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Graphical abstract

TITLE: Effect of deacetylation on the glucomannan gelation process for making
restructured seafood products.

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Abstract

This paper focuses on the relationship between network structure and physicochemical and rheological properties of aqueous glucomannan dispersions (AGD) as a function of pH, to establish optimal conditions of glucomannan gelation for making restructured seafood products. Various lots of AGD were prepared from 3% (L1) and 5% (L2) konjac glucomannan adding different amounts (0.5-5%) of 0.6N KOH to obtain samples with successively increasing degrees of alkalinity, from pH= 8.9 to =11.9 (samples L1.1-L1.6 for 3% AGD) and from pH= 7 to 11.4 (samples L2.1-L2.6 for 5% AGD). The spectra of the different AGDs were obtained by Fourier Transform Infrared spectroscopy (FT-IR) to quantify the deacetylation ratio, whose effect on the physicochemical, mechanical (puncture), viscoelastic (at both small and large time scales) and structural characteristics (scanning electron microscopy (SEM)) was analysed. A linear dependence was found between the relative area of acetyl bands of AGD and pH, showing a discontinuous region in the function or gap zone between pHs 9.3 (L1.2) and 9.8 (L1.3) for 3% AGD and between 9.2 (L2.3) and 10.7 (L2.4) for 5 % AGD. Samples before the gap zone (L1.2 and L2.3) were gels of varying degrees of weakness, becoming strong gels thereafter. The gelation conditions were best at pH~10.7 for both 3 and 5% AGDs, corresponding to high and moderate deacetylation ratio, respectively. The resulting gels possessed elastic, cohesive and time-stable networks and thus formed basic structures able to contain several ingredients for making restructured seafood products. The SEM photographs corroborated the physicochemical and rheological characteristics.

Keywords FT-IR, deacetylation ratio, restructured seafood products, glucomannan gels

1. Introduction

Restructured seafood products offer a means of using muscle by-products and developing new products that have different textures and can also contain functional ingredients. These structures are usually formed by thermostable protein gelation, but such gelation is not possible when the muscle has been previously processed and the protein is degraded. In previous works (Beatriz Herranz, Borderias, Solo-de-Zaldívar, Solas, & Tovar, 2012; B. Herranz, Tovar, Solo-de-Zaldivar, & Borderias, 2012), the authors proposed the use of Konjac glucomannan (KGM) as a thermostable gelling agent for restructuring. For that purpose an aqueous glucomannan dispersion (AGD) could be mixed with minced muscle after alkaline deacetylation to obtain thermostable gels with a texture and flavour similar to those of seafood muscle. This kind of thermostable gel is a three dimensional network, with different physical gel characteristics depending on the kind of processing. For instance, it is accepted (Maekaji, 1974; Williams, et al., 2000) that the addition of alkali strongly diminishes steric hindrance caused by the acetyl groups, causing the formation of non covalent cross-links among junction zones. The number, size and position of junctions can fluctuate with time and temperature, producing transient networks (Ross-Murphy, 1995; Herranz, Borderias, Solas, & Tovar, 2012c) involving non-covalent bonds such as hydrogen bonds, hydrophobic interactions, ionic bonds, etc., which under specific conditions may behave as permanent cross-links (Lapasin & Pricl, 1999). Hydrogen bonds in particular are considered the main interactions responsible for gel formation, although hydrophobic interactions, which gain in importance with increasing deacetylation, also seem to play an important role (Chen, Li, & Li, 2011). Moreover, when pH increases, the resulting anionic groups can form KGM chains that can change the structural function of water within the network, modifying the final properties of KGM gels. Specifically, Herranz et al. (2012a) reported differences among electrostatic interactions such as ion-dipole, depending on the type and concentration of alkali, e.g. among cations (Na^+ and K^+) with OH groups of KGM chains, and with water molecules, on mechanical and viscoelastic properties of KGM gels. The authors found that 0.6N KOH was the most suitable alkali to deacetylate KGM, thus producing KGM gels with more elastic and time-stable networks. However, the effect of pH on the deacetylation ratio and its influence on the physical properties of resulting samples has not yet been studied. This aspect is of paramount importance for designing gels with adequate textural characteristics for the particular technological purpose. In the present paper, the authors propose that strong gels capable of retaining a non-functional minced muscle as a filler and with a texture and appearance

as similar as possible to real seafood muscle, which can also be heated, would be a real solution to upgrading many different muscle by-products remaining after processes that render the muscle remain non-functional.

The objective of this study was to evaluate the effect of the percentage of acetyl groups released from KGM chains, as measured by FT-IR, on the physicochemical, mechanical, viscoelastic and the microstructural characteristics of KGM gels. The practical aim was to optimize KGM gelation conditions in line with KGM concentration, for use in the manufacture of restructured seafood products.

2. Materials and methods

2.1. Preparation of samples

3 and 5 % (w/v) aqueous glucomannan dispersions (AGD) from konjac glucomannan (Glucomannan purity 100%, Guinama, Valencia, Spain) were prepared according to the methodology previously described in Herranz et al. (2012a). For KGM gelification, 0.6N KOH (Panreac Química, S. A., Barcelona, Spain) was the alkali used to raise the pH of AGD from around 6 to around 11.9 (3% AGD) and 11.4 (5% AGD). After setting for 1 hour at 30°C and 5 hours at 5°C, they were removed from cylindrical containers (diameter 3 cm x height 3.5 cm) and Petri dishes, and placed in citrate-phosphate buffer at pH 5 (the gel:buffer proportion was 1:10) for 20 hours at 5°C in order to bring the pH of the gels down to 6.5-6.8.

