The main objective of this research has been to study the shear and extensional rheological properties of kefir suspensions in relation with other neutral polysaccharides used in food industry.

2. Experimental

2.1. Materials and sample preparation

Commercial Locust Bean Gum (LBG), Guar Gum (GG) and Methyl-Cellulose (MC) used were supplied by Sigma-Aldrich (USA), being all used as received. Both LBG and GG have a reported loss on drying and total ash content lower than 13 and 1.2%, respectively. MC has a degree of substitution of 1.3–1.9. Kefiran (K) was obtained by isolation and purification from kefir grains. The isolation and purification of kefir were performed as previously reported (Rimada & Abraham, 2006). A weighed amount of kefir grains CIDCA AGK1 was treated in boiling water (1:10) for 30 min. The mixture was then centrifuged at 10,000g for 20 min at 20 °C (Avanti J25 Beckman Coulter Inc. centrifuge, Palo Alto, California). The polysaccharide in the supernatant was precipitated by addition of two volume parts of cold ethanol and left at –20 °C overnight. The mixture was centrifuged at 10,000g for 20 min at 4 °C. Pellets were dissolved in hot water and the precipitation procedure was repeated twice. Finally the pellets were lyophilized. Samples were tested for the absence of other sugars and proteins by qualitative thin layer chromatography (TLC) and Bradford method (Bradford, 1976), respectively. The purity of kefir samples was higher than 99.9%, as determined by anthrone method (Southgate, 1991). According to TLC results, the samples did not contain simple sugar (mono or di saccharides) and the protein content determined by Bradford method was inferior to 0.01%. Kefiran sugar composition was analyzed by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-Pad, Dionex DX-300, USA), after complete hydrolysis, according to the procedure by Casabuono, Petrocelli, Ottado, Orellano, and Couto (2011), obtaining a glucose-to-galactose-ratio of 1.21:1.00. This value is similar to that found by Kooiman (1968).

For all polysaccharides, 0.5, 1 and 2% wt. solutions were prepared. Guar gum and locust bean gum by mixing the powders into Millipore water at 50 °C and continuously stirring for 5 h using a magnetic stirring. Lyophilized kefir was placed in Millipore water and dispersions were heated to 90 °C with continuous stirred until complete solubilisation. For methylcellulose solutions preparation about 1/3 of the total required volume of water was heated to 90 °C and the methylcellulose powder was added with agitation. The mixture was stirred until the particles are thoroughly wetted and uniformly dispersed. For complete solubilisation, cold water was added to decrease the temperature of the dispersion. Once the dispersion reached the temperature at which methylcellulose becomes water soluble the viscosity increases and solution were kept to 0–5 °C for 30 min. Finally the solution was stirred at room temperature for 15 h until its use.

2.2. Molecular weight determination

Molecular mass of the polysaccharides kefir was determined by gel filtration using a OH-PAK SB-805HQ gel filtration chromatography column (SHODEX, Kawasaki, Japan) in a HPLC system (Waters, Milford). Samples, dissolved in water, were eluted at room temperature, using NaNO₃ 0.1 M. The flow rate was kept constant at

0.95 ml/min (pressure 120–130 psi). Typically, 50 µl of polysaccharide solutions (0.5 g/l) were injected for each run. Prior to injection, the samples were filtered through 0.45 µm filters (Millipore, Sao Paulo, Brazil). The eluent from the column was analyzed on-line by refractive index detection in a 410 differential refractometer (Waters, Milford). Dextrans with a molecular weight (MW) range from 97,000 to 3,800,000 ALO-2770 (Phenomenex, Torrance, CA) were used as standard.

2.3. Elemental analysis

For elemental carbon-hydrogen-nitrogen-sulfur (CHNS) analysis, 10 mg samples of each polysaccharide were combusted and oxidized completely with pure oxygen to form CO₂, H₂O, N₂ and SO₂ in a CHNS elemental analyzer, model 932 (LECO, Germany). The gases obtained were transported with helium as carrier and then they were thermally desorbed and quantitatively determined. CO₂, H₂O and SO₂ were quantified by infrared and N₂ on base of its thermoconductivity. Results were expressed as percentage.

2.4. Viscoelastic properties

2.4.1. Shear rheology

Shear rheology tests were performed using an AR 2000 rheometer (TA Instruments, USA). The measurements were performed using a 60 mm (in diameter) parallel plate low inertia geometry. A gap size of 0.5 mm was used for all the shear rheological tests. Small Amplitude Oscillatory Shear (SAOS) measurements were conducted in order to obtain the linear viscoelastic properties for the polysaccharide dispersions studied as a function of frequency (between 0.01 to 10 Hz). Shear stress sweep tests were previously performed to find the linear viscoelastic range. A low gap is particularly suitable for testing flow properties over a wide range of shear rates, due to its ability to achieve much higher shear rates. During shear flow tests, the shear rate was increased step-by-step over the chosen range of shear rates and a steady state was obtained at each measurement.

