

# Novel mango bars using gellan gum as gelling agent: Rheological and microstructural studies



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## ABSTRACT

This work aimed the development of mango bars, made with fresh mango puree and gellan gum. The influence of gellan concentration as well as the ratio of LA (low-acyl) and HA (high-acyl) gellan on the rheological and microstructural properties of the mango bars was studied. Rheological outcomes showed that both LA and HA, and in mixtures, were able to produce jellified products, within a rather low maturation time. Furthermore, a synergistic effect was observed at different ratios of LA/HA, enabling the production of mango bars with intermediate viscoelastic properties. Higher values of the dynamic moduli were perceived as the LA content increased. Confocal microscopy is in agreement with the rheological results, revealing a weakening of the gel structure with the increasing percentage of HA. Results suggest that 1 g of LA/HA 50/50 per 100 g of puree produces structures with suitable mechanical properties. However, the results need to be correlated to texture and sensory analysis, before proceeding to the next steps of product development.

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## 1. Introduction

Changes in modern lifestyle and the growing awareness of the link between diet and health, as well as new processing technologies, have led to a rapid rise in the consumption of fresh fruits products (O'Shea, Arendt, & Gallagher, 2012). Ready-to-eat fresh fruit products became an important area in the food industry due to their characteristics of freshness, low caloric content, and an active promotion of fruit as a basic component of a healthy diet (Corbo, Lanciotti, Gardini, Sinigaglia, & Guerzoni, 2000). Therefore, new trends in food industry require the development of new high quality convenient fruit products compatible with a healthy diet.

Fruit and vegetables are processed into a variety of products such as sliced minimally processed fruits, juices and concentrates, pulps and dehydrated products, jams and jellies, pickles, chutneys and fruit bars (Ahvenainen, 1996; Schieber, Wieland, & Reinhold, 2000). However, it is difficult to obtain a convenient product maintaining the desired stability from a chemical, enzymatic and microbiological point of view and presenting the nutritional and sensory attributes of a fresh product. A key factor on the development of new products relies on the selection of the type of

fruits to be used, taking into account their physico-chemical and sensory properties and the characteristics of the desired final product.

Mango (*Mangifera indica* L.) is a popular and economically important tropical fruit throughout the world, due to its good texture, flavour, and high content of carotenoids, vitamin C, vitamin E, phenolic compounds, minerals and fibre, (Charles, Vidal, Olive, Filgueiras, & Sallanon, 2013; Vijaya, Sreeramulu, & Raghunath, 2010; Xiaolin et al., 2011). The consumption of mango can provide significant amounts of bioactive compounds with antioxidant activity. Its daily intake in the diet has been related to prevention of degenerative processes such as cardiovascular diseases and cancer (Alothman, Kaur, Fazilah, Bhat, & Karim, 2010; Liu, 2003; Sánchez-Robles, Rojas-Graü, Serrano-Odriozola, Gonzalez-Aguilar, & Martín-Belloso, 2009).

Mango has been processed into a large variety of products, such as juices, powders, purees and dehydrated slices (Dak, Verma, & Jaaffrey, 2007; Djantou, Mbofung, Scher, Phambu, & Morael, 2011; Ledeker, Suwonsichon, Chamber, & Adhikari, 2014; Sogi, Siddiq, & Dolan, 2015; Sriwimon & Boonsupthip, 2011). Furthermore, among the derivative mango products, mango bars or mango leathers are the most popular fruit bars in India. This mango bar is traditionally prepared by adding cane sugar, spreading the puree on bamboo mats and, drying it in the sun into

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leathery sheets (Vijayanand, Yadav, Balasubramanyan, & Narasimham, 2000). In addition, mango gels were developed using processed commercial mango juice and sodium alginate, with the addition of a calcium source (calcium orthophosphate) and a calcium sequestrant (glucono- $\delta$ -lactone) (Roopa & Bhattacharya, 2014).

Gellan gum, a polysaccharide approved as a food additive which has been used in a wide variety of food products including confectionary, jams, jellies, fabricated foods, hydrogels, pie fillings, puddings, ice cream and yogurt (Lau, Tang, & Paulson, 2000; Noda et al., 2008; Tang, Marvin, Tung, & Zeng, 1996) was chosen as gelling agent for the fresh mango bars. The native polymer, high acyl (HA) gellan, is an extracellular hydrocolloid secreted by the bacterium *Pseudomonas elodea*, and its linear structure is based on the tetrasaccharide repeating unit  $\rightarrow 4$ -L-rhamnopyranosyl-( $\alpha$ -1  $\rightarrow$  3)-D-glucopyranosyl-( $\beta$ -1  $\rightarrow$  4)-D-glucoronopyranosyl-( $\beta$ -1  $\rightarrow$  4)-D-glucopyranosyl-( $\beta$ -1  $\rightarrow$ , with O (2) L-glycerol and O (6) acetyl substituents on the 3-linked glucose (García, Alfaro, Calero, & Muñoz, 2011; Noda et al., 2008; Yamamoto & Cunha, 2007). When exposed to alkali at high temperature, the acyl groups are hydrolysed and the deacylated form, low acyl (LA) gellan, is obtained.