The different lots of gels were prepared with 3 and 5% AGD (L1 and L2 respectively) and increasing amounts of 0.6N KOH (from 0.5 to 6%) to reach a set of pH values for both lots, which were designated as shown in Table 1.

An aliquot of each sample was frozen (-80°C) and freeze dried using a VirTis Benchtop-6KB freezer (Gardiner, NY, USA), for further analysis by FT-IR spectroscopy analysis.

2.2. Analyses

2.2.1. pH and moisture

The pH was measured using a model 9165BNWP pH probe (Analítica Instrumental, S.A., Barcelona) inserted in the gel. The pHmeter was an Orion model 720A (Analítica Instrumental, S.A., Barcelona). The pH was measured after homogenization of AGD for 3 minutes immediately after addition of the necessary amount of 0.6N KOH.

Water content was determined by drying the sample to constant weight at 110°C. The results are expressed as a percentage (AOAC, 2000).

Both analyses were done in triplicate

2.2.2. Fourier Transform IR Spectra Measurement or FT-IR spectroscopy analysis

AGD (3% and 5%) was mixed with different proportions of a solution of 0.6N KOH (0, 0.5, 1, 2, 3 and 4(w/v)) to achieve different pHs and analysed by FT-IR. This range of pH values for KGM gelation was based on the reports by Thomas (1997) (pH values of 9-10), and Kohyama and Nishinari (1990) (pH values of 11.3- 12.6). In this case other lower pHs were assayed to observe the acetyl band in FT-IR spectra and its gradual disappearance with increasing pH (or alkaline addition). The freeze-dried samples were dispersed in the agate mortar. Fluorolube was used as a matrix for dispersion of samples; this only fits absorption bands in frequency range above 1360 cm^{-1} except at 2321.9 cm^{-1} , and therefore it does not interfere in the observation of the bands that were of relevance to this study. Once a very homogeneous paste had been obtained, a small quantity was placed between CaF_2 crystals; these were mounted on the supports and transmission measured in the FTIR. Prior to sample measurement, spectrophotometry was prepared by running a "background" air absorption spectrum and a Fluorolube IR spectrum, which were later subtracted from the samples to avoid potential interference.

In all cases, IR spectra were recorded by accumulation of at least 32 scans, with a resolution of 2 cm^{-1} in a frequency range of about 4000 to 100 cm^{-1} (mid-infrared spectroscopy). Measurements were carried out in triplicate. The spectral data were processed with the Grams /AI (Thermo Electron Corporation, Waltham, MA) software, which includes baseline correction, smoothing (with a nine-point Savitsky-Golay function) to reduce the noise, and band area measurement.

Because the intensity of the absorption is proportional to the concentration of the adsorbing species, quantitative analysis is possible by FT-IR spectroscopy (Wilson, Slack, Appleton, Sun, & Belton 1993)). The area under the acetyl band was measured relative to the area of the CH band. In this way, the pH of the sample was associated with the relative intensity of the acetyl bands and hence with the percentage of acetyl in the KGM chains.

Three independent spectra were taken from each sample and the area of interest was measured. The spectral data were processed with the Grams /AI (Thermo Electron Corporation, Waltham, MA) software and smoothed using the Savitsky-Golay algorithm with nine points to reduce the noise. Baseline corrections were done with a non-automatic function (multipoint correction) and smoothing with a nine-point Savitsky-Golay function to reduce the noise. Carbonyl stretching vibration ($\text{C}=\text{O}$) in Acetyl groups at $\sim 1730\text{ cm}^{-1}$, and

–CH stretching vibration at $\sim 2920\text{ cm}^{-1}$ bands area, were measured for each spectrum. The average relative areas and the mean value were then obtained and plotted versus pH by linear regression to each AGD concentration.

2.2.3. Water binding capacity (WBC)

Neutralized gels were cut into small pieces (2 g) and placed in a centrifuge tube (diameter 10 mm) with a filter paper (2 filters Whatman n° 1, diameter 90 mm). The samples were then centrifuged in a Heraeus Multifuge 3L-R (Kendro Laboratory Products, Germany) for 10 min at 3000g and room temperature. WBC was expressed as percent water retained per 100 g water in the sample prior to centrifuging. Measurements were carried out in triplicate.

2.2.4. Colour measurement

The lightness parameter (L^*) was measured five times on the surface of the gel using a colorimeter (Minolta Chroma Meter Cr-200, Japan). The colorimeter was standardized using a white calibration plate. Colour determinations were performed. Determinations were carried out on five surface points of each sample.

2.2.5. Puncture tests

Cylindrical samples (diameter 3 cm x height 3.5 cm) were pierced to breaking point using a TA-XTplus Texture Analyser (Stable Micro System Ltd., Surrey, UK) with a 5 mm–diameter round–ended metal probe. Crosshead speed was 1 mm/s, and a 5 kg load cell was used. The load as breaking force (BF) and the depth of depression as breaking deformation (BD) when the gel sample lost its strength and ruptured were recorded. The measurements were carried out at least in sextuplicate.

