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Role of gellan gum microstructure in freeze drying and rehydration mechanisms

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ABSTRACT

The role of LA (low-acyl or deacylated) and HA (high-acyl) gellan gum microstructure in freeze-drying and rehydration processes was investigated. Molecular configuration and three-dimensional network of gellan gels were evaluated in relation to the freeze-drying kinetics, dried structure and rehydration rate. Interestingly, it has been observed and not yet reported prior to this work that the freeze-drying process of LA gellan gum was considerably different from HA gellan, especially in terms of decrease in water activity over time. The former shows a higher rate in water activity reduction. The freeze-dried structures were different between the two gel types due to their molecular configuration, as indicated by total porosity and pore distribution. Overall, the freeze-dried high-acyl gellan gum gel presented slightly larger pores. Moreover, on the subsequent rehydration, LA gellan gum behaved differently from HA gellan, showing a high dependence on the polymer concentration. In this context, both the bulk and surface properties were examined.

The proposed reason for these trends refers to the different molecular and three-dimensional freeze-dried structures between the two gel types. In this light, it is the first time that a research paper reports the micro CT analysis to characterise the freeze-dried structures for both HA and LA gellan gels.

The deep understanding of the gellan behaviour in freeze-drying and rehydration processes can be applied to HA/LA gellan mixtures, especially in terms of gel structure design. Some properties of the gellan blends are intermediate to the two gel types (swelling), others are more similar to one or the other gel (drying kinetics and rehydration).

1. Introduction

The interest in the production of dried-gel structures from hydrocolloids has been increasing for applications in different sectors. In the food industry, hydrocolloids are often used in the product formulation to modulate material properties, acting as a stabiliser, thickener or gelling agent (Norton & Foster, 2002; Phillips et al., 2000; Renard, van de Velde, & Visschers, 2006; Williams & Phillips, 2002). The release of sugar or active compounds can be designed in both wet and dried systems (Lin & Metters, 2006; Nishinari & Fang, 2016; Tønnesen & Karlsen, 2002). Furthermore, dried-gel structures are widely used for pharmaceutical purposes (George & Abraham, 2006; Lee & Mooney, 2012; Tønnesen & Karlsen, 2002) and tissue engineering (Drury & Mooney, 2003; Kang, Tabata, & Ikada, 1999).

Drying allows the extension of the shelf life of products (Ratti, 2001; Van't Land, 2011) by reducing both the water content and water activity, making them ready for use when requested. Water activity (a_w) is an essential parameter to measure in drying mechanisms (Barbosa-CÃ, Fontana, Schmidt, & Labuza, 2008). It provides information about the free water in materials, available to participate in chemical, physical and microbiological reactions (Barbosa-CÃ et al., 2008; Labuza, 1980). Considering food spoilage, it is used to define a stability map (Barbosa-CÃ et al., 2008; Rahman, 2009), since the bound water to the material structure (Aguilera et al., 1999; Mathlouthi, 2001) is not involved in reactions. In addition to food preservation, transport becomes cheaper (Sagar & Kumar, 2010), due to the weight reduction of the dried product. Drying should be carried out considering potential induced alterations in terms of mechanical properties and nutrient content (Fellows, 2009). For more complex products, such as dairy, it is necessary to preserve the whole structure and all the ingredients inside them. Therefore, if additives or gelling agents are used as

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ingredients (de Vries, 2002), they need to be treated appropriately on drying, considering their high capability to retain water (Milani & Maleki, 2012). Since the dried food structure plays an important role in some product properties (e.g. colour, texture and shape) (Aguilera, 2005), dried gel systems require investigation, as they act as effective ingredients. Rehydration is also affected by the drying process and the generated structure. Since a lot of products must recover water before their use, the water uptake mechanism has been extensively studied (Lewicki, 1998; Vergeldt et al., 2014).

A widely investigated drying technique that allows both gel shape and volume to be better maintained, decreasing the occurrence of shrinkage, is freeze drying. This is made possible by the absence of the liquid – vapour interface during the process, which is based on the sublimation of water (Scherer, 1990) from the matrix. By contrast to the common air drying, it works at low temperatures (Evans, 2008; Krokida, Karathanos, & Maroulis, 1998), avoiding product damage due to thermal treatment (Avila & Silva, 1999; Ratti, 2001). Therefore, freeze-dried products can achieve a high quality structure (Ratti, 2001).

In this context, the microbial polysaccharides LA (low-acyl or deacylated) and HA (high-acyl) gellan gum are widely used hydrocolloids in the food industry (Gibson & Sanderson, 1997; Morris, Nishinari, & Rinaudo, 2012; Saha & Bhattacharya, 2010) for modifying texture and giving structure (Chandrasekaran, Millane, Arnott, & Atkins, 1988), for the preservation of flavours or taste/appearance enhancement of foods (Morris et al., 2012).

In parallel to food applications, gellan gum is widely used in tissue engineering (Gantar et al., 2014; Silva-Correia et al., 2011), microbiology and for pharmaceutical formulations (Morris et al., 2012; Shah & Jani, 2010) and for cosmetic products, such as lotions and creams, conditioners and shampoos (Kubo, Miyazaki, & Attwood, 2003).

The gellan gum molecular configuration completely affects the three-dimensional structure of the gel, having an effect on its properties. The acyl substituents (acetate and glycerate) along the HA gellan polymer chain are well-known to lead to a softer and more flexible gel (Mao, Tang, & Swanson, 2000; Phillips et al., 2000). In particular, the acetate hinders the helix aggregation, introducing an entropic barrier (McClements, 2015; Morris et al., 2012), while the glycerate enhances the stabilisation by adding new hydrogen bonds, yet leading to the disruption of the binding site for cations by orientation change of the adjacent carboxyl group (Morris et al., 2012) and consequently the junction zone alteration. The acetyl substituents do not modify the overall double helix geometry (Chandrasekaran & Thailambal, 1990; Morris et al., 2012), while the L-glyceryl groups lead to a mechanical strength drop (Morris et al., 2012).

Freeze-dried LA gellan gum gels have already been investigated in terms of generated structure (Silva-Correia et al., 2011; Tiwari, Chakkaravarthi, & Bhattacharya, 2015), although the analyses reported are only based on SEM results, without providing information about pore distribution throughout the entire bulk volume. A micro CT analysis can provide a deeper understanding at the macroscopic level. Interestingly, there is a lack of information about freeze-dried HA gellan gum structure, or a clear comparison with LA gellan gum. Furthermore, the freeze-drying kinetics for gellan gum systems has not yet been investigated, especially highlighting the role of the molecular configuration. Abramovič and Klofutar (2006) suggested that the water absorption on gellan gum polymer is strictly dependent on its molecular structure, providing a useful support for drying kinetics modelling. However, in that study these considerations are applied for a generic drying process, and without considering the 3D macrostructure. Gantar et al. (2014) investigated the rehydrated gellan gum after freeze drying in terms of final water uptake. Nevertheless, in the same work the rehydration kinetics has not been proposed and HA gellan gum has not been considered.