2.4.2. Extensional rheology

Extensional rheology tests were carried out for 2% wt. solutions of LBG, GG, MC and K using a Haake CaBER-1 extensional rheometer (Thermo Haake GmbH, Germany), equipped with two 6 mm circular parallel plates. In order to minimize the influence of gravity and shear flow during the early stages of stretch, plates were set at an initial gap (h_0) of 1.5 mm, resulting in an aspect ratio (initial length to radius, h_0/R_0) equal to 0.5. Fluid samples were carefully loaded between the plates using a pipette to ensure the absence of trapped air within the sample cylinder, or between the sample and the plates. The upper plate was suddenly raised to a pre-set height of 6 mm to create a filament and a laser beam aiming at the middle point of the filament monitored the changes in diameter.

Extensional rheology data requires measurements of surface tension of all fluid samples, which were measured in a Sigma 701 Tensiometer (KSV Instruments, Helsinki) using a Wilhelmy probe.

2.5. Statistical analysis

All experiments were performed at least in triplicate. Systat software (SYSTAT, Inc., Evanston, IL, USA) version 10.0 was used for multifactor analysis of variance. Differences were determined by Fisher's least significant difference (LSD) mean discrimination test, using $\alpha = 0.05$ as level of significance.

3. Results and discussion

3.1. Small amplitude oscillatory shear properties

Fig. 1 shows the mechanical spectra from 0.01 to 10 Hz for 2% wt. aqueous dispersions of kefiran (K) compared to three selected neutral gums used as references: methylcellulose (MC), locust bean gum (LBG) and guar gum (GG). The rheological behaviour observed for those dispersions is characteristic of low concentrated polysaccharide dispersions, in which a fluid-like behaviour is predominant at most frequencies studied, typical of weak random-coil entanglement networks, showing a tendency to reach a crossover between G' and G'' at higher frequencies. This crossover would appear at lower frequencies as the system is more structured. Thus, in Fig. 1 this crossover is only shown for those systems that present higher viscoelastic moduli (guar gum and locust bean gum). This result does not come as a surprise as those galactomannans are the polysaccharides with the greater molecular weight among the four used. The estimated molecular weights are shown in Table 1. Even if those molecular weights have errors implied as they are extrapolated, they are in good agreement with the evolution that may be found elsewhere (Chevillard & Axelos, 1997; Richardson, Willmer, & Foster, 1998; Sittikijyothin, Torres, & Goncalves, 2005; Torres, Hallmark, & Wilson, 2014; Wu, Cui, Eskin, & Goff, 2009). However, the viscous behaviour prevails over the elastic behaviour for the whole range of frequencies studied for the cellulose derivative and kefiran systems, being G' and G'' values much lower than for guar and locust bean gums. It is also noticeable how the frequency slopes for G' tend to decrease as the system gets more structured. In this sense, the neutral gums 2% solutions may be ranked, matching their molecular weights, according to their viscoelastic behaviour: guar gum > locust bean gum > methylcellulose > kefiran. It may be noticed that the sequence for the two latter gums is maintained although the difference between their molecular weights is not significant, which might be attributed to a more rigid linear structure for MC gum. Similar behaviours than those obtained in the present manuscript has been found for guar gum (Bourbon et al., 2010; Chenlo, Moreira, & Silva, 2010; Fijan, Sostar-Turk, & Lapasin, 2007; Ptaszek et al., 2009; Torres et al., 2014; Wu et al., 2009) locust bean gum (Bourbon et al., 2010; Haddarah et al., 2014; Sittikijyothin et al., 2005; Wu et al., 2009; Zarate-Ramirez, Bengoechea, Cordobes, & Guerrero, 2010), methylcellulose (Desbrieres, Hirrien, & Ross-Murphy, 2000; Kobayashi, Huang, & Lodge, 1999; Sanz, Fernandez, Salvador, Munoz, & Fiszman, 2005). Kefiran constitutes the system with the lowest values of G' and G'' , also being the system with the lowest molecular weight and reported intrinsic viscosity (Piermaria et al., 2008). It may be explained on basis of the free and independent movement of the kefiran polymeric chains, as individ-