2.2.6. Dynamic rheometry measurements

SAOS tests were performed using a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ). The measurements were obtained using parallel-plate geometry (20 mm diameter and 1 mm gap). Before measurement, the gels were tempered at ambient temperature and cut from Petri dishes into disk-shaped slices 20 mm in diameter and 1 mm thick with a 570 S.T.E slicing machine (Germany). They were then put on the lower plate of the rheometer for measurement at 25°C. Any excess sample protruding beyond the upper plate was carefully removed. Samples were allowed to rest

for 15 min before analyses to ensure both thermal and mechanical equilibrium at the time of measurement. Samples were covered with a thin film of Vaseline oil (Codex purissimum) to avoid evaporation. The temperature was controlled to within 0.1°C by a Peltier element in the lower plate which was kept at 25.0°C.

Stress sweep tests

To determine the linear viscoelastic (LVE) region, stress sweeps were run at 6.28 rad/s at 25°C with the shear stress (σ) of the input signal varying from 0.24 to 1000 Pa. 300 points on the continuous mode were used in all instances. Changes in storage modulus (G'), loss modulus (G'') and loss tangent ($\tan\delta$) were recorded. The critical (maximum) values of shear strain (γ_{max}), and shear stress (σ_{max}) on the limit of LVE range were derived by the method previously described in Campo-Deaño and Tovar, (2009).

Frequency sweep tests

Samples were subjected to stress that varied harmonically with time at variable frequencies from 10 to 0.1 Hz. The strain amplitude was set at $\gamma = 0.5\%$ within the LVE range.

Creep and recovery tests

An instantaneous stress σ_0 (30 Pa) corresponding to 0.5% shear strain within the LVE range was applied for 600 s in the creep tests and the resulting change in strain over time $\gamma(t)$ was monitored. When the stress was released, some recovery was also observed for 600s. The creep and recovery results were described in terms of the shear compliance function, $J(t) = \gamma(t)/\sigma_0$. Compliance curves generated at different linear stress levels overlap, making it possible to examine and compare the structural properties of the different food gels on larger time scales (Steffe, 1996).

All viscoelastic measurements were carried out at least in quintuplicate.

2.2.7. Scanning electron microscopy (SEM)

The 3% KGM gels, pH 9.8 (L1.3) and 10.8 (L1.4) of 3% KGM gels, and the 5% KGM gels, pH 10.7 (L2.4) and 11 (L2.5) were first subjected to a frequency sweep, then 2-3 mm cubes were cut from them for microscopic examination. The samples were then fixed (1:1 v/v) in formaldehyde (4%) and glutaraldehyde (0.2%) in 0.1M phosphate buffer (pH 7.3) and post-fixed with OsO_4 , ashed and dried in increasing concentrations of acetone, and

critical-point dried as described by Moreno, Cardoso, Solas, and Borderias (2009). They were then sputter-coated (Balzer, SCD004) with gold/palladium and examined in a Jeol Scanning Microscope (Jeol, JSC 6400, Akishima, Tokyo, Japan), at 20 kV.

2.2.8. Statistical analyses

Statistical analysis was carried out using Microsoft Excel software. Data are presented as mean values of at least five independent batches and were tested for each experiment with expanded uncertainty limit (EUL) data as the maximum and minimum deviation from the respective mean value. Trends were considered significant when means of compared sets differed at $p < 0.05$ (Student's t-test).

3. Results and discussion

3.1 FT-IR spectroscopy analysis

The FTIR spectra of 3 and 5% AGD with and without different amounts of added alkali solution (0.6N KOH) are shown in Figure 1.

In the IR spectra of samples the broad absorption band was around 3415cm^{-1} and was attributed to the stretching vibration of O–H groups (Clark and Hester 1978). This absorption band broadened and shifted to a lower wave number with increasing alkali, indicating a gradual increase of intermolecular hydrogen bonding between KGM chains.

The strong bands at about 2920cm^{-1} and 2850cm^{-1} correspond to the asymmetric and symmetric $-\text{CH}_2$ stretching modes respectively and the $-\text{CH}_2$ bend band that appears at approximately 1420 cm^{-1} (Vasconcelos, Nunes, & Vasconcelos, 2012). The band at 1730cm^{-1} is due to the stretching of $\text{C}=\text{O}$ of the carbonyl of acetyl groups in pure KGM. The band at 1645 cm^{-1} , which is attributed to in-plane deformation of the water molecule (Zhang, et al., 2001), reveals water molecule clusters with moderately strong hydrogen bonding with the network (Shen & Wu, 2003; Zhang, et al., 2001)). This band could therefore indicate the presence of intermolecular hydrogen bonds (Damodaran, 1997) established at the time of hydration.

In FTIR spectra from 3% AGD without alkali (L1.0) and treated with small quantities of alkali (L1.1 and L1.2), medium-high intensity acetyl bands in the range of 1730 cm^{-1} were found (Fig.1-a); these gradually disappeared, indicating a considerable decrease of the acetyl groups when the percentage of alkali increased (L1.3-L1.5). The deacetylation ratio was estimated as a ratio: (acetyl groups removed: total acetyl groups) $\times 100$. In the case of

3% KGM, the ratios were: sample with 1% alkali (L1.2), 26% deacetylation; 2% (L1.3), almost 75%; 3% (L1.4), 95%; and sample with 4% alkali the AGD was totally deacetylated. The 5% AGD obviously needed more 0.6N KOH to achieve more than 90% deacetylation. For 5% AGD, the ratios were: sample with 2% alkali (L2.3), 23%; 3% (L2.4); 58%; 4% (L2.5), 91%. 6% alkali (L2.6) was needed achieve total deacetylation of AGD.