In this work, the study of freeze-drying and rehydration mechanisms for both LA and HA gellan gum gels from a molecular and structural point of view is proposed. Different polymer concentrations were compared. Mixtures of LA (low-acyl or deacylated) and HA (high-acyl) gellan gum were investigated for the design of products in freeze-drying and rehydration mechanisms. Specifically, true-quiescent gels (Morris et al., 2012) were examined to clearly assess the effect of the molecular/network structures on freeze-drying and rehydration processes. However, this work can be applied to smaller aggregates as well, e.g. a fluid gel system (Banerjee & Bhattacharya, 2012; Norton, Jarvis, & Foster, 1999).

2. Materials and methods

2.1. Gel preparation

In this study, low acyl (Kelcogel F, CPKelco, UK) and high acyl (Kelcogel LT100, CPKelco, UK) gellan gum were used as gelling agents. All materials were used with no further treatment or purification. To prepare the gel solution, distilled water, obtained by a milli-Q water system, was heated up to 85 °C and then gellan gum powder was slowly added to avoid clump formation. Different polymer concentrations (1.5, 2, 2.5, 3% w/w) were used to prepare gels.

To have complete hydration, the solutions were stirred for two hours at constant temperature.

The pH was evaluated at 80 °C, equal to 5.1 ± 0.1 for LA gellan gum and 5.2 ± 0.1 for HA gellan gum, and were not dependent on the polymer percentage, from 1.5% w/w to 3% w/w.

The solutions were poured into sample moulds (22 mm in diameter and 65 mm in height) and left to cool down at room temperature (20 °C) to allow gel formation. After the gels were set, they were stored at room temperature (20 °C ± 1 °C) for 24 h.

Afterwards, the gels were cut, and from each mould, four samples were obtained (22 mm in diameter and 15 mm in height).

2.2. Freeze drying

The gel samples were put into a −18 °C freezer for 24 h to freeze, applying a 0.2 °C/min freezing rate, previously measured by using thermocouples at both the sample surface and core. Afterwards, they were placed into the freeze dryer (SCANVAC 110–4 PRO, LaboGene, UK) onto the shelf trays. The chamber pressure was lowered to 0.18 mbar by a rotary pump and the temperature of the condenser was set at −110 °C.

These process parameters were kept constant for all experiments, to highlight the effect of the gel structure.

The process was run for different times (1, 3, 6, 18, 24, 30) up to 48 h, after which the samples were stored under low vacuum conditions in a desiccator with silica gel beads until characterisation.

2.3. Water activity

Water activity is mathematically defined as the ratio of the equilibrium vapour pressure in a food or in the product (P) over the vapour pressure of pure water (P_o) at the same temperature (Labuza, 1975):

$$a_w = P/P_o$$

To measure the water activity, the Aqualab dew point water activity meter 4te (Labcell LTD, UK) was used and set at 25 °C

throughout all the experiments.

2.4. Moisture content

In this study, NMC (Normalised Moisture Content) (Brown, Fryer, Norton, & Bridson, 2010) was measured on a dry basis (Lewis, 1990) and it was used to monitor the amount of moisture throughout the drying process and also over the rehydration (eq. (1)).

$$NMC = \frac{\frac{(Md - Ms)}{Ms}}{\frac{(Mo - Ms)}{Ms}} = \frac{(Md - Ms)}{(Mo - Ms)} \quad (1)$$

Where M_d is the sample mass after drying, M_s the solid sample mass, and M_o the pre-dried sample. In this way, only water inside the material is analysed.

Before putting the gels into the freezer, they were weighed to determine M_o and numbered. According to this measurement, M_s was calculated. To find it, part of the samples was put into an oven to air dry for 24 h at 60 °C. This gentle thermal treatment could avoid potential gel degradation due to the high temperatures, yet assuring a moisture content plateau is reached (24 h). When the solid mass percentage of these samples from the same batch is found, it was used to assess M_s.

Brown et al. (2010) suggested a value of NMC < 0.1 as the goal to achieve to have negligible moisture content.

2.5. Drying modelling

Drying kinetics was evaluated by comparing two models (Table 1), the Newton model (Akpinar, 2006; Jain & Pathare, 2004) and the Page model (Akpinar, 2006; Belghith, Azzouz, & ElCafsi, 2016). The regression analysis is based on the Least Squares method.

The drying kinetics models chosen were based on both the actual moisture content (M_d) and at the equilibrium point (M_{eq}), defined by the Moisture Ratio, as expressed in equation (2):

$$MR = \frac{Md\,(t) - Meq}{Mo - Meq} \quad (2)$$

In order to find M_{eq} and the desorption isotherm curves, the Oswin model (Table 1) was used to have the relationship between water activity and the moisture content at the equilibrium, based on experimental data for both LA and HA gellan gum. Abramovič and Klofutar (2006) have argued that the Oswin model better fits the experimental data in all the range of water activity (a_w).

2.6. Optical light microscopy and micro computed tomography (mCT)

Investigation of the dried gel structure was performed by using a light microscope (Brunel SP300-fl Brunel Microscopes Ltd., UK) fitted with an SLR camera (Canon EOS 133 Rebel XS, DS126 191). Objective lens up to 4 × magnification was used.

In combination with optical microscopy, micro computed tomography (Bruker microCT, SkyScan 1172) was carried out to have a quantitative analysis of the total porosity. This system allows visualisation of 2D cross-sections and to have a complete 3D structure reconstruction without any chemical fixation and sample preparation. The acquisition mode can be set at a maximum current of 96 μA and voltage of 100 kV. Qualitative and quantitative analysis was performed using a CT-analyser (1.7.0.0), after binarisation into black and white images, obtaining porosity information not only about the surface, but also about the entire sample.

2.7. Static contact angle

Measurements were performed at room temperature (20 °C ± 1 °C) using the KRÜSS Drop Shape Analyser – DSA 100. A 500 μL glass syringe with a 0.5 mm needle diameter was used to deposit a 5 μl distilled water drop onto the dried gel. The freeze-dried samples were previously compressed into circular tablets (1 cm in diameter and 3 mm in height) to obtain a flat surface, applying 10 tons in a hydraulic press for 10 s.

The static sessile drop method was used to measure in triplicate the distilled water contact angle. These values were collected 2 s after the drop deposition.

2.8. Rehydration and swelling

The water uptake was evaluated by measuring the sample weight every 6 min for 30 min and after 24 h to determine the rehydration end point and swelling behaviour. The gel samples were laid into a distilled water bath (Vergeldt et al., 2014), below the water-air interface, at room temperature (20 °C ± 1 °C) and were carefully blotted before weighing to remove surface water.

Regression analysis was carried out using both linear and polynomial fitting. Furthermore, the rehydration trend was modelled by the Peleg model (Goula & Adamopoulos, 2009) (Table 2).

Swelling capability was assessed by measuring the gel weight (Khare & Peppas, 1995) at the rehydration plateau after 24 h and was normalised with the original weight.