HA gellan hydration is inhibited by the presence of sugars and high pH values; therefore, the hydration must be carried out in presence of less than 40 °Brix total soluble solids content (TSS) and at pH values below 4. By the contrary, LA gellan hydrates easily in the presence of sugars (up to 80 °Brix TSS), being its hydration much more dependent on the ions concentration, and generally it is not complete at pH values below 4 (Philips & Williams, 2009, chap. 9).

The gelation process of both gellan types is generally considered to involve two separate steps. In aqueous solutions, upon heating, the molecules of gellan are in a disordered coil state (single chain). Upon cooling the molecules adopt an ordered double helical conformation followed by associations between the helices through weak interactions such as hydrogen bonds and van der Waals forces (Matsukawa & Watanabe, 2007; Nickerson, Paulson, & Speers, 2003; Picone & Cunha, 2010). In the case of LA gellan, the helices aggregation are also promoted by pH reduction or mediated by cations, either by site binding between pairs of carboxylate groups on neighbouring helices or by suppressing electrostatic repulsion by binding to individual helices (Morris, Nishinari, & Rinaudo, 2012).

Generally, HA gels are thermally reversible without thermal hysteresis and set at temperatures around 70–80 °C; while the gel setting temperature of LA gels is around 30–50 °C showing significant thermal hysteresis, which extent is dependent on the nature and concentration of cations in solution (Philips & Williams, 2000, chap. 7; Williams & Philips, 1999; Rocha, Gonçalves, Bicho, Martins, & Silva, 2014).

One of the major advantages of gellan gum relies on the ability of LA and HA to produce gels with quite different characteristics. HA gellan usually produces elastic, soft, non-brittle and opaque gels and LA gellan enables the formation of non-elastic, hard, brittle and transparent gels (Ogawa, Takahashi, Yajima, & Nishinari, 2006; Philips & Williams, 2009, chap. 9). Therefore, a wide range of structures, with varied rheological properties, appealing textures and good flavour release, may be designed by controlling the acyl content. The development of texturized fruit bars involves the understanding of synergism between gellan gum and mango puree. The final structure and textural properties of these systems are strongly dependent on gellan physicochemical properties (e.g. acylation degree), pH and composition of the puree (e.g. sugars, cations and fibre content), as well as on processing conditions such as gellan concentration, LA/HA ratio and temperature.

The influence of the addition of gellan gum (different concentrations and LA/HA ratios) to the mango puree was evaluated through analysis of the rheological (small amplitude oscillatory

shear measurements) and microstructural (confocal laser scanning microscopy) properties in order to develop a new fresh mango bar.

## 2. Materials and methods

### 2.1. Materials

Two types of commercial gellan gum were kindly supplied by CP Kelco Corporation, Wilmington, USA: low-acyl gellan gum (Kelcogel® F) and high-acyl gellan gum (Kelcogel® LT). The composition of gellan gum powder was supplied by the manufacturer. LA gellan: carbohydrate (dietary fibre) 82 g/100 g; moisture 7 g/100 g; Ca<sup>2+</sup> 252 mg/100 g; P 115 mg/100 g; Fe<sup>2+</sup> 4 mg/100 g; Mg<sup>2+</sup> 91 mg/100 g, Na<sup>+</sup> 478 mg/100 g and K<sup>+</sup> 4650 mg/100 g. HA gellan: carbohydrate (dietary fibre) 78 g/100 g; moisture 6 g/100 g; Ca<sup>2+</sup> 259 mg/100 g; P 233 mg/100 g; Fe<sup>2+</sup> 2 mg/100 g; Mg<sup>2+</sup> 98 mg/100 g, Na<sup>+</sup> 510 mg/100 g and K<sup>+</sup> 1930 mg/100 g.

Mature mangoes (*M. indica* L. cv. Palmer) were purchased from a local supermarket in Lisbon, Portugal and selected based on firmness and integrity.

### 2.2. Methods

#### 2.2.1. Preparation and analysis of fresh mango puree (control)

Mangos were stored at 5 ± 1 °C until processing (within 24 h). The fruit was then washed under running water, and manually peeled with a knife, cut into small pieces, and pureed in a food blender Vorwerk Thermomix TM-31 at 134 × g for 4 min at 22 ± 2 °C.

**2.2.1.1. Determination of ash content.** The total ash content was determined according to AOAC Official Method 923.03 (AOAC, 1990). A mass of 5 g of mango puree was weighed into a shallow dish and subjected to a temperature of 550 °C until light a grey ash was produced. The dish was then cooled in a desiccator and weighed soon after reaching room temperature. The total ash content was calculated using eq. (1):

$$\% \text{ Total ash} = \frac{\text{ash weight}}{\text{original sample weight}} \times 100 \quad (1)$$

**2.2.1.2. Determination of mineral content.** The ash obtained according the method described previously, was dissolved in 3 mL concentrated nitric acid and then diluted to 25 mL with deionized water. The solution obtained was used to determine the mineral content using an atomic absorption spectrophotometer (UNICAM M series, UK). Six types of minerals were determined, potassium, calcium, magnesium, sodium, and iron. Phosphorus was assayed colorimetrically based on the reaction of phosphate with molybdo vanadate complex (Windham, 1995). The minerals content was expressed in mg/100 g edible portion. The analysis was carried out in triplicate.