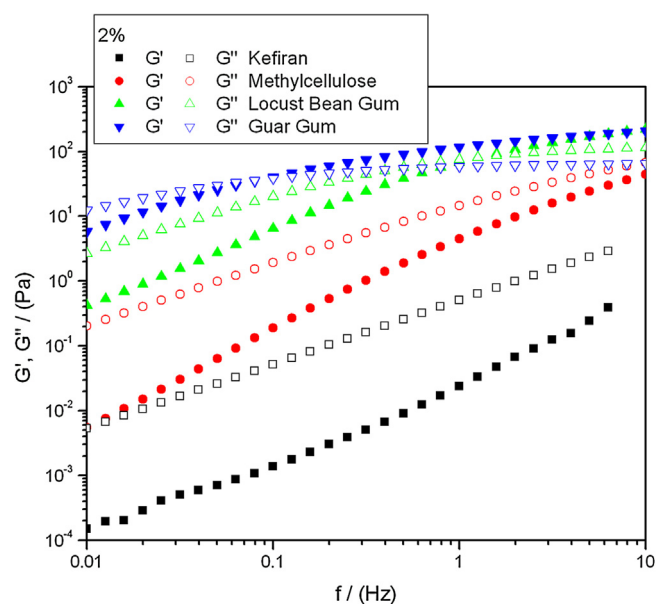


Fig. 1. Elastic (G' , filled symbols) and viscous (G'' , open symbols) moduli evolution with frequency for 2% wt. neutral gum solutions of kefiran (■), methylcellulose (●), locust bean gum (▲) and guar gum (▼).

ual polysaccharide coils are well separated from each other, which results in very weak polymer–polymer interactions. Thus, at a 2% wt. solution, kefiran is the polysaccharide solution with the presumably smallest relaxation time, showing viscoelastic properties in the terminal region of the relaxation spectrum, with both moduli increasing with frequency by more than three orders of magnitude. An increase of the same magnitude has been previously reported for other random-coil polysaccharide solutions (Bourbon et al., 2010; Torres et al., 2014).

Table 1 also shows the effect of concentration on the mechanical properties of the neutral gum aqueous dispersions. It may be noticed how an increase in the gum concentration from 0.5 to 2% wt. generally yields a strengthening of the structure reflected in the evolution of some viscoelastic parameters. Thus, it is clear how generally G^* at 1 Hz (G^*_1) gets higher, as $\tan\delta_1$ gets lower when gum concentration increases from 0.5 to 2% wt., independently of the gum nature. Thus, the increase in gum concentration results in an increase in the elastic modulus of the samples. The greater viscoelastic behaviour of the galactomannans studied is reflected in their higher G^* and lower $\tan\delta$ at all concentrations studied and may be related to their reported high water binding capacity, which allows them to form highly viscous solutions at low concentrations

Table 1

Molecular weight and SAOS parameters for aqueous neutral gum solutions at different concentrations. Different letters in the same column indicate a significant difference for each polysaccharide ($p < 0.05$).

Gum	MW (kDa)	% (w/w)	G^*_1 (Pa)	$\tan\delta_1$	$f_{\text{crossover}}$ (Hz)
Guar gum (G)	$2.2 \times 10^4 \pm 3 \times 10^3$	0.5	0.250 ± 0.008^a	8.3 ± 0.9^a	>10.0
		1	15 ± 2^b	0.90 ± 0.02^b	0.9 ± 0.1^a
		2	130 ± 24^c	0.500 ± 0.007^c	0.09 ± 0.0^b
		0.5	0.92 ± 0.02^a	9.4 ± 0.4^a	>10.0
Locust Bean Gum (LBG)	$1.00 \times 10^4 \pm 6 \times 10^2$	1	10 ± 1^b	2.60 ± 0.01^b	9 ± 1^a
		2	101 ± 4^c	1.1 ± 0.2^c	1.1 ± 0.2^b
		0.5	0.25 ± 0.06^a	73 ± 49^a	>10.0
Methylcellulose (MC)	$4.1 \times 10^3 \pm 3 \times 10^2$	1	1.3 ± 0.9^b	10.1 ± 0.8^b	>10.0
		2	15.2 ± 0.9^c	3.20 ± 0.04^c	10.00 ± 0.01
		0.5	0.074 ± 0.050^a	85 ± 30^a	>10.0
Kefiran (K)	$6.0 \times 10^3 \pm 3.6 \times 10^3$	1	0.17 ± 0.05^a	80 ± 32^a	>10.0
		2	0.51 ± 0.04^b	21 ± 2^b	>10.0