2.9. Micro DSC

Thermal transitions were investigated by using a micro DSC 3 evo (Seteram Instrumentation). All the experiments were carried out from 5 °C to 90 °C with a scan rate of 1 °C min^{-1} and in each experiment two heating/cooling cycles were performed. Isothermal periods were applied to prevent the system being impacted from the thermal history effect.

Table 1
Models used for freeze drying.

Model	Model's Equation	Parameters
Oswin (Abramovič and Klofutar, 2006)	$Meq = A\left[\frac{a_w}{(1-a_w)}\right]B$	LA gellan: A = 0.136; B = 0.446 HA gellan: A = 0.106; B = 0.478
Page (Akpinar, 2006)	$MR = e^{-K^{t^n}}$	K: empirical coefficient n: number constant
Newton (Akpinar, 2006)	$MR = e^{-Kt}$	K: empirical coefficient n: number constant

Table 2
Peleg model for rehydration.

Peleg (Goula & Adamopoulos, 2009)	$X(t) = X_0 + \frac{t}{\alpha + \beta t}$ $X_{eq} = X_0 + \frac{1}{\beta}$	X: Moisture content at time t Xo: Initial moisture content Xeq: Equilibrium moisture content t: time β: Peleg capacity constant α: Peleg rate constant

2.10. Statistical analysis

All the experiments were carried out in triplicate and error bars represent plus/minus a single standard deviation. The experimental results used to investigate and model both the drying and rehydration kinetics are the average values related to the previous set of experiments.

3. Results and discussion

3.1. Freeze drying

A series of experiments was carried out to investigate the drying process in terms of water activity as a function of time, highlighting the effect of the polymer concentration (1.5, 2, 2.5, 3% w/w) for both LA and HA gellan gum (Fig. 1a–b).

The water activity decrease over time on freeze drying was completely different between LA and HA gellan gum. The former reaches a value below the microbial growth threshold (0.6) faster than the latter. This means that at a given timescale, the amount of free water is higher for HA gellan, leading to a higher water activity value. The final values of water activity (48 h) are in agreement with those for a freeze-dried product (Beuchat, 1981).

In general, it was observed and confirmed that water activity was highly dependent on the material, rather than the polymer concentration, since no specific trends were noticed by changing the solid mass percentage in the studied range.

The different behaviour in gellan gum systems is related to the different molecular structure and, consequently, differences in moisture content in the monolayer are expected. What could effectively change the water holding and therefore a_w, are both the morphological and the chemical aspects. Considering the former, the steric hindrance of acetyl groups along the HA gellan chain, which are not present along the LA one, keep the polymer network more open (Mao et al., 2000) and obstruct the aggregation of the double helices (Morris et al., 2012). A more open gel network may reduce the probability for water molecules to interact with the molecular strands, lying in a larger region between the chains and resulting in a higher free water content. From a chemical point of view, the glycerate substituents tend to stabilise the double helix aggregation, since more hydrogen bonds are created within the strands and between the helices (Morris et al., 2012). Apparently, this could suggest that more interactions with water are to be expected for the HA gel. However, from the water activity results it seems that the combination of these two factors results in a higher free water content at the HA sample. To further investigate this observation, wettability for both the gel types was assessed by measuring the static contact angle with distilled water after two seconds after the drop deposition. For LA gellan gum the value was 78.5° ± 1.2°, while HA gellan was less wettable, as the contact angle is 97.2° ± 2.2°. In effect, the presence of the L-glyceryl groups changes the orientation of the carboxyl groups (Morris et al., 2012), which are directly related to the overall polymer hydrophilicity (Prezotti, Cury, & Evangelista, 2014). Since this is concerned with

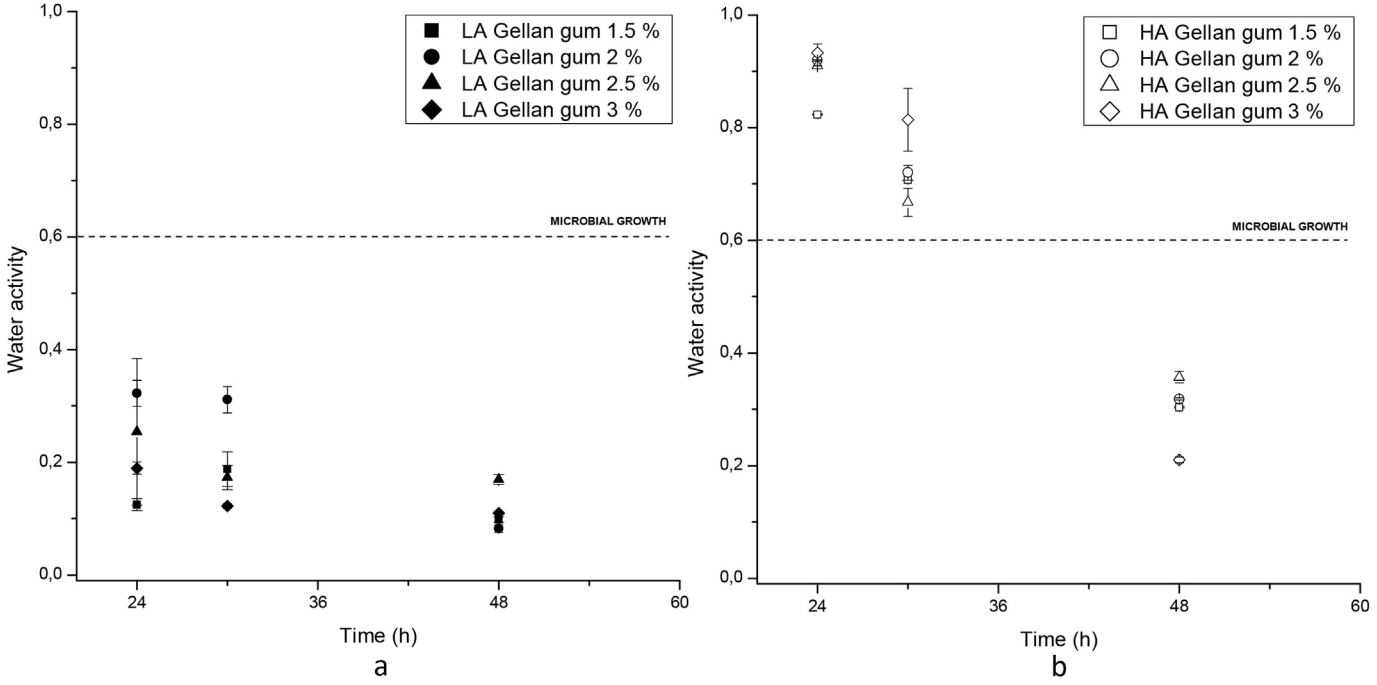


Fig. 1. a–b. Water activity (a_w) as a function of drying process time and LA (a), HA (b) gellan gum concentration.

