



High acyl gellan as an emulsion stabilizer

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ABSTRACT

High acyl gellan (0.01–0.2% w/w) was used as stabilizer in oil in water emulsions containing 30% (w/w) of sunflower oil and prepared under different process conditions. Stable emulsions to phase separation could be obtained using high acyl gellan (HA) content above 0.05% (w/w), while low acyl gellan (LA) prepared at the same conditions could not stabilize emulsions. Emulsions properties depended on the process used to mix the oil and gellan dispersion since high pressure homogenization favored stabilization while very high energy density applied by ultrasound led to systems destabilization. Emulsions prepared using high pressure homogenization showed zeta potential values ranging from -50 up to -59 mV, suggesting that electrostatic repulsion could be contributing to the systems stability. Rheological properties of continuous phase were also responsible for emulsions stabilization, since HA gellan dispersions showed high viscosity and gel-like behavior. The high viscosity of the continuous phase could be associated to the presence of high acyl gellan microgels/aggregates. Disentanglement of these aggregates performed by ultrasound strongly decreased the viscosity and consequently affected the emulsions behavior, reducing the stability to phase separation.

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1. Introduction

Emulsions can be defined as systems composed by spherical droplets of one liquid (dispersed phase) spread out in another liquid (continuous phase) which are at least partly immiscible between them. Usually, the diameters of the droplets in food emulsions are between 0.1 and 100 μm (Dickinson, 1992; McClements & Weiss, 2004; Tadros, 2013). In an attempt to achieve kinetic stability of these thermodynamically unstable systems, in general, two types of ingredients can be added, emulsifiers or stabilizers (texture modifiers). The first one acts by covering the interface between these two phases (generally water–oil) to form a protective coating that prevents the droplets aggregation and also by decreasing the interfacial tension, which facilitates the disruption of droplets during homogenization and allows the formation of smaller droplets in the emulsions (McClements, 2007; Walstra, 2003). Emulsifier agents used in food products can be low-molecular weight surfactants (such as polysorbates, lecithin or monoglycerides), macromolecular emulsifiers (as proteins and some polysaccharides) and solid particles (Dickinson, 2003, 2012; Pickering, 1907; Tadros, 2013).

On the other hand, stabilizer compounds usually act via viscosity increase or gelation of the continuous phase, slowing down the movement of droplets due to gravity or Brownian motion,

preventing their aggregation. Stabilizers are also important to provide characteristic textural attributes to the product. Different polysaccharides and proteins can be used as thickening or gelling agents in food emulsions (Dickinson, 2009; Desplanques, Renou, Grisel & Malhiac, 2009; Lorenzo, Zaritzky & Califano, 2013; McClements, 2007; Perrechil & Cunha, 2010).

High acyl gellan (HA gellan) is the natural or native form of this polysaccharide excreted by the bacterium *Sphingomonas elodea*. It is a linear anionic chain composed by a tetrasaccharide repeated unit (two molecules of D-glucose, one of L-rhamnose and one of D-glucuronic acid) that contains two acyl substituents, acetate on approximately 50% of the glucose residues and L-glycerate groups on all of the same glucose residues (Kuo, Dell, and Mort, 1986; Sanderson, 1990). Gellan gum can differ by the proportion of units with acyl substituents attached to the glucose molecule (degree of acylation) and the distribution of these acyl groups along the polysaccharide chain. Commercial samples are usually described as exhibiting a 'high' or 'low' degree of acylation. In low acyl gellan gum the acetyl groups were removed by alkaline treatment. The amount of acyl substituents strongly affect the properties of systems that contain this polysaccharide, in particular their rheological properties (Bajaj, Survase, Saudagar & Singhal, 2007; Fialho et al., 2008; Huang, Tang, Swanson & Rasco, 2003).

Biopolymers with emulsifier ability are amphiphilic and show surface-active properties. If it is a hydrocolloid, it must contain hydrophobic groups that are numerous enough and sufficiently accessible on a short timescale to allow the adsorbing molecules

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to adhere to and spread out at the oil–water interface, protecting the recently formed droplets (Dickinson, 2003). Polysaccharides are examples of stabilizers that are predominantly hydrophilic and usually do not show any particular surface activity. Exceptions include arabic gum, some galactomannans, methylcellulose or hydroxypropyl cellulose and acetylated and depolymerised citrus pectin (Akhtar, Dickinson, Mazoyer & Langendorff, 2002; Dea & Madden, 1986; Endreß & Rentschler, 1999; Gaonkar, 1991). The surface activity of these hydrocolloids is usually associated to non-polar character of chemical groups attached to the hydrophilic polysaccharide backbone or the presence of a protein component linked covalently or physically to the polysaccharide (Dickinson, 2003, 2009). High acyl gellan, despite its acyl group content, has not been studied as the unique stabilizer/emulsifier compound in systems composed by oily and aqueous phases. To the best of our knowledge, only one work studied an emulsified system containing HA gellan. Gelation of the aqueous phase was promoted by HA gellan which was used together with an emulsifier (Tween® 80) to form stable emulsions (Lorenzo et al., 2013).

Therefore the purpose of this work was to investigate emulsions containing only water, oil and HA or LA gellan gum. Emulsion properties such as kinetics stability, mean droplet size, morphology and flow behavior were determined. Although low acyl gellan gum (LA gellan) did not present any potential to stabilize emulsions in the used conditions, some experiments were done in an attempt to evaluate the role of structural properties of these two polysaccharides on the emulsions stabilization capacity.