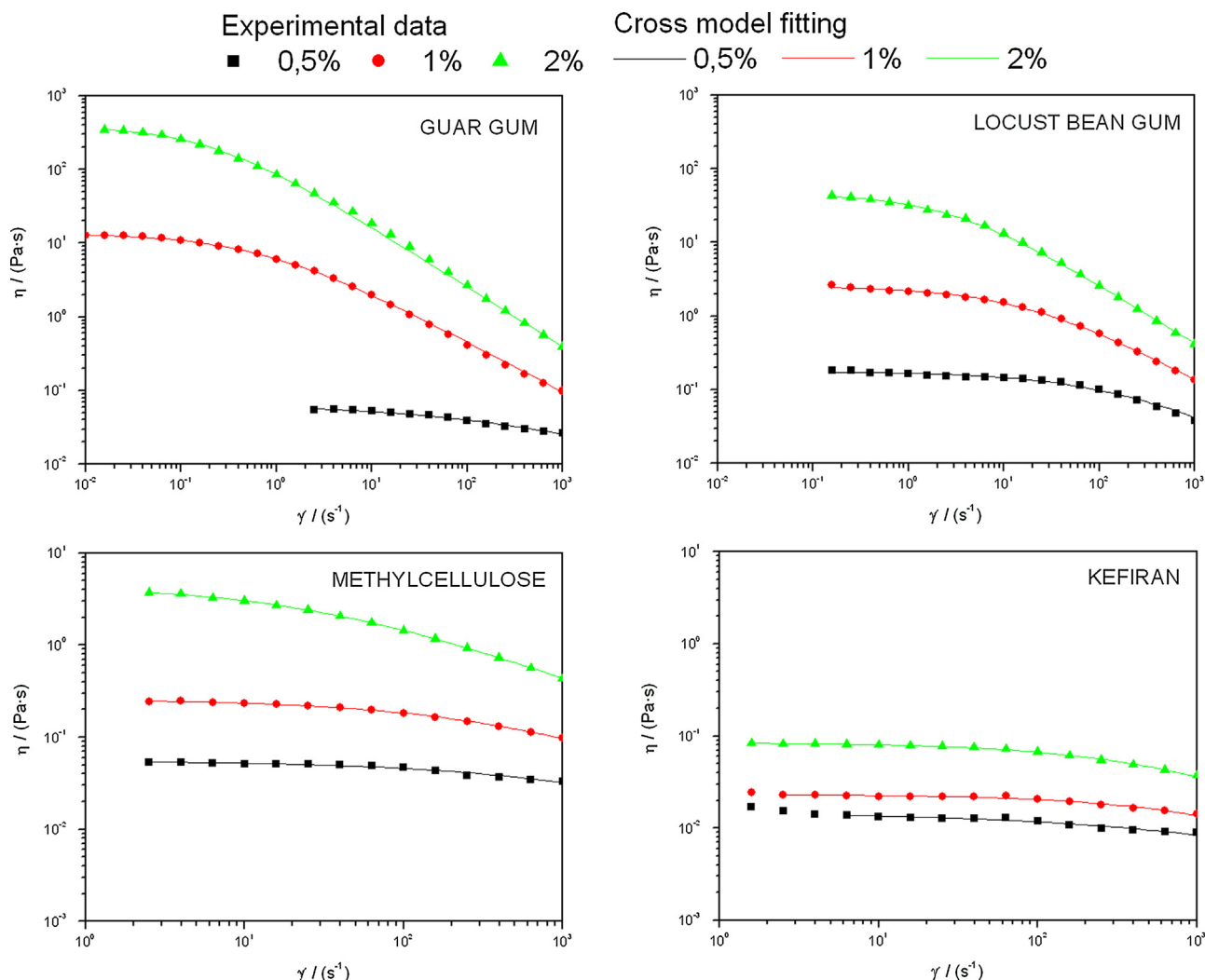


Fig. 2. Flow curves and Cross model fitting for neutral gum dispersions at concentrations 0.5 (■), 1 (●) and 2% wt. (▲).

(Wu et al., 2009). For those gums, the elastic behaviour increases strongly with concentration, as the entanglements are favoured. The evolution of the frequency at which the G' - G'' crossover takes place ($f_{\text{crossover}}$), moves to lower values as the galactomannan concentration increases, as a consequence of increasing relaxation times. This has been found for several other random-coil polysaccharide solutions (Bourbon et al., 2010; Brummer, Cui, & Wang, 2003; Sittikijyothin et al., 2005). It is not possible to observe the same evolution for methylcellulose and kefirin in the range of concentrations studied, as no crossover is clearly observed such that they are weakly structured systems.

Even if kefirin solution at 2% wt. displays a mechanical spectrum with G' and G'' values close to those obtained with methylcellulose solution at 1% wt. or LBG and GG solutions at 0.5% wt., the prevalence of the viscous component over the elastic one for kefirin in the concentration range studied is apparent as $\tan\delta$ values point out.