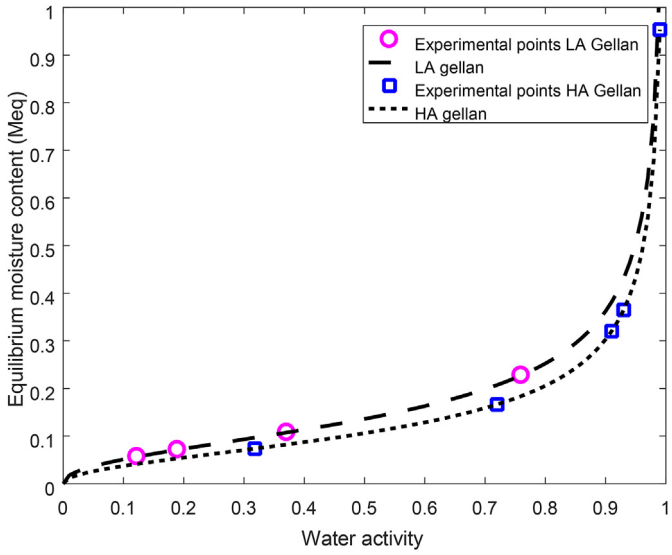


Fig. 2. Desorption isotherms at 25 °C: (○) LA gellan 2% w/w and (□) HA gellan 2% w/w.

the interactions between the water molecules and the material, it is confirmed that in general water interacts less with HA gellan gum.

In effect, Abramovič and Klofutar (2006) suggested that LA gellan gum binds more water per gram of material, 0.0683 g/g against 0.0594 g/g for HA gellan, since the number of available active sites is larger. Furthermore, Abramovič and Klofutar (2006) mathematically reported that the area of the monomolecular layer per gram of LA gellan is 242 m^2/g against 211 m^2/g for HA gellan. Specifically, this monolayer in a dynamic equilibrium depends on the rapid interaction (less than 10^{-11} s) between the water molecules and the chemical substituents of the polymer chains (Barbosa-CÃ et al., 2008).

In this study, it seems that polymer concentration does not significantly affect the results. It is well-known that more polymer leads to the distance reduction between the helices, increasing the possibility to have junction zones, and therefore increasing the mechanical properties (Banerjee & Bhattacharya, 2011). However, in terms of freeze drying this effect was negligible in the range between 1.5 and 3% w/w. Moreover, since the pH of the gel solution is constant for both the materials as a function of the polymer amount, the overall molecular charge, which might affect the water activity within the material, is likely to remain constant in this range.

The freeze-drying process parameters influenced the drying rate, but they were kept constant for all the experiments to highlight the effect of the material properties. After 48 h drying all the samples were below the moisture content limit (NMC < 0.1), set as a reference (Brown et al., 2010).

The collected data for both the gels were used to model both the desorption isotherms at 25 °C and the drying kinetics. The former were found by using the Oswin model (Fig. 2). Since the water activity is not dependent on the polymer concentration, the experimental values used in the model were the a_w related to 2% w/w gellan gum, assumed as a reference.

From this comparison, at a given generic equilibrium moisture content the water activity was lower for LA than for HA gellan, in agreement with Abramovič and Klofutar (2006), especially in the linear region of the isotherm, which describes water absorbed in multilayers (Mathlouthi, 2001). This is likely to be due to the relatively higher amount of free water in HA gellan gum, since the water content in the monolayer region (Mathlouthi, 2001) is slightly higher for LA gellan.

Once the M_{eq} and MR are worked out, the freeze-drying kinetics was found by fitting the experimental data with the Newton and the Page models (Fig. 3a–b). Interestingly, the freeze-drying kinetics was not affected by the specific gel type, since the trends are similar.

For both materials, it was found that the Page model better fits the experimental points, as the R^2 is higher. It might suggest that a first-order kinetics model (Newton) is not the most suitable to fit the experimental data for freeze-drying.

Polymer concentration should affect the drying kinetics, since a more packed and entangled three-dimensional network is expected as the concentration rises. However, no significant discrepancies were noted between the concentrations, as discussed for water activity.

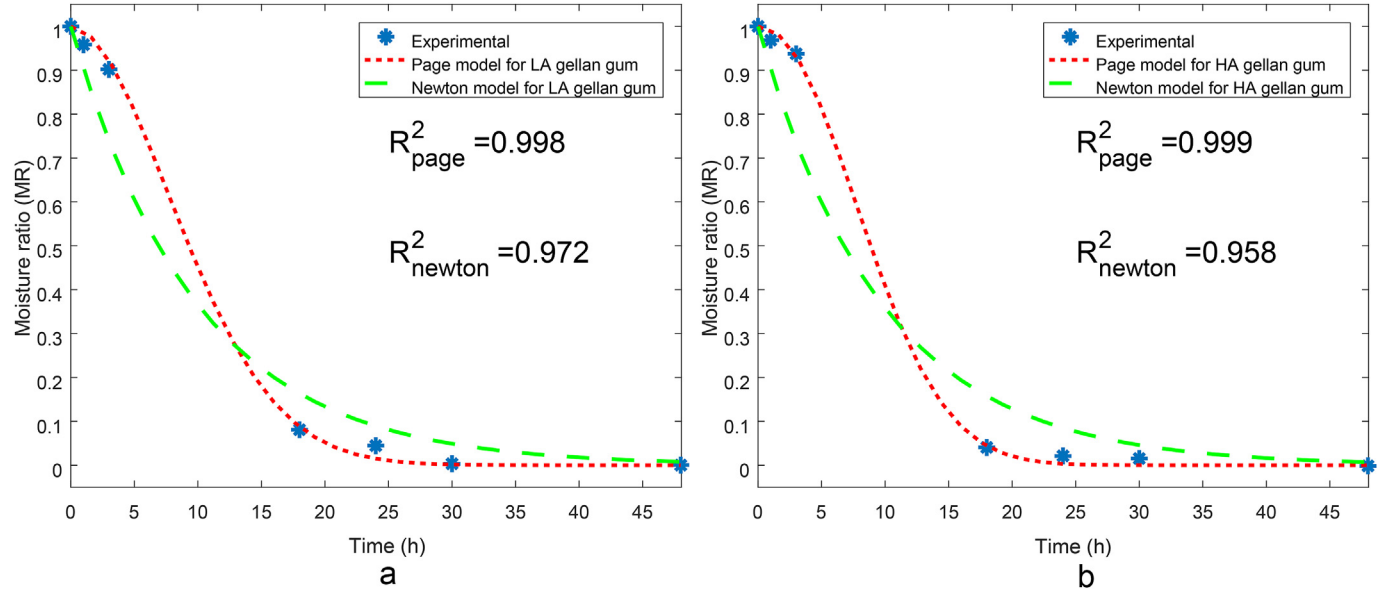


Fig. 3. a–b. Moisture content ratio (MR) for LA gellan gum (a) and HA gellan gum (b) both at 2% w/w as a function of drying process time.