The acetyl band in FTIR spectra was barely perceptible within the pH range 10.8–11.4 in the case of 3 % AGD and 11.0–11.4 in the case of 5 % AGD. Within these pH ranges the percentage of acetyl released by KGM reached the point where structural changes take place, producing a gel suitable for our practical purposes. These are analysed in subsequent sections. The acetyl band was imperceptible and the area was not measurable within the spectrum of 3% AGD at pH 11.9 (L1.6).

As a consequence of the alkali addition there was also a significant increase in the intensity of the band in the region $\sim 1560\text{cm}^{-1}$ (Figure 1), which may be attributed to the acetate anion ($\text{CH}_3\text{-COO}^-$) since this band was not found in L1.0 and L2.0 (AGD without alkali) at concentrations of either 3 or 5% AGD, respectively. The intensity of this characteristic band (1560 cm^{-1}) increases with increasing pH, i.e. when KGM chains are being deacetylated. The $\text{CH}_3\text{-COO}^-$ anion has two resonance forms which are both equivalent, providing more energy stabilization for the acetate group (Allinger, 1988). The energy of this resonance hybrid is less, so that the acetate group can be recognized by its vibration frequency (1560 cm^{-1}), which is lower than that of carbonyl (1730 cm^{-1}) in the acetyl group prior to deacetylation.

The increase of KGM concentration did not influence the characteristic position of different FTIR bands, as the IR spectra were similar at 3% and 5% KGM.

Figure 2 shows the linear dependence between the relative area of acetyl bands of AGD and pH. There was a visible zone of sharp discontinuity (gap zone) in the linear trend between pHs 9.3 (L1.2) and 9.8 (L1.3) in the case of 3% AGD and, 9.2 (L2.3) and 10.7 (L2.4) in the case of 5 % AGD. These gap zones showed the pH ranges that induced faster alkaline deacetylation and separated the samples in terms of the physical, rheological and structural properties of interest in this study. The increase in the absolute value of the slope at both KGM concentrations indicates that per cent deacetylation was more sensitive to alkalinity level after the gap region, although this had no real impact at rheological level since the gel was already formed.

3.2. Influence of the deacetylation ratio on moisture, WBC and colour

All KGM gel samples showed very high moisture content (96-97%) indicating the ability of KGM to capture up to 100 g of water per g of sample (Koroskenyi & McCarthy, 2001).

Given that the purpose of this gel is to retain the texture in a network containing the muscle, and given that the WBC of this muscle is very low, these high WBC values of deacetylated KGM are essential to bind enough water to preserve juiciness during any heating process, e.g. cooking.

Table 2 shows the water binding capacity (WBC) of samples L1.3-L1.5 and L2.3-L2.5 together with lightness (L^*) values of samples L1.1-L1.5 and L2.1-L2.5.

WBC values were all greater than or equal to 71% in weight (Table 2). In samples L1.4, L1.5 and L2.5, where the deacetylation ratio exceeded 90%, WBC rose significantly. When pH increased, so did the quantity of K^+ cations and OH^- anions in the KGM network, thus raising the density of the electrostatic charge and strengthening the ion-dipole interactions. This electrostatic charge reduced the mobility of the water molecules and facilitated the formation of clusters by hydrogen bonding among water molecules themselves and with OHs of KGM.

The Lightness (L^*) values of gels were greater than 44 in all cases. This is similar to the value recorded in a white seafood muscle (Sánchez-Alonso, Careche, Moreno, González, & Medina, 2011), which is interesting if these gels are intended for use in seafood restructurates. The greater the deacetylation, the greater is L^* (Herranz et al., 2012a). L^* values in samples L1.2 and L1.3 differed significantly ($p < 0.05$) (Table 2). This is associated with a sharp change of the slope in the linear fit between the area values of the acetyl band and pH (Figure 2). However, with a KGM concentration of 5 %, L^* increased (although not significantly) between L2.3 and L2.4, but did increase significantly ($p < 0.05$) between L2.4 and L2.5, with the highest value of L^* in L2.5. This high L^* value is consistent with the similarly high WBC value and may be related to the strong ion-dipole interaction reported above, so that the water was linked better and the light scattered through the different crystalline regions distributed locally through the network.

At a similar pH, L^* did not differ significantly among gels with different concentrations of KGM; significant differences were found only at high pHs (L1.5 and L2.5). Gels with the higher KGM concentration (5%) had a high density of cross-links between chains with more numerous and extended junctions, resulting in a denser network that would scatter the light more intensively and thus increase L^* values (Herranz et al., 2012b).

3.3. Influence of the deacetylation ratio on puncture data

The values of breaking force (BF) and breaking deformation (BD) measured at 25°C are shown in Table 3.

The trend of pH dependence was similar in 3 and 5% KGM gels. There was a large, significant increase in BF between L1.2 and L1.3, and L2.3 and L2.4, in 3% and 5% KGM gels respectively. Levels were similar in L1.4 and L1.3, and slightly lower in L2.5 than in L2.4, indicating that where cross-link density increased most noticeably was immediately after the gap zone, mainly through hydrogen bonding between KGM chains, which improved mechanical properties of their networks like BF making them more rigid. However, neither pH nor KGM concentration caused any differences in BD values. This may be because fracture deformation is controlled by the shape of junctions and their ability to change form or extend during applied force (Leksrisompong, Lanier, & Foegeding, 2012). The basic interactions within the network are electrostatic, principally hydrogen bonds, together with coulombic, van der Waals and hydrophobic interactions (before and after the gap zone). Thus, molecules associated by interactions of this kind possess a basic mechanism of inter-chain association based on the same physical principle (in both states: before and after gap zone), so the capacity to alter form during applied force is similar. This standardizes their mechanical response to applied stress, so that BD is practically constant in all samples (Table 3). Therefore, if we take the BF and BD data together, we find a considerable increase in the net rigidity of samples after the gap zone, due to enhancement of the number and size of the junction zones produced by more intense deacetylation.