3.2. Steady shear flow properties

When submitted to steady shear, all gums show a shear-thinning behaviour (Fig. 2), consequence of modifications in macromolecular organisation in the solution as the shear rate changes. First, a Newtonian plateau region, characterized by a zero-shear rate apparent viscosity, η_0 , is found at low shear rates. In

this region, the disruption of entanglements that takes place when shear is applied is balanced by the formation of new ones, resulting in a constant viscosity. This region is broader in the shear rate range studied when the gum concentration is lower (e.g. 0.5% wt.). When increasing shear rate, the disruption predominates over the formation of new entanglements, and then, molecules get aligned in the direction of flow and the viscosity decreases (Bourbon et al., 2010; Sittikijyothin et al., 2005). As may be observed in Fig. 2, the shear-thinning behaviour for all gums studied increases with concentration, since the critical shear rate ($\dot{\gamma}_c$) at which the flow behaviour becomes shear-thinning decreases.

The Cross model (Cross, 1965) has been used to describe the shear-thinning behaviour of all the neutral gum solutions studied

$$\eta = \eta_{\infty} + \frac{(\eta_0 - \eta_{\infty})}{[1 + (\tau\dot{\gamma})^m]} \quad (1)$$

where $\dot{\gamma}$ is the shear rate (s^{-1}), η is the viscosity (Pa·s), η_0 is the zero-shear rate viscosity (Pa·s), η_{∞} is the infinite-shear rate viscosity (Pa·s), τ (s) is a characteristic relaxation time at which the apparent viscosity reaches half de value of η_0 , and m is a dimensionless constant. Since η_{∞} was never reached in the present study, the equation above was simplified, assuming $\eta_0 \gg \eta_{\infty}$

$$\eta = \frac{\eta_0}{[1 + (\tau\dot{\gamma})^m]} \quad (2)$$

Table 2

Cross model-fitting parameters for the flow curves of different concentrations of aqueous neutral gum solutions. A horizontal shift time-concentration factor, a_c , is also included. Different letters in the same row indicate a significant difference for each polysaccharide ($p < 0.05$).

	C (%)	η_0 (Pa s)	$\dot{\gamma}_c$ (s^{-1})	m	R ²	a_c
Kefiran	0.5	0.013 ± 0.001 ^a	1868 ± 442 ^a	0.45 ± 0.12 ^a	0.9514	1.6
	1	0.022 ± 0.002 ^b	1675 ± 243 ^a	0.72 ± 0.10 ^b	0.9686	2.5
	2	0.084 ± 0.001 ^c	659 ± 21 ^b	0.71 ± 0.02 ^b	0.9976	1
Methylcellulose	0.5	0.053 ± 0.004 ^a	1800 ± 236 ^a	0.54 ± 0.07 ^a	0.9747	39.8
	1	0.24 ± 0.02 ^b	432 ± 10 ^b	0.61 ± 0.01 ^a	0.9995	12.6
	2	4.0 ± 0.7 ^c	31 ± 2 ^c	0.64 ± 0.01 ^b	0.9995	1
Locust Bean Gum	0.5	0.200 ± 0.006 ^a	147 ± 17 ^a	0.40 ± 0.06 ^a	0.9833	79.4
	1	2.6 ± 0.1 ^b	17 ± 1 ^b	0.70 ± 0.03 ^b	0.9947	7.9
	2	50.00 ± 0.01 ^c	2.5 ± 0.2 ^c	0.74 ± 0.02 ^b	0.9992	1
Guar Gum	0.5	0.07 ± 0.01 ^a	312 ± 79 ^a	0.39 ± 0.03 ^a	0.9917	7080
	1	12.3 ± 1.9 ^b	0.73 ± 0.03 ^b	0.69 ± 0.01 ^b	0.9994	8.9
	2	381 ± 22 ^c	0.20 ± 0.01 ^c	0.82 ± 0.02 ^c	0.9995	1

The values of these parameters are displayed in Table 2 as function of gum type and concentration.

Fig. 2 and Table 2 show how the simplified Cross model properly describes the experimental data of the kefiran solutions, as well as the rest of the neutral gums studied, as indicated by the magnitudes of the regression coefficient, R², also shown in Table 2.

The shape of all the flow curves suggests that it is possible to obtain a master flow curve, after performing a concentration-dependent shift using the 2% wt. solution as the reference for each gum (Fig. 3). It may be observed how this time-concentration superposition is valid for these gums in the concentration range studied. A vertical and a horizontal shift were used to obtain the superposition of the flow curves: the vertical shift is given by a normalization with the zero-shear viscosity of each solution considered, whereas the horizontal shift is carried out by means of a time-concentration factor, a_c . The values of a_c parameter are also included in Table 2,

where the unity was always assigned to the reference solution (2% wt.).