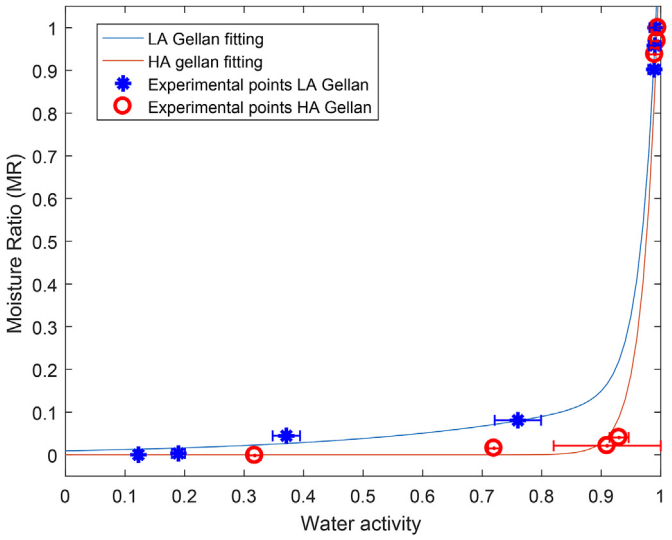


Fig. 4. Moisture ratio (MR) vs water activity for LA (blue stars) and HA (red circles) gellan gum. Concentration at 2% w/w. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

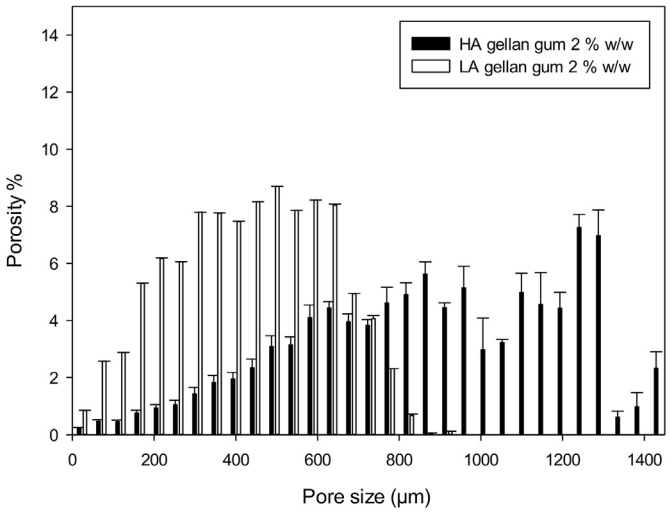


Fig. 6. Pore distribution after freeze drying. Percentage is referred to the total pore volume.

In Fig. 4, the MR values as a function of water activity are shown (HA and LA gellan gum at 2% w/w). As discussed in Fig. 1a–b, there is a considerable gap between HA and LA gellan gum in terms of water activity, especially at low moisture content values.

The experimental data were fitted with a two-term exponential function. R^2 is 0.999 and 0.992 respectively for LA and HA gellan gum.

3.2. Freeze-dried structure

The freeze-dried gel structure is deeply affected by the crystal nucleation and growth during the freezing step, leading to a highly porous structure. Therefore, the freezing rate was kept constant throughout the experiments and the final temperature was set at −18 °C.

Ice crystal size and shape are related to the gel formulation and to the material properties. The latter can affect the ice crystal growth (Caldwell, Goff, & Stanley, 1992; Regand & Goff, 2003; Voitkovskii, 1962). On the other hand ice crystal distribution is not affected by the material properties (Sutton & Wilcox, 1998). Consequently, a softer material might more easily accommodate the ice crystals, leading to larger pores after freeze-drying, since the gel network is able to support more stretch (Scherer, 1990).

In this context, the generated freeze-dried gel structure was investigated by micro computed tomography (Fig. 5a–b).

These micrographs suggest that the produced pores during the ice crystal sublimation are interconnected, forming paths for water to be reabsorbed in the following rehydration process.

For 2% w/w LA gellan the total porosity was 84.8 ± 4.2%, while for HA gellan there was a slight increase up to 90.9 ± 1.9%. Polymer concentration affects the freeze-dried structure, making it more packed. A drop in total porosity was noticed at 3% w/w. LA gellan reduces it to 74.7 ± 0.8%, while HA to 84.3 ± 0.4%.

The collected results show larger pores for the freeze-dried HA gellan. Since it is softer and less rigid than LA gellan, the lower mechanical resistance can encourage the ice crystal growth. It may also be correlated to the higher amount of free water, as water molecules could aggregate onto the ice crystals more easily, being less influenced by the polymer chains. Moreover, the slightly higher monovalent and divalent ion concentration (Na^+, K^+, Mg^{++}, Ca^{++}) in the gel formulation for LA gellan, 5.02 w/w % (Amici, Clark, Normand, & Johnson, 2000) compared to 3.12 w/w % for HA

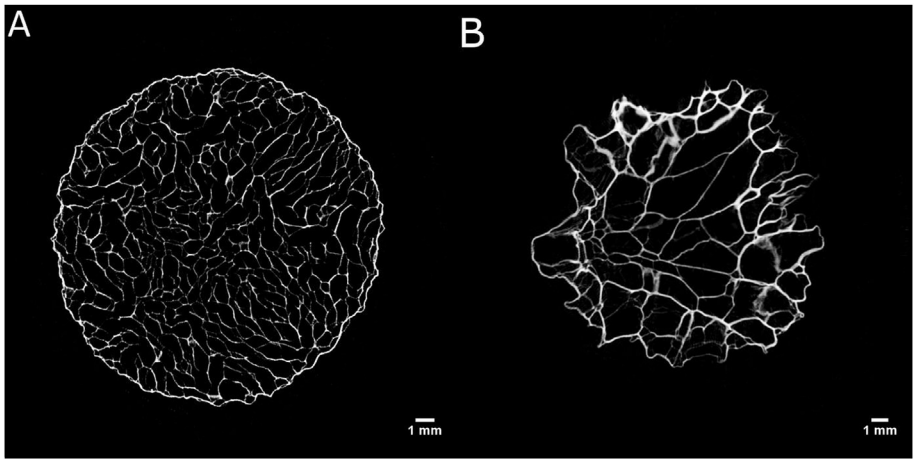


Fig. 5. Microstructures by micro CT: 2% w/w LA gellan gum (A) and HA gellan gum (B).