With respect to increased KGM concentration, comparison of samples at similar pH shows that gels with 5% KGM were naturally firmer and stronger with higher breaking force (BF) and similar BD values, because inter-chain associations are more numerous at high polymer concentrations.

3.4. Influence of the deacetylation ratio on SAOS measurements

As in large-deformation measurements (section 3.3), it was decided to characterize the viscoelastic behaviour only of the samples located next to the gap zone (Figure 2), i.e. before (L1.2) and after the gap zone (L1.3 and L1.4) for 3% KGM concentration, and likewise for the 5% KGM concentration: before (L2.3) and after the gap zone (L2.4 and L2.5).

3.4.1. Stress sweeps

The effect of the deacetylation percentage at both KGM concentrations (3 and 5%) on stress and strain amplitudes (σ_{max} , γ_{max}), complex modulus (G^*) and loss factor ($\tan\delta=G''/G'$) within the LVE range was examined (Table 4). In the case of 3% KGM, both amplitudes (σ_{max} , γ_{max}) significantly increased ($p<0.05$) between samples L1.2 (pH= 9.3) and L1.3 (pH=9.8), i.e. before and after the gap zone, respectively (Figure 2). This indicates that when there was a considerable increase in the percentage of deacetylation (from 26% to ~75%), there was a strong increase of interchain attractions among KGM molecules, so that numerous large junctions were formed, resulting in a denser and more flexible physical network with higher σ_{max} and γ_{max} values (Mezger, 2006, chap. 8). Moreover, in these samples G^* increased and $\tan\delta$ decreased, both significantly (Table 4), indicating that the increase of rigidity (higher G^* values) was caused by the increased elasticity of the samples ($G'>G''$), and hence $\tan\delta$ diminished. For that reason, connectivity was noticeably enhanced after the gap region, so that the L1.3 network was more elastic and compact than the L1.2 network.

In the case of 5% KGM, the analogous gap zone which was found between pH values 9.2 (L2.3) and 10.7 (L2.4) was reflected in a different change in the internal structure of samples, transiting from a weak gel (high $\tan\delta=0.66$) in L2.3 sample to a strong gel in L2.4 sample (low $\tan\delta<0.1$) (Table 4). The weak-gel character of L2.3 can also be seen in the high σ_{max} , γ_{max} values (Table 4), indicating that the network was more porous and weakly interconnected with smaller junctions; this produced some structural stabilization, basically through hydrogen bonding, enhancing the conformational flexibility of the L2.3 sample at pH= 9.2 and 5% KGM concentration. This is a first step in physical gelling. After the gap zone (L2.4), a strong gel was obtained, as evidenced by a considerable increase in G^* (268%) and a decrease in σ_{max} , more particularly in γ_{max} values between L2.3 and L2.4 samples. This result indicates that between 23% (L2.3) and 58% (L2.4) of deacetylation a continuous network was formed, having larger junctions produced by hydrogen bonds and hydrophobic interactions (Chen, et al., 2011), with a packing effect that increased at pH> 10.7 (L2.4 and L2.5 gels). Increased aggregation of KGM chains (5% KGM) made for tighter networks with high water-retaining ability (Herranz et al., 2012a) as reflected in the fact that the highest values of WBC were recorded in L2.5 gels (Table 2). Also, the network was highly compacted, making for a less ordered structure with more boundaries to scatter light (Herranz et al., 2012b), as reflected in the fact that L^* values were highest in L2.5 gel.

3.4.2. Frequency sweeps

The mechanical spectra of the gels with 3% and 5% KGM are shown in Figure 3a and 3b respectively. In the case of 3% KGM gels, although all samples were more elastic than viscous, L1.2 differed considerably in this respect from L1.3 and L1.4 samples. In both L1.3 and L1.4 (after the gap zone) the relative difference between G' and G'' was greater ($G' \gg G''$) and there was minimum frequency dependence. These mechanical spectra are characteristic of “strong (true) gels” (Rao, 2007). In both L1.3 and L1.4, G' was practically frequency-independent and G'' increased slightly, particularly at low frequencies (Figure 3a); this rheological response is characteristic of KGM gels made at 25°C at the highest pH=12 (Herranz et al., 2012a) and stronger gels obtained at high temperature (Herranz et al., 2012c). L1.3 and L1.4 presented greater deacetylation, 75 and 95% respectively (section 3.1.), which promoted the formation of larger, more numerous junctions making for denser and more elastic networks, particularly in the case of L1.4 gel. However, the mechanical spectrum of L1.2 sample was different, firstly in that the relative difference between G' and G'' values over the frequency range was smaller, and secondly in that G' and G'' were considerably more frequency dependent than in L1.3 and L1.4 samples. This was particularly so in the case of G'' modulus, in the low frequency range (Figure 3a), where after a plateau region (from 62 to ~10 rad/s) G'' values decreased continuously down to the lowest frequency (0.62 rad/s). Such behaviour may be summarized by simple power law relations (equation 1):