**2.2.1.3. Determination of moisture content.** A mass of 3 g of the mango puree ( $w_1$ ) was placed in the oven for 6 h at 105 °C (in triplicate). After drying, the dried sample was weighed ( $w_2$ ), and the moisture content was calculated using the following equation:

$$\text{Moisture content (\%)} = \frac{w_1 - w_2}{w_1} \times 100 \quad (2)$$

**2.2.1.4. Evaluation of pH and total soluble solids.** The pH was determined using a pH meter BASIC 2° CRISON, Spain, calibrated

with standard buffers at 6.89 and 4.01. A digital refractometer PAL-1, USA, calibrated with distilled water was used to determine total soluble solids (TSS). Each analysis was carried out in triplicate.

### 2.2.2. Rheological measurements

To perform the rheological measurements, sample preparation consisted on dispersing the required gellan powder into 20 g of puree, under stirring for 10 min on a magnetic plate stirrer at room temperature ( $22 \pm 2$  °C). Then, the sample was transferred to the rheometer plate where the jellification, gels maturation and mechanical properties determination took place. To study the effect of gellan concentration on the rheological properties of texturized mango puree, samples were prepared without gellan addition (control) and with 0.25, 0.5, 1.0 and 2 g of gellan per 100 g of puree, either using LA or HA gellan.

To evaluate the effect of the LA/HA ratio on the rheological properties of the mango puree, samples were prepared in the same way without gellan (control) and with 1 g of gellan per 100 g of puree at LA/HA ratios: 100/0 (LA), 75/25, 50/50, 25/75 and 0/100 (HA).

Small amplitude oscillatory shear measurements were performed with a controlled Stress Rheometer, RS 75 Rheostress HAAKE, Germany, connected to a thermostatic bath for the temperature control. The samples were placed between two parallel stainless steel serrated plates with a diameter of 35 mm, maintaining a constant gap of 1.0 mm. Liquid paraffin was applied along the border of the plates to prevent sample dehydration.

To evaluate the gel setting temperature, the experimental procedure consisted on subjecting the samples to 90 °C for 1 min, followed by temperature sweep of oscillatory measurement from 90 °C to 20 °C at a rate of 1 °C/min,  $f = 0.1$  Hz and  $\tau = 1.0$  Pa. In the case of HA gellan, the temperature range was between 95 °C and 20 °C in order to detect the gelling temperature. Immediately after the temperature sweep, time sweep of oscillatory measurements was used to investigate the change and the stability of the gels structure as a function of time. The maturation of the gels formed was monitored at 5 °C during 2 h at constant stress ( $\tau = 1.0$  Pa) and frequency ( $f = 0.1$  Hz).

The samples were tested over a range of shear stresses. The stress sweep was applied under oscillatory shear of  $f = 0.1$  Hz at 5 °C within a stress range between  $\tau = 10^{-1}$  and  $10^4$  Pa.

Afterwards, to determine the mechanical spectra, a new proportion of the same formulations were poured into the rheometer plate, first subjected to the temperature and time sweep tests described previously with the same oscillatory conditions. Frequency sweeps were conducted at 5 °C, for frequency ranging from  $10^{-2}$  to  $10^2$  Hz, with a constant shear stress within the linear viscoelastic region.

The frequency dependency was quantified from the  $\beta$  exponent values obtained by fitting the power law regression  $G'(G'') = \alpha f^\beta$  (Valdez, Acedo-Carrillo, Rosas-Durazo, Lizardi, & Goycoolea, 2006). Each experiment was carried out in triplicate and reproducible results were obtained.

### 2.2.3. Confocal laser scanning microscopy analysis

For confocal laser scanning microscopy analysis, mango bars were also prepared without gellan (control) and with 1g of gellan per 100 g of puree at LA/HA ratios: 100/0 (LA), 75/25, 50/50, 25/75 and 0/100 (HA).

The procedure consisted on transferring 80 g of the puree obtained into a 100 mL beaker, immersed in a boiling water bath (to prevent heat burn of the puree) on a heater/magnetic stirrer, and homogenizing it by stirring at  $134 \times g$ . When the puree reached a temperature of  $88 \pm 2$  °C, the required gellan gum was added, followed by increasing the rotation speed up to  $1640 \times g$  for 30 s in

order to have a good dispersion and dissolution of the gellan powder. Afterwards, for the non-covalent labelling, fluorescent probe Fluorescein (0.1 g fluorescein/500 g) was added under stirring. The sample was then placed into rectangular silicon moulds ( $W \times H \times L = 50 \times 10 \times 25$  mm) and allowed to set at room temperature ( $22 \pm 2$  °C), afterwards the samples were stored at 5 °C for another 30 min.

Confocal laser scanning microscopy (CLSM) of mango puree and thin slices of mango fruit bars with different LA/HA ratio was performed with a Leica TCS SP5, (Leica Microsystems, Mannheim, Germany) inverted confocal microscope using a  $63\times$  apochromatic water immersion objective lenses with a NA of 1.2 and an Argon laser for excitation. Fluorescence was analysed using a 488 nm excitation wave length and emission was collected between 510 and 670 nm. The fluorescent probe, Fluorescein standard (Fluka, USA) was used for the non-covalent labelling of the mango puree and mango puree/gellan gum mixtures. The autofluorescence of mango puree was detectable in the same conditions. Microscopic observations were made at 20 °C and digital image files were acquired with  $2048 \times 2048$  pixels for a typical sample area of  $246 \times 246$   $\mu\text{m}$ .