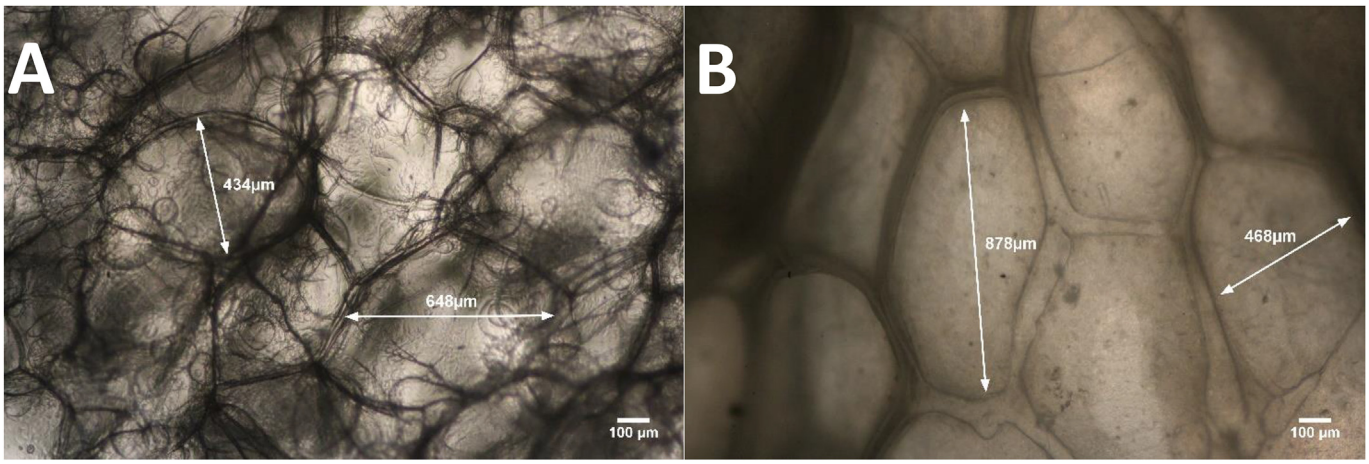


Fig. 7. a–b. Microstructures by optical microscope: 2% w/w LA gellan gum (A) and HA gellan gum (B).

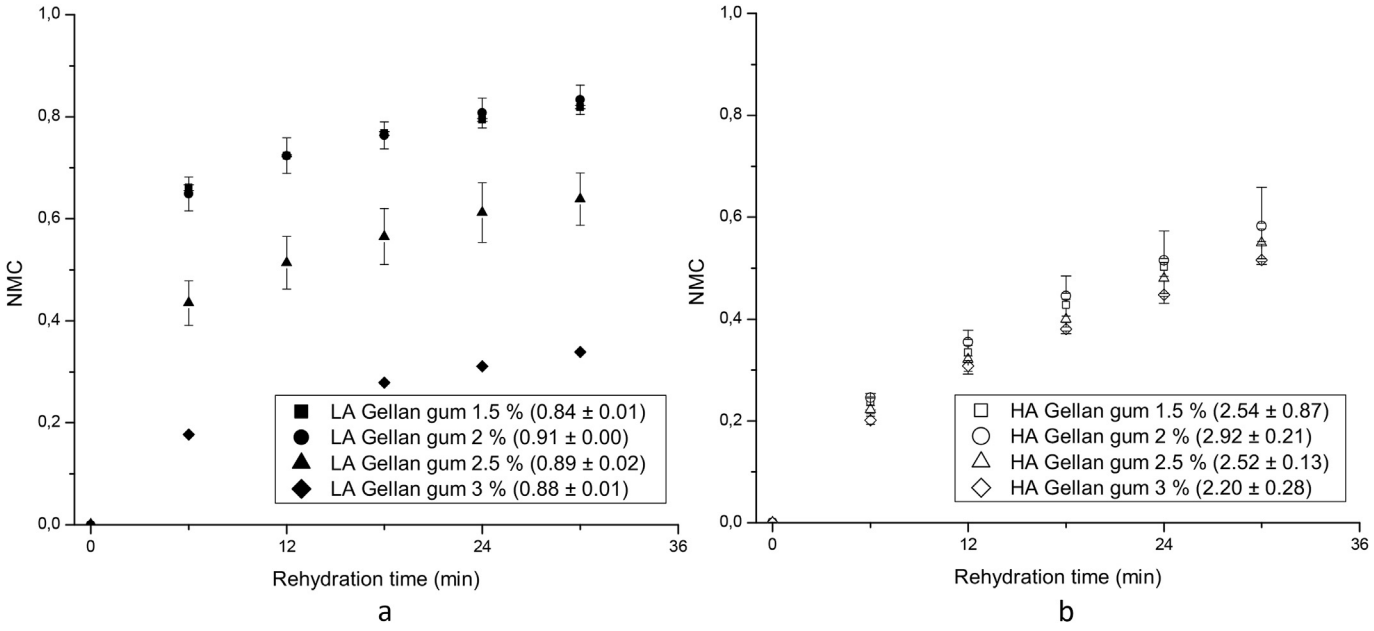


Fig. 8. a–b. Rehydration expressed as NMC as a function of rehydration time and LA (a), HA (b) gellan gum concentration. The final value of rehydration, after 24 h, is expressed in the leg end.

gellan (Huang, Singh, Tang, & Swanson, 2004), may affect both the ice crystal nucleation and growth. These cations are mainly added as chlorides (CPKelco, 2007). As a consequence, the pores are formed by thicker walls in the case of HA gellan gum.

The pore size distribution is reported in Fig. 6 and it shows that for HA gellan gum the void size was shifted towards larger values. Tiwari et al. (2015) found similar results for LA gellan gum, reporting a pore size range between 219 and 600 µm.

The optical microscope observations (Fig. 7a–b) show larger pores in agreement with the values collected by mCT.

The resulting cryogel (Job et al., 2005; Pajonk, Repellin-Lacroix, Abouarnadasse, Chaouki, & Klavana, 1990; Tamon, Ishizaka, Yamamoto, & Suzuki, 2000) is a macroporous material and it can be considered like a sponge (Kumar, Mishra, Reinwald, & Bhat, 2010; Lozinsky et al., 2003), where the solvent, such as water, can penetrate and fill the structure. The polymer chains are forced to align and to associate along the ice crystals edges in a so-called side-by-side mechanism (Zhang, Zhang, & Wu, 2013). For this reason, Zhang et al. (2013) suggested that new junction zones might potentially form during the freezing step. These junctions may persist once formed (Dea et al., 1977). If rehydrated, these cryogels are comparable to the gel structures obtained by freezing and thawing weak gels (Giannouli & Morris, 2003; Richardson & Norton, 1998).

3.3. Rehydration and swelling

Both gel type samples in different concentrations were left to rehydrate up to 30 min (Fig. 8a–b). In particular, for LA gellan it was noticed that increasing the polymer concentration, and therefore having a more packed and entangled the structure, the rehydration was slower.