$$G' \propto \omega^p, G'' \propto \omega^q \quad (1)$$

where the exponents p and q distinguish the different classes of weak gels, in this case at low frequency range (from 7 to 0.6 rad/s). Thus, the values of p (0.209) and q (0.178) in L1.2 sample are typical of weak-gels (Lapasin and Prici, 1999). The difference between p and q was relatively small (15%), suggesting that the transience of the physical network (L1.2) cannot be determined precisely on the present low time scales. It is therefore analysed using creep and recovery tests (next section), which are more time-consuming. The reason for this rheological response is that less acetyl groups were released (25%) since L1.2 sample was an initial step in the 3% KGM gelling process. Thus, there were still enough acetyl groups in the KGM chains to prevent chain associations, making the L1.2 network weaker and less stable. This result is consistent with the lowest BF measured by puncture test (Table 3), and the lowest σ_{max} and γ_{max} and highest $\tan\delta$ from stress sweeps (Table 4), as discussed earlier. Moreover, the fact that there were more acetyl groups in

the KGM chains in L1.2 sample increased steric hindrance (Chen, et al., 2011), causing a decrease of WBC.

In the case of 5% KGM gels, the difference in the mechanical spectra before (L2.3) and after the gap zone (L2.4 and L2.5) was even more marked than in the case of 3% KGM. Thus, L2.4 and L2.5 were both strong (true) gels, with high elasticity (they were indistinguishable in that respect), while G' was approximately 1 or ~2 orders of magnitude larger than G'' , and both moduli were practically frequency-independent over the frequency range analysed. L2.3 sample (23% deacetylation) presented a frequency dependence profile that was qualitatively similar to that of L1.2 (26% deacetylation), but in this case the decrease of both G' and G'' with frequency was greater, particularly in the low frequency range, converging at the lowest frequency (0.1 Hz) (Figure 3b). Thus, the values of p and q were higher ($p=0.462$ and $q=0.226$) and the difference (50%) was greater than in 3% KGM gel, so that both elasticity and viscosity decreased with increasing oscillation time (lower frequencies). However, the loss of elasticity was noticeably faster than the loss of viscosity, indicating that the viscoelastic response of L2.3 shifted towards one typical of concentrated solutions, as generally occurs when the system is close to the sol/gel limit (Lapasin & Prici, 1999).

Therefore, in order to form a denser, packed network with a high degree of connectivity capable of holding seafood particles, higher values of pH are needed, regardless of KGM concentration. At these high pH values, a large number of acetyl groups may be released, forming junction zones that serve to build highly-packed structures.

3.4.3. Creep and recovery tests.

The transient characteristics of KGM gels can be analysed on longer time scales than are associated with oscillatory tests, by means of creep-recovery experiments.

Figure 4a and b show creep-recovery compliances $J(t)$ for 3% and 5% KGM samples respectively. These data serve to derive the relaxation modulus $G(t)$ (Ferry, 1980), which provides the *gel strength* (S) and relaxation exponent (n) by means of equation 2.

$$G(t) = S \cdot t^{-n}. \quad (2)$$

In the case of 3% KGM concentration, as occurred in mechanical spectra, the creep-recovery response of L1.2 sample was different from that of L1.3 and L1.4 gels since the $J(t)$ values for L1.2 over the creep time were considerably higher than those of L1.3 and L1.4. However $J(t)$ values (L1.2) decreased noticeably during the recovery stage and were similar to those of L1.3 gel at the end of the assay (Figure 4a), indicating that the L1.2

network was softer (creep stage), with greater creep-recovery ability at the end of the test. This result may be explained by a low deacetylation percentage (26%) making for small junctions. This would reduce the inter-aggregate forces (low S values) within the network, permitting molecular rearrangements among the KGM chains after the load was removed (high elasticity) (Table 5).

Conversely, L1.4 gel had the lowest $J(t)$ values during both creep and recovery stages, and its network had little resilience, being more rigid due to the high deacetylation ratio (95%). Thus, more KGM chains were associated into larger junctions (high S value) and permanent cross-linking increased (low n exponent), making the network less elastic (Table 5). The creep-recovery response of L1.3 gel was qualitatively similar to L1.4, with higher $J(t)$ values (Figure 4a) during both creep and recovery times; this is because the deacetylation ratio was a little lower (75%) than that of L1.4, resulting in a rather less cohesive (low S values) and more time-dependent network (high n exponent) (Table 5).

In the case of L2.4 and L2.5 (58% and 91% deacetylation, respectively), the higher KGM concentration (5%) produced stronger gels so that $J(t)$ values were considerably lower than in 3% KGM (L1.3 and L1.4) Figure 4b. Thus, when KGM concentration increased, resulting in more dense and rigid networks (Herranz et al., 2012d), it did so particularly in the case of L2.5 because of the high deacetylation ratio, which produced larger junctions resulting in a less flexible network (low % elasticity). For instance, at longer experimental times, it can easily break into short chain fragments, which could explain why the n value was higher than in L2.4 gel (Table 5).

However, in the case of L2.3 (23% deacetylation), the $J(t)$ values for both creep and recovery times were considerably higher than in L2.4 and L2.5 gels (under stresses within the LVE range). L2.3 is a peculiar sample in that two factors converge that contribute to the connectivity in the network in opposite ways: on the one hand it has less cross-links among chains due to its low deacetylation (low S value). And, on the other hand, it has more KGM chains (5% KGM), which naturally increase the number of polymer-polymer associations but contain enough acetyls to exert a considerable steric effect that tends to separate the KGM chains. Thus, the viscoelastic response of L2.3 is essentially time-dependent, as evidenced by the large increase of $J(t)$ during creep time (Figure 4b), resulting in a more flexible network with high resilience, as reflected by high elasticity (Table 5).

