Carbohydrate Polymers 192 (2018) 111–117

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Role of process variables on the formation and *in vitro* digestion of gellan gels

Tatiana Porto Santos, Rosiane Lopes Cunha*

Department of Food Engineering, Faculty of Food Engineering, University of Campinas (UNICAMP), R. Monteiro Lobato, 80, 13083-862, Campinas, SP, Brazil

ARTICLE INFO

Keywords:
Gellan gum
Hardness
Gelation rate
Water holding capacity
In vitro digestion

ABSTRACT

Gellan gels were produced using different approaches forming structures with varied potential applications. Gels were characterized from appearance, mechanical properties, water holding capacity (WHC) and microstructure. In addition, *in vitro* digestibility of these gels was evaluated to understand the effect of gastrointestinal environment on their structure stability. All gels presented high WHC (> 80% w/w) but gels were stronger with salt or acid addition, which was associated to the reduction of double helices repulsion of the negatively charged carboxyl groups of gellan. Moreover, low gelation rate induced a more controlled gellan helices aggregation, strengthening even more gels structure. Gellan gels presented resistance to digestion conditions but hardness of these gels during digestion mainly depended on the gelation rate. Based on these findings it would be possible to tune gel properties for a specific application as texture modifier or even as a faecal bulk formation assistant and an "ileal break" inducer.

1. Introduction

Gellan gum is a negatively charged extracellular polysaccharide produced by the bacteria *Sphingonomas elodea*. This polysaccharide is composed of a linear tetrasaccharide repeating unit containing a carboxyl side group (1,3-β-D-glucose, 1,4-β-D-glucuronic acid, 1,4-β-D-glucose, 1,4-α-L-rhamnose) (Jansson, Lindberg, & Sandford, 1983; O'Neill, Selvendran, & Morris, 1983). Gellan shows the capability to form hydrogels and therefore this gum is widely used in food products as texture agent. However, its high resistance to gastric pH makes this polysaccharide suitable to be used in encapsulation systems and reduced-fat products. Nevertheless, gellan gels characteristics, mainly mechanical properties, depend on the polymer concentration, pH and ionic strength of the medium (Sanderson, 1990).

Gelation of gellan follows a two-step mechanism (Milas & Rinaudo, 1996). Firstly, heated aqueous gellan solution is subjected to cooling, which promotes a polysaccharide conformational transition from random coil molecules to double helices. After that, two different models can explain the aggregation of gellan double helices or junction zones formation mediated by cations, and consequently the gelation of gellan solutions (Morris, Nishinari, & Rinaudo, 2012). In the first model lateral association of double helices can form aggregates by cations presence, which are named "cation-mediated aggregates". These helices aggregates coexist with stretches of disordered gellan chains and interactions can occur between the aggregates and the non-aggregated helices. (Crescenzi, Dentini, & Dea, 1987; Morris et al., 2012; Robinson et al., 1991; Yuguchi, Urakawa, & Kajiwara, 1997). The second model describes the formation of long filaments under non-gelling conditions, and helices aggregates are called "crystalline junction zones". These "filaments aggregates" or "clusters" are described by association of double helices that cannot form a continuous network. The main difference between the two models is that the first one presents stretches of disordered gellan chains between the aggregated and/or the non-aggregated double helices, while these disordered regions do not appear in the second model (Gunning & Morris, 1990; Morris et al., 2012).

In general, an increase of ionic strength by monovalent cations or acid addition to the medium reduces the repulsion between gellan helices because of the reduction of the carboxyl negative groups, promoting a stronger interaction between gellan double helices (Funami et al., 2008; Yamamoto & Cunha, 2007). Despite the effect caused in the negatively charged groups due to acid or monovalent salts addition, divalent cations can also decrease the distance between neighboring gellan double helices by binding their carboxyl groups, increasing even more gel strength (Morris et al., 2012; Tang, Tung, & Zeng, 1996). Gelation rate also exerts a great influence on gellan gels formation besides of the physicochemical environmental conditions (Yamamoto & Cunha, 2007). Lower gelation rate can promote more organized intermolecular interactions between gellan carboxyl groups and added ions, impacting positively on gels strength (Cavallieri & Cunha, 2008; Vilela, Cavallieri, & Cunha, 2011).