## 3. Results and discussion

### 3.1. Composition of mango puree

Among the minerals identified in mango puree, potassium was the one at higher concentration:  $157.6 \pm 2.3$  mg/100 g. The concentration of the other minerals was: calcium  $12.5 \pm 1.6$  mg/100 g; magnesium  $9.4 \pm 0.8$  mg/100 g; phosphorus  $8.4 \pm 2.3$  mg/100 g; sodium  $2.2 \pm 1.8$  mg/100 g and iron  $2.6 \pm 0.6$  mg/100 g. The ash content was  $300 \pm 25$  mg/100 g. The cation content of the mango puree and of both LA and HA gellan gum was enough to promote the gelation, as can be seen in the following sections. As such, there was no addition of external cations to the gellan/mango puree mixtures.

The water content was high ( $81 \pm 0.03\%$ ), therefore, the hydration of gellan was promoted without the addition of water.

The TSS content of the mango puree used was  $18.0 \pm 0.5$  °Brix whereas the pH was  $4.3 \pm 0.3$ . These values are in line with ideal conditions for the hydration of both LA and HA gellan: minimum pH value of 4 for LA gellan and TSS content below 40 °Brix for HA gellan (Philips & Williams, 2009, chap. 9).

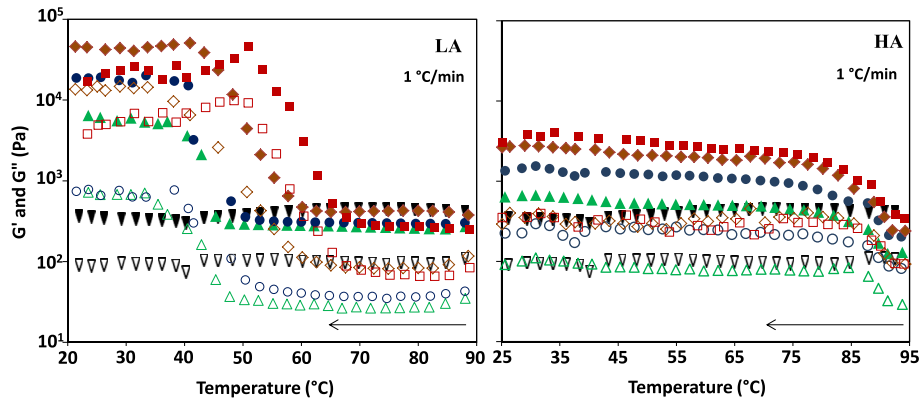
### 3.2. Viscoelastic properties of the control and mango puree/gellan systems

This section is focused on the kinetics of gel network formation bearing in mind the different physicochemical characteristics of the two types of gellan referred in the literature (Huang, Tang, Swanson, & Rasco, 2003; Mao, Tang, & Swanson, 2000).

#### 3.2.1. Effect of concentration and type of gellan on the gelation kinetics and viscoelastic properties of texturized mango puree

The effect of type of gellan and its concentrations on the variation of the storage ( $G'$ ) and loss ( $G''$ ) moduli with temperature of mango puree/gellan mixtures is shown in Fig. 1.

Temperature sweeps indicated that all evaluated samples exhibited  $G'$  larger than  $G''$  in whole temperature range, with no crossover between the moduli. The initial gelation temperature was defined as the one at which an increase of both moduli is observed, around  $45 \pm 1.2$  °C for 0.25 g/100 g of LA and  $64 \pm 1.0$  °C for 2 g/100 g of LA.  $G'$  and  $G''$  continued to increase with decreasing temperature achieving a maximum value equivalent to the final gelation temperature around  $37.0 \pm 1.3$  °C for 0.25 g/100 g of LA and



**Fig. 1.** Temperature sweeps of mango puree with different LA and HA gellan concentration, full symbols – storage modulus ( $G'$ ), open symbols – loss modulus ( $G''$ ); ( $\blacktriangledown$ ) control, ( $\blacktriangle$ ) 0.25 g/100 g, ( $\bullet$ ) 0.5 g/100 g, ( $\blacklozenge$ ) 1 g/100 g, ( $\blacksquare$ ) 2 g/100 g.

50 ± 1.2 °C for 2 g/100 g of LA. The initial and the final gelation temperatures became higher as the gellan concentration increased. In fact, the increase in polymer concentration offers more facility to establish intermolecular interactions, which is also in correlation with the increase of the dynamic moduli. For all gellan concentrations, the values of the moduli tend to a plateau after reaching a maximum value; this may be attributed to the formation of a fully developed internal network. It is interesting to note that a decrease of  $G'$  and  $G''$  for temperatures below 50 °C for mango puree in the presence of 2 g/100 g of LA gellan was observed (Fig. 1). This can be justified by the presence of fracture within the sample or slippage between the sample and the measuring geometry. Richardson and Goycoolea (1994) observed similar a behaviour with deacylated konjac glucomannan where the values of  $G'$  and  $G''$  decreased during gelation. Further experiments are needed in order to confirm the presence of fracture and/or slippage.