As may be seen in Fig. 2, Table 2 and Fig. 3 (insert), an increase in concentration results in an increase in the zero-shear viscosity, which may be regarded as a macroscopic representation of the microstructural nature of the biopolymers (Bourbon et al., 2010), related to the number of entanglements formed, and depending on the molar mass and on interchain interactions. Moreover, Fig. 3 (insert) shows that a power-law fitting may be properly applied (R²: 0.8944–0.9996), resulting in slopes of 1.03, 3.11, 4.00 and 6.34 for kefiran, methylcellulose, locust bean gum and guar gum, respectively. Some authors have tried to relate this slope to the flexibility of the backbone of the polymer, being more rigid as the slope is higher. There is no chance to distinguish any critical concentration defined by transitions between dilute-semidilute regions (C*) or semidilute-concentrated regions (C**) as other authors did (Sittikijothin et al., 2005). Possibly, methylcellulose was in the

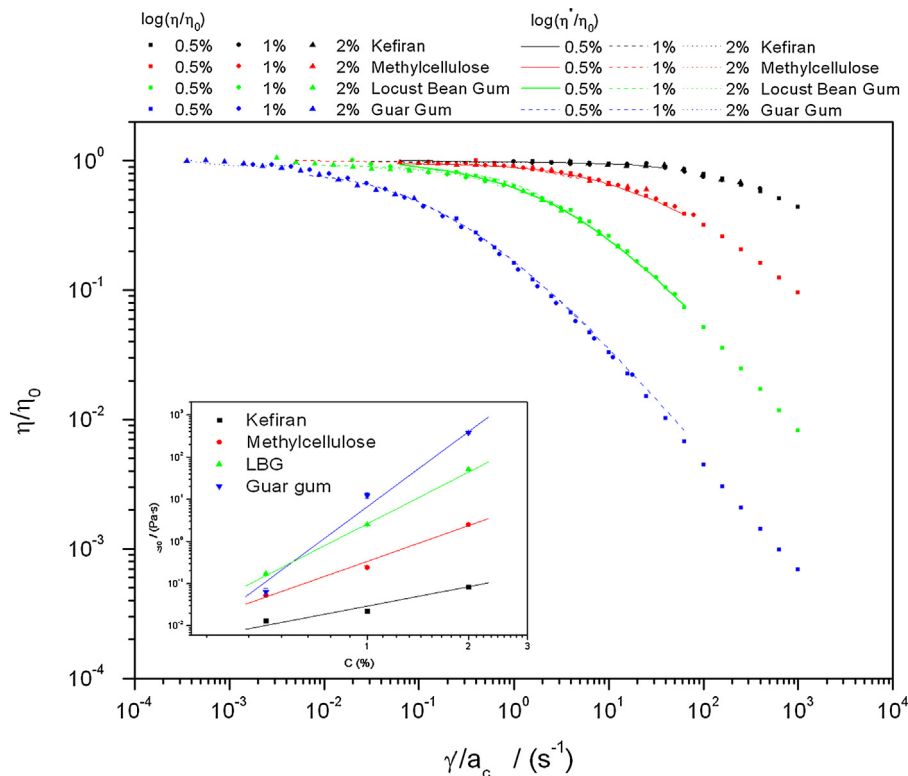


Fig. 3. Master flow curve for kefiran, methylcellulose, locust bean gum and guar gum, after performing a concentration-dependent shift (η_0 , a_c) using the 2% wt. solution as the reference for each gum. *Insert:* Experimental and power-law fitting data for the evolution of zero-shear rate viscosity (η_0) with concentration.

semidilute region at 0.5% wt. and in the concentrated region at 1 and 2% wt. (Koliandris, 2010), but no clear estimation of C^{**} could be possible with the data obtained. As observed, kefiran, which shows the lowest MW (Table 1), also shows the lowest values for η_0 at all concentrations studied, as well as the lowest slope. Typical slopes for biopolymers range from 3 to 5 (Launay, Cuvelier, & Martinez-Reyes, 1997; Wu et al., 2009), though some deviations have been found (Kapoor, Milas, Taravel, & Rinaudo, 1994). The low values found for kefiran are in agreement with a system where the individual polysaccharide coils are free to move independently as they are well separated from each other. Kefiran solutions may possibly be in a different regime (dilute or semi-dilute) than the rest of the gums. Anyway, in agreement with the results obtained by Sittikijyothin et al. (2005) the rate of formation of new entanglements seems to diminish as either the molecular weight or the concentration of the gum increases. Thus, the characteristic relaxation time (τ) increases with concentration from 0.5 to 2% wt., due to an increase of the chains' entanglement density for methylcellulose, locust bean gum and guar gum. This is related to a progressive restriction of the movement of the chains that eventually results in an increase in the time needed to form new entanglements. Even if the fitting to the model is acceptable, it is noticed in Fig. 2 how kefiran solutions at 0.5 and 1% wt. and methylcellulose solution at 0.5% wt. show an almost Newtonian behaviour in the shear rate studied, only displaying the onset of the shear-thinning flow, which would explain the lack of an evolution in τ with concentration. As for parameter a_c , it always increases with concentration (Table 2), which is consistent with the increase of entanglements in the system (Sittikijyothin et al., 2005).