In short, all the foregoing results indicate that both L1.2 and especially L2.3, are weak gels (more acetylated) that prevent the formation of the numerous large junction zones needed to make strong, time-stable networks, and so they are not suitable for our technological purposes.

3.5. *Scanning electron microscopy.*

Fig. 5 shows scanning electron microscopy (SEM) images at 3500x magnification of the 3% (L1.3 and L1.4) and 5% (L2.4 and L2.5) KGM gels after the gap zone. In general, the samples showed well-formed and homogeneous networks containing little holes embedded and appeared like true gels under microscopy.

In the case of 3% KGM gels, sample L1.3 (Fig. 5a) showed some discontinuous networks, formed mainly by coarse particles with small “spongy zones” (circled in red). This lack of a continuous spongy matrix (Fig. 5a) is consistent with the fact that both G' (mechanical spectrum) and S (creep test) were lower than in L1.4 gel. Moreover, the coarse particles (L1.3) suggest less connectivity in the network and may explain why WBC was lower than in L1.4 since the irregular network is more time-dependent, as evidenced by the n parameter, which was higher than in L1.4. However, L1.4 gel (Fig. 5b) showed well-defined spongy networks with holes homogeneously distributed throughout the networks, allowing the water molecules to settle inside them (Herranz et al., 2012a). This makes for a more homogeneous structure (higher S values) which can better retain water molecules (high WBC), lending the network more elasticity (higher G' moduli in mechanical spectrum) and also more time-stability (lower n parameter) in creep tests. These results (L1.4) were the consequence of a higher percentage of acetyl release (95%), as discussed in previous sections.

In the case of 5% KGM gels, L2.4 and L2.5 (Fig. 5c and 5d respectively) showed denser, more compact spongy networks than L1.4 (fig. 5b) because the higher KGM concentration naturally provided more numerous and larger junctions, much as reported previously for 5% KGM gels at pH=12 (Herranz et al., 2012d). Some differences were observed between the two samples (L2.4 and L2.5). L2.4 (Fig. 5c) showed a more “open” spongy matrix, while the L2.5 matrix (Fig. 5d) was slightly compacted. This increased aggregation of the L2.5 network due to the high deacetylation ratio (95%) is consistent with the very high values of S and G^* , together with the rigidity of the network indicated by the smaller force required to reach the failure point. This rigidity (L2.5) indicates good ability to transfer the breaking energy from one filament to another (Foegeding, González, Hamann & Case,

1994). It is also apparent in the higher n , and in the lower elasticity. This is consistent with the fact that the WBC value was the highest, since a tighter network can better retain the water molecules in the gel, thus reducing the rupture force.

Thus, from all the rheological and structural analyses, it seems that with 3% KGM concentration a very high ratio of acetyl release (95%) is needed to obtain more cohesive, elastic and time-stable networks. With a higher KGM concentration (5%), on the other hand, a moderate degree of deacetylation (58%) would be enough to obtain gels with acceptable rheological characteristics.

4. Conclusions

The addition of different quantities of 0.6N KOH to 3 and 5% AGD was effective in enhancing the gelling ability of KGM in different ways. The difference was essentially dependent on the required deacetylation ratio.

Increased KGM concentration produced two essentially different rheological effects on the internal structure of the samples, depending on the gap found in the linear representation of the relative area of acetyl bands versus pH at either KGM concentration (3 and 5%). At low pH (~9.2) (before gap) the higher KGM concentration (5%) made physical networks less elastic (high $\tan\delta$), much more flexible (high γ_{max}), more frequency-dependent (convergence between G' and G'' values at low frequencies in mechanical spectrum) and less time-stable (high n value) than samples with the lower KGM concentration (3%). Conversely, at high pH (~10.7) (after gap), samples containing either 3 or 5% KGM were all true gels, with little frequency dependence and with comparable conformational flexibility (similar γ_{max}), although 5% samples were naturally more rigid (high values of BF, S and G^*). Therefore, it is reasonable to conclude that the gap zone separates the two basic behaviours of physical networks in terms of the possibility of transition from a gel to a sol state. This is greater in the case of 5% KGM, whose weaker network at pH ~9.2 would qualify as a reversible gel that may revert to a sol over longer times.

Therefore, at similar alkaline pH~10.7, a 3% KGM concentration produced suitable gels with a very high ratio of acetyl release (95%). On the other hand, with the higher KGM concentration (5%), moderate deacetylation (58%) was enough to obtain gels suitable for technological purposes.

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Table 1. Nomenclature of samples containing 3 and 5% glucomannan at different pHs.

pH			
GM 3%		GM 5%	
L1.0	5.8	L2.0	5.5
L1.1	8.9	L2.1	7
L1.2	9.3	L2.2	8.1
L1.3	9.8	L2.3	9.2
L1.4	10.8	L2.4	10.7
L1.5	11.4	L2.5	11
L1.6	11.9	L2.6	11.4

Table 2. WBC (%) and lightness values (L*) of 3 and 5% glucomannan gels at different pHs.