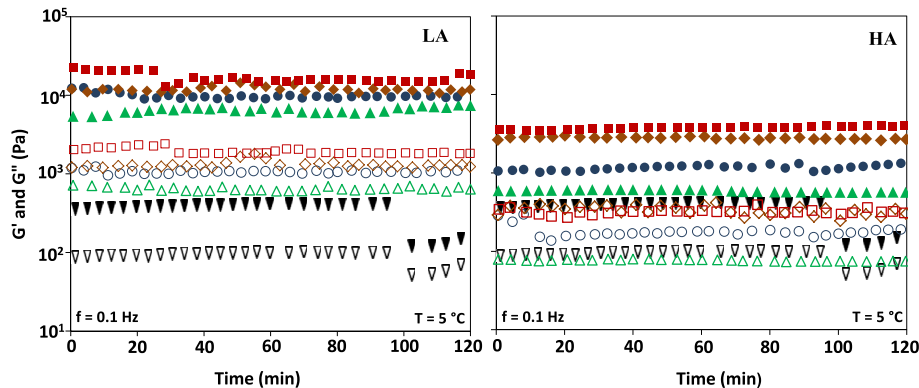
The pectin molecules present in the mango puree are expected to play an important role on the gel formation kinetics and on the final gel characteristics. This may happen because both LA gellan and high methoxyl pectin form gels which properties are influenced by the presence of cations (e.g. calcium) (Basu & Shivhare, 2010; Miyoshi & Nishinari, 1999). Cations may suppress the electrostatic repulsions between the negative charge of pectin and gellan respectively, promoting gelation, and eventually the formation of a denser polymer network. However, further studies strictly focused on the interactions between both gellan types and pectin molecules in the presence of calcium are needed, to fully

comprehend how the tridimensional carbohydrate network is formed within the complex system that is the mango puree.

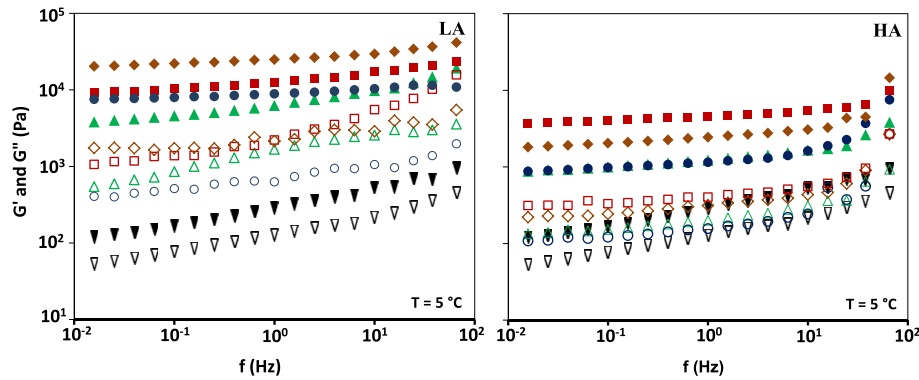
When HA gellan was used as gelling agent, a slight increase of the moduli was noticed around 90 °C corresponding to the gelation temperature. A well-defined plateau is perceived for temperatures below 65 °C for all HA gellan concentrations tested, with the values of the moduli becoming higher as the polymer content increased (Fig. 1). For mango puree/HA systems,  $\text{Ca}^{2+}$  didn't play a similar role as in the case of LA gellan. More acyl groups lead to a greater inter-chain repulsion, therefore, less dense network formation with reduced values of the moduli was noticed (Basu & Shivhare, 2010).

Immediately after the temperature sweep, samples were cooled down to 5 °C at a rate of 1 °C/min and the gel maturation kinetics of the samples were monitored through the evolution with time of  $G'$  and  $G''$  moduli (Fig. 2). All the formulations with gellan gum reached the steady-state revealing constant values of both moduli. This information indicates that the gels formed have a stable and fully developed structure in 30 min of maturation. This fact is quite important, because it may increase significantly the fruit bars production rate at an industrial scale.

The structure of the mango puree (control) changes with time, the dynamic moduli shows a tendency to decrease after almost 90 min of maturation. This may be attributed to the thixotropic properties of mango puree where the increasing degree of structural breakdown with time is due to shearing (Bhattacharya, 1999). For this type of soft solids, rheological properties change not only with shear stress but also with time of shearing (Tiu & Boger, 1974).



**Fig. 2.** Time sweeps of mango puree with different gellan concentration, full symbols – storage modulus ( $G'$ ), open symbols – loss modulus ( $G''$ ); ( $\blacktriangledown$ ) control, ( $\blacktriangle$ ) 0.25 g/100 g, ( $\bullet$ ) 0.5 g/100 g, ( $\blacklozenge$ ) 1 g/100 g, ( $\blacksquare$ ) 2 g/100 g.



**Fig. 3.** Frequency sweeps of mango puree with different gellan concentration, full symbols – storage modulus ( $G'$ ), open symbols – loss modulus ( $G''$ ); ( $\blacktriangledown$ ) control, ( $\blacktriangle$ ) 0.25 g/100 g, ( $\bullet$ ) 0.5 g/100 g, ( $\blacklozenge$ ) 1 g/100 g, ( $\blacksquare$ ) 2 g/100 g.

**Fig. 3** shows the mechanical spectra of the mango puree (control) and texturized samples with different gellan concentrations. For the control,  $G'$  and  $G''$  increased with increasing frequency with a high frequency dependence, where  $G'$  values are greater than  $G''$  values through the whole range of frequencies studied. The ratio of  $G''$  to  $G'$  ( $\tan \delta$ ) is greater than 0.1 (results not shown) meaning that the mango puree could be characterized as having a behaviour of a weak gel. This type of behaviour has been reported by other authors for mango pulp (Iagher, Reicher, & Ganter, 2002) and rice flour dispersions (Chun & Yoo, 2004).