Fig. 3 also shows how the Cox-Merz rule (Cox & Merz, 1958) was satisfactorily applied to correlate dynamic and steady shear properties of the four systems. Thus, the magnitude of the apparent viscosity in steady shear, $|\eta(\dot{\gamma})|$, and the magnitude of the complex viscosity in oscillatory shear, $|\eta^*(\omega)|$, were compared at equal values of shear rate and frequency. Moreover, it has been possible to successfully apply analogous vertical and horizontal shifts to the complex viscosities to obtain a master curve for every gum system that includes both the apparent and complex viscosities. Then, it seems that all neutral gums considered behave like ordinary polysaccharides. Similar results were previously obtained for cellulosic, galactomannan and chitosan systems (Andrade, Azero, Luciano, & Goncalves, 1999; Calero, Munoz, Ramirez, & Guerrero, 2010; Haque & Morris, 1993; Sittikijyothin et al., 2005; Torres et al., 2014).

3.3. Extensional properties

Fig. 4 displays the profile of mid-filament diameter, D_{mid} , normalised against its initial value, D_0 , versus time for the 2% wt. solutions of the four neutral gums studied (kefiran, methylcellulose, locust bean gum and guar gum). Surface tension (Γ) of the solutions was always in the range of 0.055–0.060 Nm^{-1} . This parameter is considered for the estimation of the Bond number (Bo)

$$Bo = \frac{\rho g D_0^2}{4\Gamma} \quad (3)$$

where ρ is the density of the solution, which may be approximated to the density of water, g is the gravitational constant, and D_0 is the initial sample diameter. The values of Bo estimated according to Eq. (3) are around 1.47–1.60, being consistent with the typically established condition $\frac{h_0}{D_0} \leq \frac{1}{\sqrt{Bo}}$, maintained in order to keep the initial configuration close to cylindrical (Rodd et al., 2005).

It may be observed how in all cases the polymer filament passes through four stages (rest-stretch-relaxation-breaking). Sim-

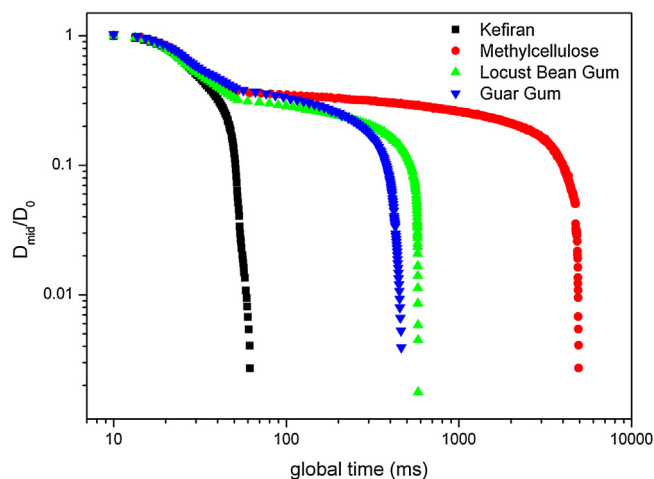


Fig. 4. Evolution of normalised filament diameter (D_{mid}/D_0) along extensional time for 2% wt. gum solutions. Kefiran (■), methylcellulose (●), locust bean gum (▲) and guar gum (▼).

ilar behaviour has been found previously (Bourbon et al., 2010; Torres et al., 2014). In any case, it is apparent that very large differences exist between the different polysaccharides. The break-up time (t_f) ranged from 62 ms (kefiran) to 4936 ms (methylcellulose), whereas galactomannans solutions displayed values of a few hundred ms. It is also apparent that the relaxation phase is much less pronounced for kefiran than for the rest. The reason of this may be in the possible mechanisms that oppose to the capillary forces after stretching. Solutions containing 2% wt. galactomannans or methylcellulose show an evolution of the mid-filament that is basically controlled by the balance between surface tension, which tends to destabilize the filament, and viscous/elastic forces, showing a stabilizing effect. In comparison, the 2% wt. solution of kefiran approaches a nearly Newtonian fluid behaviour, with the diameter decaying almost linearly after the stretch stage.