2. Materials and methods

2.1. Materials

High acyl gellan (Kelcogel LT-100) and low acyl gellan (Kelcogel F) were donated by Kelco Biopolymers (San Diego, CA) and used without further purification. The nitrogen content of HA and LA gellan powder were 1.75 ± 0.14 and $0.32 \pm 0.02\%$ (w/w), respectively (wet basis), which was determined by a Flash 2000 CHNS/O Analyzer (Thermo Scientific, Waltham, USA). The ions content of the gellan gums was determined by Inductively Coupled Plasma Optical Emission Spectrometry. HA gellan showed the following composition (w/w): calcium (0.23 ± 0.04), magnesium (0.097 ± 0.01), potassium (2.0 ± 0.20) and sodium (1.5 ± 0.2). The ions content of LA gellan was (w/w): calcium (0.25 ± 0.03), magnesium (0.074 ± 0.01), potassium (3.60 ± 0.05) and sodium (0.9 ± 0.2). Sunflower oil (Liza, Cargill, EUA) was purchased from the local market.

2.2. Preparation of gellan aqueous dispersions

HA or LA gellan was dispersed in Milli-Q water under magnetic stirring (MS). Part of gellan dispersions was subjected to one step in a rotor–stator Ultra-Turrax model T18 (IKA, Germany) at 14,000 rpm for 4 min, which was one of the conditions used to prepare gellan emulsions. Rheological properties (Section 2.5.2) of these dispersions were evaluated, before (MS) and after the rotor–stator step (RS). The effect of ultrasonic treatment (UT) was also evaluated, in which HA and LA gellan dispersions were subjected to ultrasonic processor model QR750 (Ultronique, Campinas, Brazil) operating at 20 kHz frequency with power of 750 W and operating surface area of $1.32 \times 10^{-4} \text{ m}^2$. Ultrasonic treatment was done with the aid of a jacketed beaker connected to a water bath set to 24 °C, and each 100 g of sample were processed for 15 min. Zeta potential (Section 2.5.5), pH and rheological properties (Section 2.5.2) of these dispersions before and after ultrasonic treatment were evaluated. Interfacial tension between

sunflower oil and HA or LA gellan dispersions was also measured (Section 2.5.6).

2.3. Effect of additional mechanical treatment in HA aqueous dispersion prior to emulsions preparation

The effect of rotor–stator step or ultrasonic treatment on HA gellan dispersion prior to emulsion preparation was determined. HA gellan aqueous dispersion prepared by magnetic stirring (MS) with fixed concentration of 0.14% w/w was used to prepare emulsions containing 0.1% (w/w) of polysaccharide and 30% (w/w) of sunflower oil. ‘Emulsion 1’ was prepared by mixing HA gellan dispersion and oil using only a step in rotor–stator (14,000 rpm/4 min) and was used as a control sample. ‘Emulsion 2’ was prepared in the same way, but gellan dispersion was subjected to an additional rotor–stator step before emulsion preparation. In ‘Emulsion 3’ the gellan dispersion was subjected to ultrasonic treatment (using the same ultrasonic process conditions described in Section 2.2) before being mixed with oil in the rotor–stator. ‘Emulsion 4’ was prepared using ultrasonic process after a pre-mixing in rotor–stator. In all steps the same amount of material was used in order to maintain a constant applied energy/volume in each device.

2.4. Emulsions preparation

Oil-in-water emulsions were prepared by mixing appropriate amount of HA or LA gellan, sunflower oil and Milli-Q water, using a rotor–stator Ultra-Turrax model T18 (IKA, Germany) at 14,000 rpm for 4 min. The sunflower oil concentration was always 30% (w/w), while the gellan concentration varied from 0.01 to 0.2% (w/w). All concentrations were calculated in relation to the whole emulsion. HA emulsions were also prepared using a rotor–stator step (14,000 rpm/4 min) followed by high pressure homogenization performed in a Panda 2K NS1001L double-stage homogenizer (Niro Soave, Italy). The homogenization pressure was 300 bar in the first and 50 bar in the second stage. Morphology (Section 2.5.3) and rheological properties (Section 2.5.2) were evaluated after 24 h. Droplets size distribution (Section 2.5.1) and phase separation profile (Section 2.5.4) were evaluated after 24 h and 7 days to determine the stability of the droplets to coalescence.

2.5. Evaluation of gellan aqueous dispersions and emulsions

2.5.1. Particles size distribution and mean diameter

Droplet-size distributions of the emulsions were determined by laser diffraction technique using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). Mean droplet sizes were characterized in terms of the volume moment mean (De Brouckere Mean Diameter – D_{43}) (Eq. (1)), surface area moment mean (Sauter Mean Diameter – D_{32}) (Eq. (2)) and $D_{50\%}$ (maximum particle diameter below which 50% of the sample volume exists).

$$D_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (1)$$

$$D_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (2)$$

where n_i is the number of particles with diameter d_i . Span, parameter related to the width of a distribution was also obtained according to Eq. (3).

$$\text{Span} = \frac{D_{90\%} - D_{10\%}}{D_{50\%}} \quad (3)$$

where $D_{10\%}$, $D_{50\%}$ and $D_{90\%}$ are the equivalent volume diameters at 10, 50 and 90% of cumulative volume, respectively. Each sample

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