In the presence of gellan, all formulations exhibited a typical strong gel spectra, i.e.  $G'$  was always greater than  $G''$  with a slight increase of the moduli as the frequency increased. In particular, both  $G'$  and  $G''$  increased as LA concentration increased, indicating a reinforcement of the gel structure. The frequency dependence of the dynamic moduli tended to decrease as the LA concentration increased, which was quantified from the  $\beta$  exponent values obtained by fitting the power law regression to experimental data (Table 1). Similar behaviour was observed for samples with HA gellan. For identical concentrations of LA and HA, lower dynamic values were observed for HA gels, which is consistent with their softer structure.

Overall, the addition of either LA or HA gellan to the mango puree improved significantly the viscoelastic properties, from the unstable and weak gel structure of the mango puree to a fully developed network with higher dynamic modulus and small frequency dependence.

Based on the mechanical spectra obtained for LA or HA/mango puree mixtures, the concentration of 1 g/100 g enables the formation of gels with better viscoelastic properties than those with 0.5 g/100 g of gellan. The values of the moduli for 1 g/100 g LA/mango puree mixtures were slightly higher than 2 g/100 g LA/mango puree, probably due to an incomplete hydration of gellan gum when 2 g/100 g LA was used. The water and cations content of the puree, along with the time of heating at 90 °C, may not have been enough to completely hydrate the polymer, with consequences on the viscoelastic properties of the gel formed. Therefore, the gellan

concentration of 1 g/100 g was selected to study the effect of LA/HA blends on the viscoelastic properties of the texturized product.

### 3.2.2. Effect of LA/HA ratios on the gelation kinetics and viscoelastic properties of mango puree

A synergistic interaction exists between the native and deacylated gellan molecules upon gel formation when both gellan types are present in the system. Mao et al. (2000), reported that the weight ratio of LA/HA played a more important role in the textural properties of mixed gellan gels than the overall polymer concentration. Moreover, Morris, Gothard, Hember, Manning, and Robinson (1996) studied the LA/HA mixtures by Differential Scanning Calorimetry and observed two separate conformational transitions at temperatures characteristic of individual components. As such, there is the evidence of the establishment of zones within the gel network composed mainly by LA or HA molecules, connected to each other, enabling overall texture properties that cannot be obtained with only one type of gellan.

In order to design a wider range of mango bar samples with dissimilar textural properties, formulations containing varied LA/HA ratios, maintaining constant the overall polymer concentration (1 g/100 g) were studied. The temperature sweeps of mango puree and LA/HA mixed gels (Fig. 4) presented similar behaviour to those described in Section 3.2.1. All the formulations showed  $G'$  larger than  $G''$  in the whole temperature range studied. The initial gelation temperature for LA/HA gellan blends was around  $60 \pm 1.1$  °C for 75/25,  $53 \pm 1.3$  °C for 50/50 and  $50 \pm 1.0$  °C for 25/75.  $G'$  and  $G''$  continued to increase with decreasing temperature reaching a maximum value equivalent to the final gelation temperature,  $31 \pm 1.1$  °C for 75/25,  $41 \pm 1.0$  °C for 50/50 and  $40 \pm 1.1$  °C for 25/75.

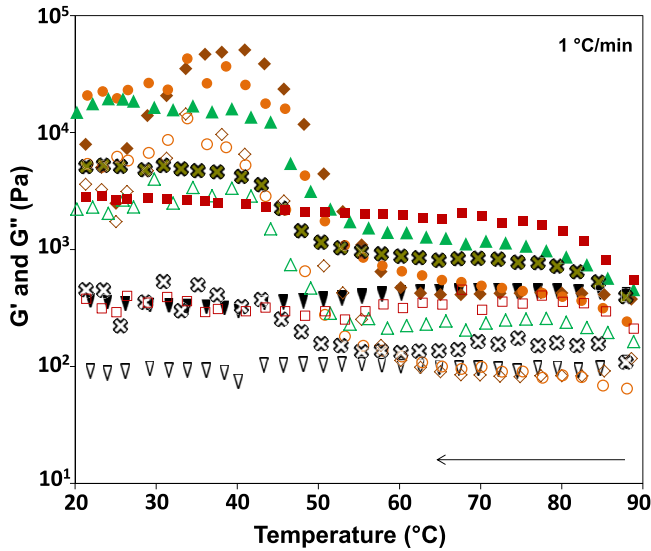
Time sweeps at  $T = 5$  °C carried out immediately after the temperature sweeps, are presented in Fig. 5. All samples exhibited constant dynamic moduli during the entire time range studied showing rapid gel maturation.

**Fig. 6** shows the mechanical spectra of mango puree gels prepared with the LA/HA blends. The increase of LA/HA ratio led to the increase of both  $G'$  and  $G''$  while maintaining their slight dependency with the frequency, as illustrated by the small variation of  $\beta$  exponent values,  $0.06 < \beta (G') < 0.09$  and  $0.11 < \beta (G'') < 0.13$  (Table 2). For all ratios used, texturized mango puree became less dependent with the frequency than the control. These mechanical spectra indicate gels with intermediate viscoelastic properties between those obtained with only LA or HA gellan.