* Corresponding author.
E-mail addresses: t093034@dac.unicamp.br (T.P. Santos), rosiane@unicamp.br (R.L. Cunha).

https://doi.org/10.1016/j.carbpol.2018.03.062
Received 28 November 2017; Received in revised form 26 February 2018; Accepted 18 March 2018
Available online 19 March 2018
0144-8617/

Mechanical properties of gellan gels have been extensively studied evaluating the salt effect (Evageliou, Karantoni, Mandala, & Komaitis, 2010; Mao, Tang, & Swanson, 1999; Mao, Tang, & Swanson, 2001; Moritaka, Nishinari, Taki, & Fukuba, 1995; Tang, Lelievre, Tung, & Zeng, 1994; Tang, Tung, & Zeng, 1995) or pH conditions (Moritaka et al., 1995; Norton, Cox, & Spyropoulos, 2011; Picone & Cunha, 2011; Yamamoto & Cunha, 2007). However, these previous works only focused on separated process effects and, to our knowledge there are no published studies evaluating and comparing gellan gels structure produced from different processes and mainly subjected to gastrointestinal *in vitro* conditions. Gellan gels *in vitro* gastrointestinal studies could be an interesting innovation since the introduction of cations after gels formation can still act reinforcing their structure. Moreover, evaluation of gastrointestinal effects on the ingredients has been a widespread interest considering their possible applications.

Therefore, this study aimed at investigating the effect of biopolymer concentration, final pH, ionic strength and gelation rate on the structure of gellan gels before and after gastrointestinal *in vitro* digestion process. Gels were characterized from visual appearance, mechanical properties, water holding capacity (WHC) and structure by scanning electron microscopy (SEM). Linear and non-linear relationship between stress and strain were evaluated in order to understand how different magnitudes of deformation affected gels mechanical properties. Hardness and structure of gellan gels after simulated gastrointestinal digestion were also evaluated, making possible to assess potential applications.

2. Material and methods

2.1. Material

Deacylated gellan gum powder (Kelcogel® F) with moisture content of 2.5% (w/w) was kindly provided by Kelco Biopolymers (San Diego, CA). Ions content of gellan was determined by atomic absorption spectroscopy and presented the following composition (mg/kg): calcium (0.42), sodium (0.61), magnesium (0.11) and potassium (3.9). Carbon (36.95% w/w) and nitrogen content (0.077% w/w) was determined by a CHNS/O Analyzer Flash 2000 Thermo Fisher Scientific Inc (Delft, Netherlands). Glucono-δ-lactone (GDL) was purchased from Sigma–Aldrich Corporation (St. Louis, USA) and other reagents were of analytical grade.

2.2. Preparation of gellan gels

Gellan gum was firstly dispersed in deionized water under magnetic stirring for 10 min at room temperature. Then temperature was increased, and this mixture was kept at 80 °C for 30 min under agitation. After the heat treatment, gellan aqueous solution was used to prepare six different gels with two concentrations (0.9 and 1.5% w/w) and varied process conditions (Table 1). In the first condition, heated gellan solution was only poured into plastic cylinders (GELNAT) previously lubricated with silicon oil (approximately 20 mm inner diameter × 20 mm height), cooled to 25 °C and incubated for 48 h. Sodium hydroxide (NaOH), hydrochloric acid (HCl) or calcium chloride ($CaCl_2$) was directly added into the hot gellan solution before to be transferred to cylinders and processed as described above, forming GEL7, GEL3D and GELCAD respectively, which are gels formed under direct ions addition. To form gels with indirect ions addition, gellan aqueous solution was firstly subjected to a temperature decrease at 50 °C, before the gluconic acid from GDL (GEL3I) or $CaCl_2$ indirect addition (GELCAI). The slow decrease of pH promoted by GDL hydrolysis and the controlled diffusion of calcium ions by dialysis membrane were considered indirect ions addition. GDL amount was adequate to decrease the pH to 3 ± 0.5 (Yamamoto & Cunha, 2007). Samples with GDL followed the same steps as the others, while an indirect addition of $CaCl_2$ was performed using a dialysis membrane (Dialysis tubing cellulose membrane D9652 Sigma Aldrich). In the latter process, gellan aqueous solution was poured into a dialysis membrane and calcium chloride solution as surrounding medium for 48 h at 25 °C. All gels were prepared in three replicates.