WBC (%)				L*			
GM 3%		GM 5%		GM 3%		GM 5%	
L1.1	n.a. *	L2.1	n.a. *	L1.1	44.6 ± 3.9 abA	L2.1	46.8 ± 2.1 dA
L1.2	n.a. *	L2.2	n.a. *	L1.2	45.0 ± 0.9 aB	L2.2	45.7 ± 4.1 dA
L1.3	71.9 ± 1.1aA	L2.3	71.4 ± 1.5 cA	L1.3	48.9 ± 0.7 bcC	L2.3	45.9 ± 2.5dBC
L1.4	76.2 ± 2.5 bB	L2.4	72.1 ± 1.9 cB	L1.4	49.2 ± 1.0 bcD	L2.4	48.0 ± 0.4 dD
L1.5	77.3 ± 1.9 bD	L2.5	79.4 ± 1.9 dD	L1.5	49.9 ± 0.3 cE	L2.5	54.0 ± 0.7 eF

Values are given as mean ± expanded uncertainty limit (EUL)

a-e Different small letters in the same column indicate significant differences among different pHs at fixed glucomannan concentration (p <0.05)

A-F Different capital letters in the same row indicate significant differences between 3 and 5% glucomannan concentration (p <0.05) at similar pH.

(*) n.a. Sample not analysed

Table 3. Effect of pH, from the beginning to total deacetylation, on breaking force (BF) and breaking deformation (BD) values of glucomannan gels at 3% and 5% glucomannan concentration . T=25°C

BF (N)				BD ($\cdot 10^{-3}$ m)			
GM 3%		GM 5%		GM 3%		GM 5%	
L1.2	0.21 $\pm$ 0.02 aA	L2.3	1.92 $\pm$ 0.24 dB	L1.2	9.53 $\pm$ 0.56 gA	L2.3	9.84 $\pm$ 0.2 hA
L1.3	1.62 $\pm$ 0.09 bC	L2.4	4.10 $\pm$ 0.18 eD	L1.3	9.50 $\pm$ 0.27 gB	L2.4	10.00 $\pm$ 0.1 hB
L1.4	1.63 $\pm$ 0.12 bE	L2.5	3.18 $\pm$ 0.55 fF	L1.4	9.26 $\pm$ 0.18 gC	L2.5	9.37 $\pm$ 0.50 hC

Values are given as mean $\pm$ expanded uncertainty limit (EUL)

a-h Different small letters in the same column indicate significant differences among different pHs at fixed glucomannan concentration (p <0.05)

A-F Different capital letters in the same row indicate significant differences between 3 and 5% glucomannan concentration (p <0.05) at similar pH.

Table 4. Limit values of linear viscoelastic (LVE) range: stress (σ_{max}) and strain (γ_{max}) amplitudes, complex modulus (G^*) and loss factor ($\tan\delta$) of glucomannan gels among pHs from the beginning to total deacetylation at 3 and 5% glucomannan concentration $\nu=1\text{Hz}$, $T=25^\circ\text{C}$

	σ_{max} (Pa)	γ_{max} (%)	G^* (kPa)	$\tan\delta$
GM 3%				
L1.2	20±2 aA	1.05±0.09 fG	1.99±0.16 iK	0.316±0.019 mQ
L1.3	78±7.8 bC	1.96±0.28 eI	4.08±0.54 jM	0.178±0.063 nS
L1.4	86±8.6 b E	2.42±0.57 eJ	3.77±0.90 jO	0.183±0.078 nU
GM 5%				
L2.3	352±35 d B	12.6±1.4 gH	2.85±0.35 kL	0.657±0.058 oR
L2.4	249±25 c D	2.44±0.46 hI	10.5±1.8 iN	0.072±0.006 pT
L2.5	261±26 c F	2.21±0.28 hJ	12.0±1.6 iP	0.087±0.021 pU

Values are given as mean $\pm$ expanded uncertainty limit (EUL)

a-p Different small letters in the same column indicate significant differences among different pHs at each glucomannan concentration ($p < 0.05$)

A-U Different capital letters in the same column indicate significant differences between 3 and 5% glucomannan concentration ($p < 0.05$) at similar pH.

Table 5. Power-law parameters of equation 2 for glucomannan gels among pHs from the beginning to total deacetylation at 3 and 5% glucomannan concentration, T= 25°C.

	S (kPa)	n	r²	% elasticity
GM 3%				
L1.2	0.85±0.01	0.219±0.004	0.972	40
L1.3	3.70±0.05	0.302±0.008	0.931	16
L1.4	5.77±0.10	0.252±0.01	0.852	22
GM 5%				
L2.3	1.20±0.01	0.498±0.003	0.996	47
L2.4	13.38±0.19	0.262±0.009	0.901	37
L2.5	21.30±0.40	0.320±0.012	0.888	16

Captions for Figures

Figure 1. FTIR spectra of two series of konjac glucomannan with different deacetylation ratio for (a) 3 %(w/v) and (b) 5%(w/v) glucomannan at different pHs.

Figure 2. Acetyl standardized band (FTIR) area of (a) 3 %(w/v) and (b) 5%(w/v) glucomannan samples at various pH. Bars represent expanded uncertainty limit (EUL) from five replications.

Figure 3. Mechanical spectra data of (a) 3% (w/v) and (b) 5 % (w/v) glucomannan gels deacetylated with different pH. Open symbols G'' , closed symbols G' . $T=25^{\circ}\text{C}$.

Figure 4. Evolution of creep and recovery compliances of several aqueous glucomannan dispersions (a) 3% and (b) 5% glucomannan at different values of pH.

Figure 5. Scanning electron micrograph (magnification: x3500) of L1.3 (a), L1.4 (b), L2.4 (c) and L2.5 (d) gels. L1.3 = KGM gel at pH = 9.3, L1.4 = KGM gel at pH = 10.8, L2.4 = KGM gel at pH = 10.7 y L2.5 = KGM gel at pH = 11.0.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5