Pectic polysaccharides are one of the prominent structural constituents in the mango fruit (Tharanathan, Yashoda, & Prabha, 2006) and together with gellan gum can contribute to the stable

**Table 1**  
 $\beta$  Values of the power law regression for different gellan concentrations.

Gellan (g/100 g)	LA		HA	
	$\beta (G')$	$\beta (G'')$	$\beta (G')$	$\beta (G'')$
0	0.23	0.23	0.23	0.23
0.5	0.16	0.21	0.06	0.09
1	0.10	0.12	0.10	0.09
2	0.11	0.22	0.04	0.10



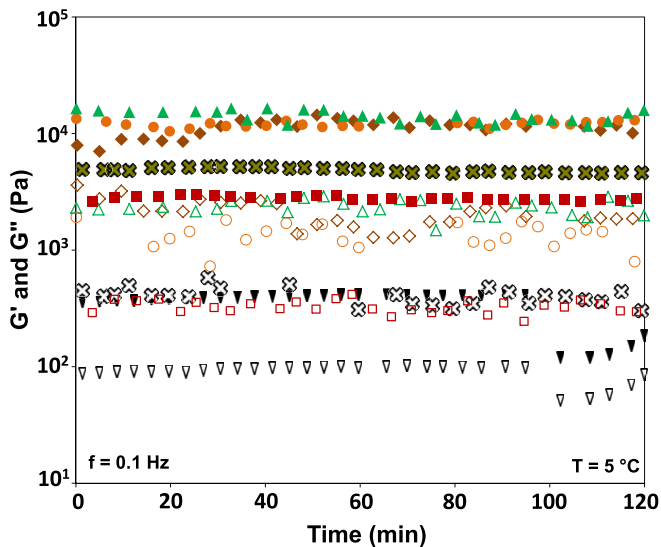
**Fig. 4.** Temperature sweeps of mango puree with LA, HA and different LA/HA gellan ratios for a fixed overall gellan concentration (1 g/100 g); full symbols – storage modulus ( $G'$ ), open symbols – loss modulus ( $G''$ ); (▼) control, (◆) LA, (●) 75/25, (▲) 50/50, (⊗) 25/75, (■) HA.

and well developed structure for the texturized mango puree. The addition of both gellan gum types into the mango puree overcomes the thixotropic character of the mango puree.

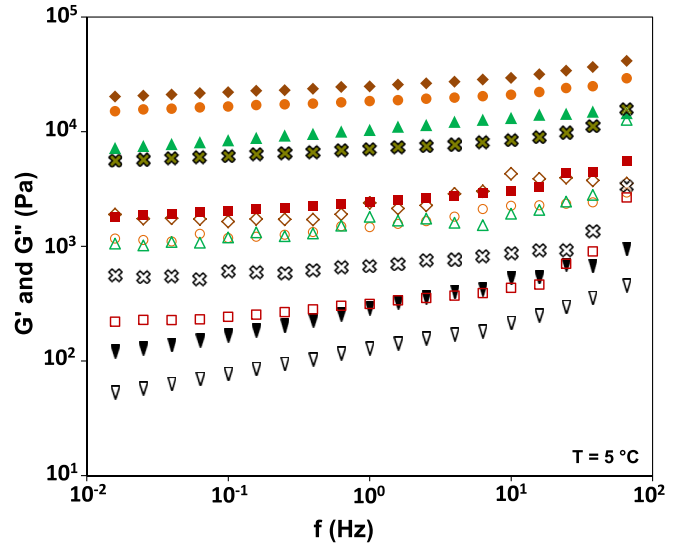
Overall, the results obtained from the small amplitude oscillatory shear measurements suggested that it is possible to create jellyfied mango bars, either by using different ratios of LA/HA gellan or LA and HA alone.

3.3. Confocal laser scanning microscopy observations

The structural organisation of the mango puree and mango bars was visualized by confocal laser scanning microscopy (CLSM). Microscopic observations of the mango puree with and without a fluorescent probe and that of stained mango puree/gellan gum



**Fig. 5.** Time sweeps of mango puree with LA, HA and different LA/HA gellan ratios for a fixed overall gellan concentration (1 g/100 g); full symbols – storage modulus ( $G'$ ), open symbols – loss modulus ( $G''$ ); (▼) control, (◆) LA, (●) 75/25, (▲) 50/50, (⊗) 25/75, (■) HA.



**Fig. 6.** Frequency sweeps of mango puree with LA, HA and different LA/HA gellan ratios for a fixed overall gellan concentration (1 g/100 g); full symbols – storage modulus ( $G'$ ), open symbols – loss modulus ( $G''$ ); (▼) control, (◆) LA, (●) 75/25, (▲) 50/50, (⊗) 25/75, (■) HA.

mixtures (1 g of gellan per 100 g of puree) with LA, HA and LA/HA (75/25, 50/50 and 25/75) ratios were performed at 20 °C.