It is worth mentioning the exceptional behaviour shown by the methylcellulose solution in extensional flow, with a break-up time much higher than the rest, even though their shear flow properties are lower than those of the galactomannans systems.

Fig. 5 shows the results obtained when D_{mid}/D_1 , being D_1 the diameter of the filament when first formed after the stretching stage, was plotted along global time normalised against t_f for the four systems studied in the present work. Previously, different authors have reported a so-called “master curve” when the mid-filament diameter normalised against D_1 was plotted against t/t_f for dilute polymeric solutions at either different concentrations or molecular weights (Anna & McKinley, 2001; Torres et al., 2014). The shape of the necking profile obtained for the systems containing MC, LBG or GG is similar to those reported by former authors, displaying an initial linear region followed by a sharp decay at t_f . All the three systems result in a matching initial linear region and an exponential decay along time around almost 2 orders of magnitude in D_{mid}/D_1 . The linear region has been previously related to the viscoelastic behaviour of the polymeric solutions. On the other hand, kefiran seems to deviate from that common behaviour, tending to a lineal decay, rather than an exponential one. The deviation found for the system containing kefiran does not necessarily relate to the lower molecular weight of the gum, as Anna and McKinley (2001) reported that the elasto-capillary thinning behaviour exhibits a very weak dependence on molecular weight. Kefiran system is closer to a Newtonian fluid than to an elastic fluid, with significantly lower times to break up. Several authors have predicted linearly decreasing diameters for Newtonian filaments, whereas fluids where the viscous stresses are assumed to

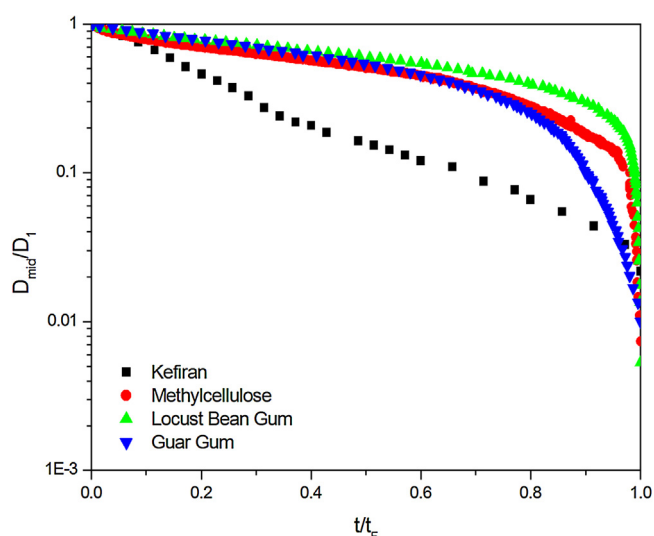


Fig. 5. Dimensionless filament diameter profiles with dimensionless time for 2% wt. gum solutions. Kefiran (■), methylcellulose (●), locust bean gum (▲) and guar gum (▼).

be negligible, a predominant elasto-capillary behaviour characterized by filaments that decrease exponentially with time is found. The different behaviour observed for the kefir system is also evident when the Trouton ratio (N_{Tr}) is compared at the same Hencky strain for all the systems. The Hencky strain, ϵ , is defined using the mid-filament diameter,

$$\epsilon = 2 \ln \left(\frac{D_1}{D_{mid}(t)} \right) \quad (4)$$

and it is the strain experienced by the sample at the axial mid-plane at time t . These values are governed by the self-thinning of the filaments, not the response of an imposed shear rate. As the molecular weight of the system increases, a decrease in N_{Tr} is found: from a high value around 60 for kefir to 3 for locust bean gum and guar gum, at a Hencky strain equal to 6. Galactomannans show the lowest Trouton number values, as chain–chain interactions are expected to be higher than in the other gums.

4. Concluding remarks

This study has shown that kefir solutions show a flow behaviour in shear qualitatively similar than other representative neutral gums, like methylcellulose, locust bean gum or guar gum, obtaining a flow master curve at different concentrations (0.5–2% wt.) similar to the one obtained for the rest of the gums. Nevertheless, it presents much lower viscosity values, as well as elastic (G') and viscous (G'') moduli in oscillatory measurements, which is explained on terms of its microstructure. This also would explain that kefir solutions do not show important extensional properties at 2% wt., displaying a behaviour close the Newtonian at low Hencky strains.

Thus, regarding the potential of kefir as functional additive in food products, it may be necessary to consider in some cases the addition of another ingredient into the formulation if higher viscoelastic properties are to be achieved. Future research should be focused in this direction.

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