By contrast to the drying results, this observation suggests that rehydration of LA gellan gum was sensitive to the polymer concentration. On the other hand, HA gellan shows overlapping rehydration curves. Furthermore, for the former it was possible to distinguish two main rehydration rates, corresponding to the first

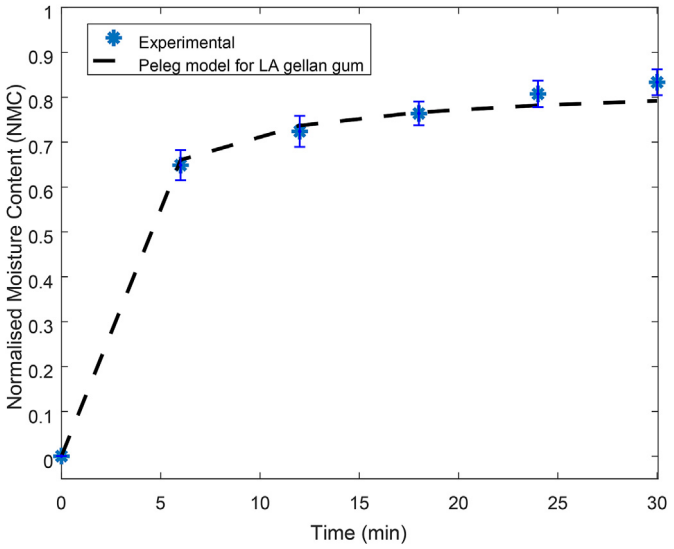


Fig. 9. Rehydration expressed as NMC (X(t) in Table 2) as a function of rehydration time for LA gellan gum 2% w/w. The model used is Peleg.

period (up to six minutes) and the second period (from six to thirty minutes). Specifically, a fast water uptake was noticeable at short timescale. For 2% w/w LA gellan gum the initial rate is 0.110 min^{-1}, while the second rate for longer periods decreases to 0.006 min^{-1}. This last value is constant for all the concentrations, equal to 0.006 ± 0.001 min^{-1}. On the other hand, HA gellan gum did not present a faster initial step and the second rate is slightly higher, equal to 0.012 ± 0.001 min^{-1}.

In terms of trend, a polynomial fitting was more suitable for HA gellan gum, since R^2 is 0.989 using a parabolic fitting, while a linear regression gives R^2 equal to 0.942. This confirms that the overall water uptake tends to slow down over time. Similarly, a parabolic fitting was suitable for LA gellan gum in the second period (R^2 equal to 0.996). However, LA gellan gum in the entire measured range, from 0 to 30 min, is better fitted by the Peleg model (Goula & Adamopoulos, 2009), which takes into consideration an initial quicker step (Fig. 9).

The collected data suggests that this model will reproduce the LA gellan gum behaviour (R^2 = 0.99), while it is not ideal for HA, with R^2 = 0.54.

Rehydration is essentially affected by two main factors: surface and bulk structure (Aguilera et al., 1999). The molecular configuration and three-dimensional network of the gel are correlated to these factors, raising the discrepancies noted during the water uptake.

Rehydration for LA gellan is firstly governed by a quicker water uptake, mainly due to the affinity with water (higher wettability), which makes the penetration into the porous structure faster. The first rate is highly dependent on the polymer concentration, while the second rate was considerably lower, since most of the water is already absorbed. For HA gellan there was not the initial quick uptake, since the walls around the voids in the dried structure were thicker and less hydrophilic. However the rate was slightly higher compared to the second rate for LA gellan, as the porosity was higher with bigger voids. Overall, it seems that HA gellan is considerably less sensitive to the variation in polymer concentration compared to LA gellan.

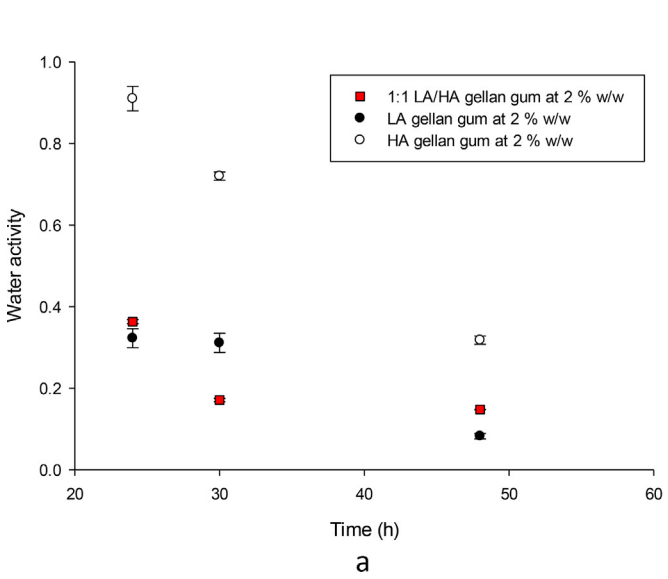
In terms of swelling, HA gellan significantly increases its weight after 24-hour rehydration, mainly due to its softer more flexible mechanical behaviour and different molecular properties (Aguilera et al., 1999; Tanaka & Fillmore, 1979).

3.4. HA/LA gellan mixture

The understanding of the freeze-drying and rehydration mechanisms allows the prediction and design of new dried structures made from a mixture of the single gels. The 1:1 HA/LA blend at 2% w/w was chosen as a model system. Fig. 10a–b show the desorption isotherm and the freeze-drying kinetics.

In terms of drying, the results were closer to the LA gellan behaviour. On the other hand, the rehydration results (Fig. 11) showed an evident drop in rehydration rate, especially if they are compared to the rehydration curves with 2% w/w for the single gels.

As previously discussed, both surface and bulk properties need to be considered. In this case, the total porosity was 84.8 ± 4.2%, closer to LA gellan gum. Considering the presence of the acyl substituents along the HA gellan chains, the material becomes less wettable. These two parameters together decrease the rehydration



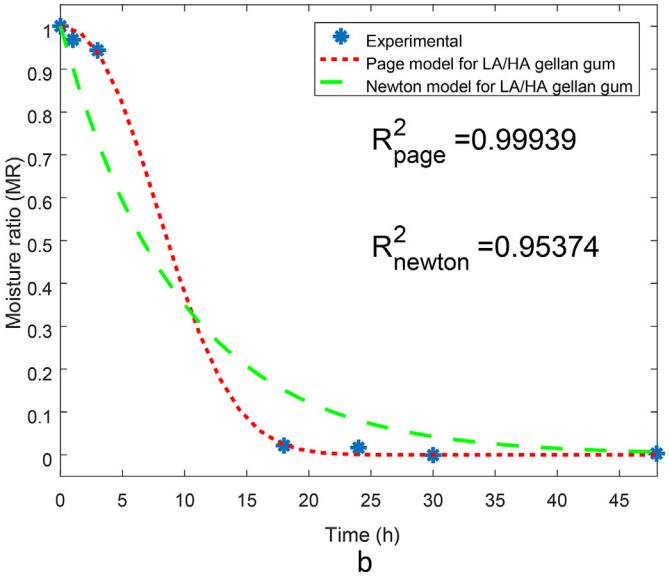


Fig. 10. a–b. 1:1 HA/LA mixture at 2% w/w: water activity vs time (left), drying kinetics (right).

