Carbohydrate Polymers 152 (2016) 97–104

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Shear and extensional properties of kefiran

Judith Piermaría[a], Carlos Bengoechea[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

ARTICLE INFO

Article history:
Received 21 April 2016
Received in revised form 8 June 2016
Accepted 15 June 2016
Available online 23 June 2016

Keywords:
Kefiran
Neutral gum
Rheology
Cross
Extensional viscosity

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 pharmaceutics 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.

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 synthetized 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 (Piermaria, de la Canal, & Abraham, 2008; Zavala, Roberti, Piermaria, & Abraham, 2014) and edible films with good mechanical and barrier properties (Piermaria et al., 2011; Piermaria, Pinotti, Garcia, & 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: cbengoechea@us.es (C. Bengoechea).

http://dx.doi.org/10.1016/j.carbpol.2016.06.067
0144-8617/

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 kefiran 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 kefiran 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 10,000*g* 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 10,000*g* 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 kefiran 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 kefiran 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 kefiran 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 $NaNO_3$ 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 CO_2, H_20, N_2 and SO_2 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. CO_2, H_20 and SO_2 were quantified by infrared and N_2 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.