Mango fruit is rich in dietary fibre (DF), such as insoluble dietary fibre (IDF): cellulose, hemicellulose, lignin; and soluble dietary fibre (SDF) mainly pectin (Tharanathan et al., 2006; Vergara-Valencia et al., 2007). Many publications have confirmed that different plant living cells containing insoluble dietary fibre (IDF) might exhibit intrinsic fluorescence (Roshchina, 2003). Fig. 7A show the microstructure of unstained mango puree with relatively weak intrinsic fluorescence where less aggregates with a bright green colour (in the web version) can be detected corresponding probably to the insoluble dietary fibre (IDF). When fluorescein was added to the samples, a contrast by differences in fluorescence, with intense fluorescent zones equivalent to several insoluble carbohydrates and less intense fluorescence zones, corresponding to the overall gel network of the mango bar may be observed.

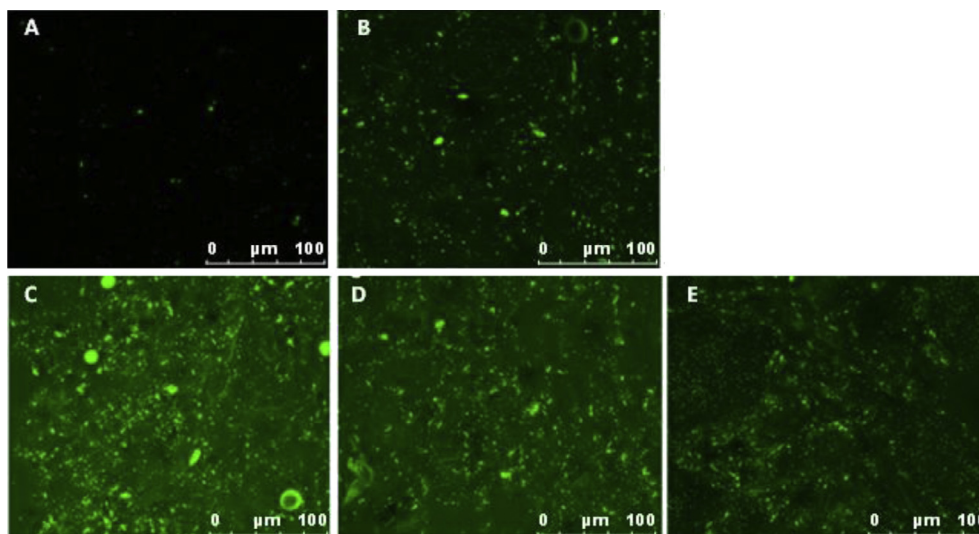
For samples with LA, and, the mixture LA/HA 50/50, the distribution observed shows a similar pattern (Fig. 7C and D), resulting from the presence of aggregates surrounded by a continuous fluorescent phase, although there are large zones irregularly spread over the focal plane. This type of structure was also observed in the samples with formulations LA/HA 75/25 and 25/75 (results not shown) and represents a relatively homogeneous and well aggregated system.

The addition of 100% HA gellan to the mango puree resulted in a major change in the structural organisation of the sample. Fig. 7E shows a less dense network of the fluorescent mango puree/HA sample, indicating a weaker gel structure. Fig. 7B, corresponding to the stained mango puree, also shows a weak, less structured and deficiently aggregated gel structure. These results are in agreement

**Table 2**

$\beta$  Values of the power law regression for polymer at 1 g/100 g with different LA/HA ratios.

LA/HA ratio	$\beta$ ( $G'$ )	$\beta$ ( $G''$ )
100/0	0.06	0.12
75/25	0.06	0.11
50/50	0.10	0.09
25/75	0.07	0.08
0/100	0.09	0.11



**Fig. 7.** CSLM micrographs; Mango puree (control): A – autofluorescence, B – containing non-covalently labelled fluorescein; Mango purée with (1 g/100 g) gellan gum containing non-covalently labelled fluorescein C – LA, D – LA/HA 50/50, and E – HA gellan.

with rheological measurements where lower values of  $G'$  and  $G''$  moduli were observed for these two systems. Clearly, the addition of LA gellan to the mango puree, results in more homogeneous and well aggregated systems which corresponds to the desirable structure for the product under development.

#### 4. Conclusions

In this work, the gel forming kinetics and gel properties of gellan gum/mango puree mixtures were studied. The results show that chemical characteristics of the mango puree in terms of cations and water content, pH and TSS content are compatible with the hydration and gelation conditions required for the gelling agent. As referred in the literature (Bhattacharya, 1999; Iagher et al., 2002), mango puree shows thixotropic behaviour by decreasing the moduli with the lapse of time. The addition of LA, HA or LA/HA ratios to the mango puree improved its structure and produced texturized mango puree with interesting viscoelastic properties. Low maturation time (30 min) was needed for both LA and HA to reach a fully developed gel structure. For 1 g/100 g gellan concentration, LA gellan forms stronger structures than HA gellan. By using different LA/HA ratios for the same overall gellan concentration (1 g/100 g), it was possible to produce texturized mango puree samples with intermediate viscoelastic properties, with a progressive rise of the dynamic moduli as the LA content increased.

CLSM observations also highlighted the formation of a denser structure for gels obtained with 100% LA and LA/HA in different ratios, having the gels with only HA a more loosened structure. The overall results obtained suggest that it is possible to create mango bars structures with interesting designed rheological and microstructural properties by using LA/HA gellan mixtures. For the final development of the mango bars, these properties still need to be correlated to inputs from texture analysis and sensory evaluation as well as further microscopy techniques at smaller distance scales in order to obtain more microstructural and morphological information about these systems.

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