Table 1
Process conditions used to prepare gellan gels.

Nomenclature	Gel-inducing strategy				
	Agent	Process of agent addition	Temperature of addition	Gelation Rate	Final pH
GELNAT	–	–	–	–	5.3
GEL7	NaOH	Direct	80 °C	High	7.0
GEL3D	HCl	Direct	80 °C	High	3.0
GEL3I	GDL	Indirect	50 °C	Low	3.0
GELCAD	$CaCl_2$	Direct	80 °C	High	5.3
GELCAI	$CaCl_2$	Indirect	50 °C	Low	5.3

GELNAT: Pure gellan gel, GEL7: Gel with NaOH addition until pH 7, GEL3D: Gel with HCl addition until pH 3, GEL3I: Gel with GDL addition until pH 3, GELCAD: Gel with $CaCl_2$ direct addition, GELCAI: Gel with $CaCl_2$ indirect addition.

The pH measurement was carried out using a penetration glass electrode at different points of the gels. Uniaxial compression, water holding capacity (WHC), visual appearance and scanning electron microscopy (SEM) measurements of gels were evaluated after 48 h of storage. Structure and hardness of gels (0.9% w/w gellan) subjected to an *in vitro* gastrointestinal digestion process were evaluated. *In vitro* static digestion process was performed in two replicates.

2.3. In vitro static digestion

Simulated gastrointestinal *in vitro* digestion was carried out according to Minekus et al. (2014). To sum up, samples were added in an adequate dilution of simulated salivary fluid (SSF). After that, simulated gastric fluid (SGF), porcine pepsin and calcium chloride were added, and the pH was adjusted to 3. Finally, this mixture was subjected to static digestion in an orbital shaker (TE-420 model, Tecnal, Piracicaba, SP, Brazil). An adjustment of pH was performed during all the process. After two hours of gastric digestion, simulated intestinal fluid (SIF), pancreatin, calcium chloride and bile extract were added to the sample. *In vitro* digestion was performed for two more hours at pH 7. Gels samples were removed at different times to be evaluated. Stress at rupture of gels was measured after each digestion phase and gels structure was also evaluated using scanning electron microscopy after the end of the process.

2.4. Mechanical properties

Mechanical properties were measured from uniaxial compression measurements. These measurements were carried out using a TA-XT-Plus Texture Analyzer (Stable Microsystems Ltd, Surrey, UK) equipped with an acrylic cylindrical plate (80 mm diameter) lubricated with silicon oil in order to minimize friction between gel and probe.

Hencky or true stress (σ) and strain (ε) were calculated from the force-deformation data (Steffe, 1996) according to the Eqs. (1) and (2).

$$\sigma = F(t) \times [H(t)/H_0A_0] \quad (1)$$

$$\varepsilon = -\ln[H(t)/H_0] \quad (2)$$

where F(t) is the force at time t, A_0 and H_0 are the initial area and height of the sample, respectively, and H(t) is the height at time t.

Young or elasticity modulus (E) was obtained from the slope of the initial linear region of the stress-strain curve up to 5% strain. Bulk modulus was obtained from the slope of linear region over 10% strain (Nussinovitch, 2004). Rupture properties were obtained from the first maximum point of the stress–strain curve. Stress at rupture (σ_{rup}) was