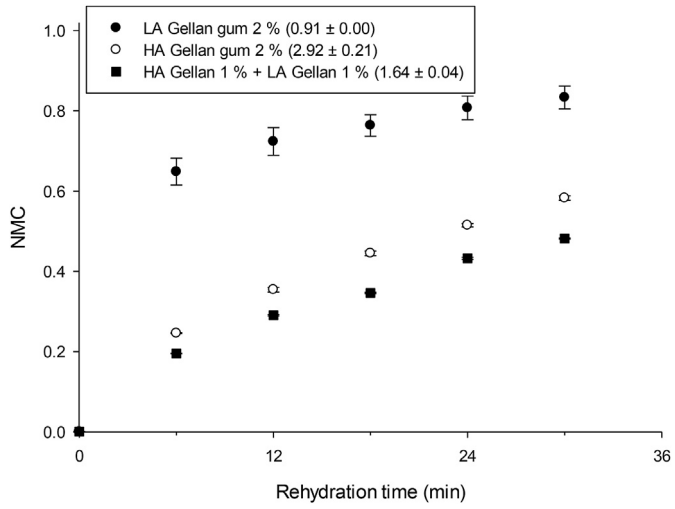


Fig. 11. Rehydration expressed as NMC as a function of rehydration time. LA gellan 2% w/w (●), HA gellan 2% w/w (○), LA + HA 1:12% w/w (■). The final value of rehydration, after 24 h, is expressed in the legend.

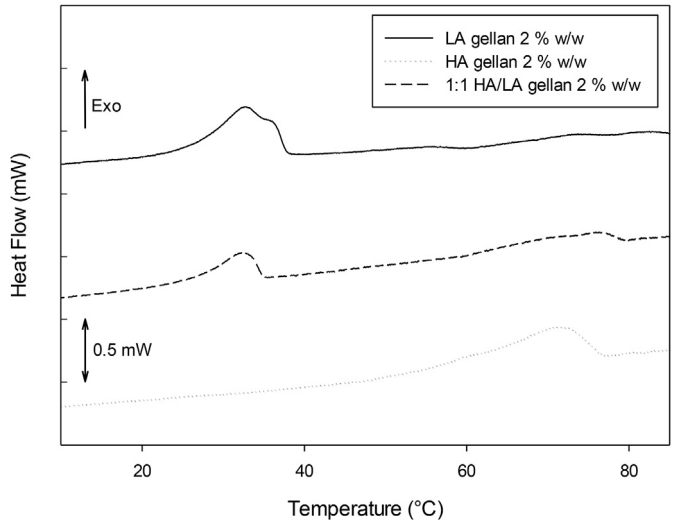


Fig. 12. DSC curves for LA, HA gellan and 1:1 mixture at 2% w/w.

rate of the mixture.

The gel-system mixing may affect these mechanisms. Gelling agents may homogeneously mix or phase-separate. For LA and HA gellan it has been reported that double helices do not include strands of the two gel types (Morris et al., 2012), as the DSC peaks show separate thermal transitions for the mixture (Kasapis et al., 1999). Fig. 12 confirms these results, since for the DSC curve for the mixture both peaks are noticeable, yet less pronounced.

From a macroscopical perspective, micro CT micrographs did not show any bulk phase-separation, since areas with different density (noticeable at the mCT as darker or brighter regions) were not present for both the pre-dried (before freeze drying) and dried gels (Fig. 13a–b). This can suggest an interpenetrating three-dimensional gel network (Mao et al., 2000).

Interestingly, the swelling mechanism was intermediate (Table 3), since the final weight, after leaving the gel 24 h in water, was around 64% greater than the pre-dried gel weight. By contrast, in terms of weight LA gellan showed a decrease of around 9%, while HA gellan a gain of 192%, likely due to the presence of acetyl groups (Wareing, 1997). It seems that LA and HA gellan form two networks, more rigid for the former. On rehydration HA gellan swells, yet the swelling is limited by the presence of the stiffer LA gellan network. Presumably, on swelling, the LA gellan network was broken, allowing some expansion but not to the same extent as a pure HA gellan sample of equivalent concentration.

The study of the freeze-dried mixture of LA and HA gellan gum gels may advance the knowledge in the production of improved additives to design the food formulation. It can be beneficial for the modulation of the product texture and properties over time (rehydration) for both dried products, to be rehydrated before consumption (e.g. instant food) or directly eaten.

The investigation of the moisture content/water activity over time, may be used to optimise the freeze-drying process and to extend the product shelf life.

4. Conclusions

In this study, the role of both molecular and macroscopic structures for HA and LA gellan gum on freeze-drying and rehydration mechanisms were investigated. After focusing on the single gellan gum gel type, the properties of their mixture were assessed.

It was reported for the first time that the water activity decrease during freeze drying was considerably larger for LA gellan, since the molecular structure more effectively binds water molecules. On the other hand, drying kinetics in terms of moisture content is similar

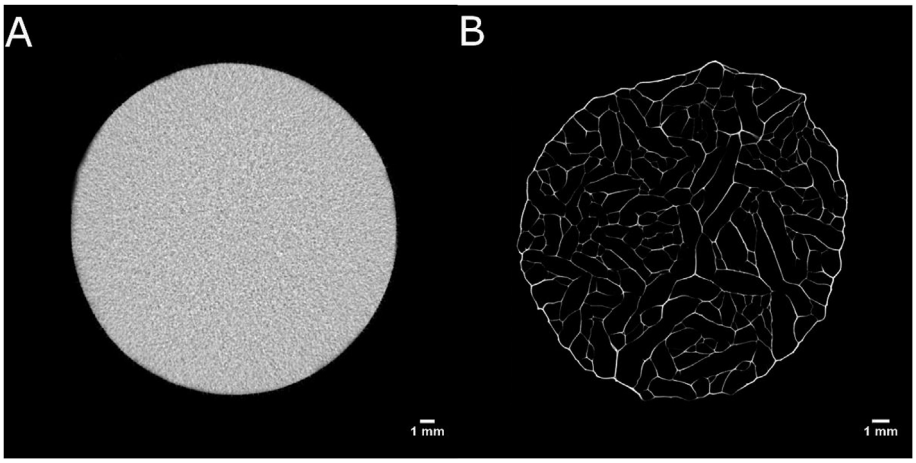


Fig. 13. Microstructure by micro CT: 1:1 HA/LA mixture at 2 w/w. Pre-dried gel (a), freeze-dried gel (b).

Table 3
Gel swelling (weight) after 24 h in distilled water. Values are compared to the initial gel weight, before freeze drying.

Gel system	Swelling
LA gellan gum 2% w/w	−9%
HA gellan gum 2% w/w	+192%
1:1 HA/LA blend at 2% w/w	+64%

between the gels.

The dried structure for LA and HA gellan had comparable porosity, with slightly higher values for the latter. In addition, the pore size distribution for HA gellan was moderately shifted towards larger values.

Nevertheless, the rehydration was considerably higher for LA gellan, since it was found to be more hydrophilic. Designing the gel formulation, it is possible to predict and modulate the water uptake rate, accordingly to the specific application.

Understanding a more complex system, such as a mixture of the two polymer types, provides a further method to modulate the dried-gel properties. In this context, it has not yet been reported in the literature to date the properties of the HA/LA gellan blends on freeze-drying and rehydration. Specifically, it was shown that the mixture has an intermediate behaviour in some properties, such as swelling capability, while others are more similar to either HA gellan (rehydration) or to LA gellan (drying kinetics and water activity reduction).

A further investigation on hydrocolloids with similar and different molecular structure and gel network, such as k-carrageenan or gelatin, may be useful to complete the work as well as a the study of a more complex gel system with the presence of additives, such as sugars.

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