TITLE: Effect of alkalis on konjac glucomannan gels for use as potential gelling agents in restructured seafood products

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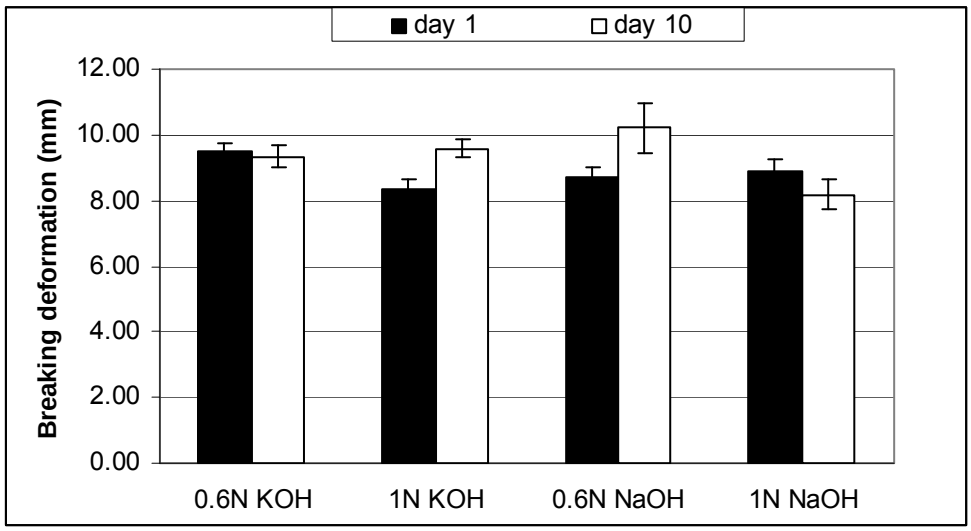
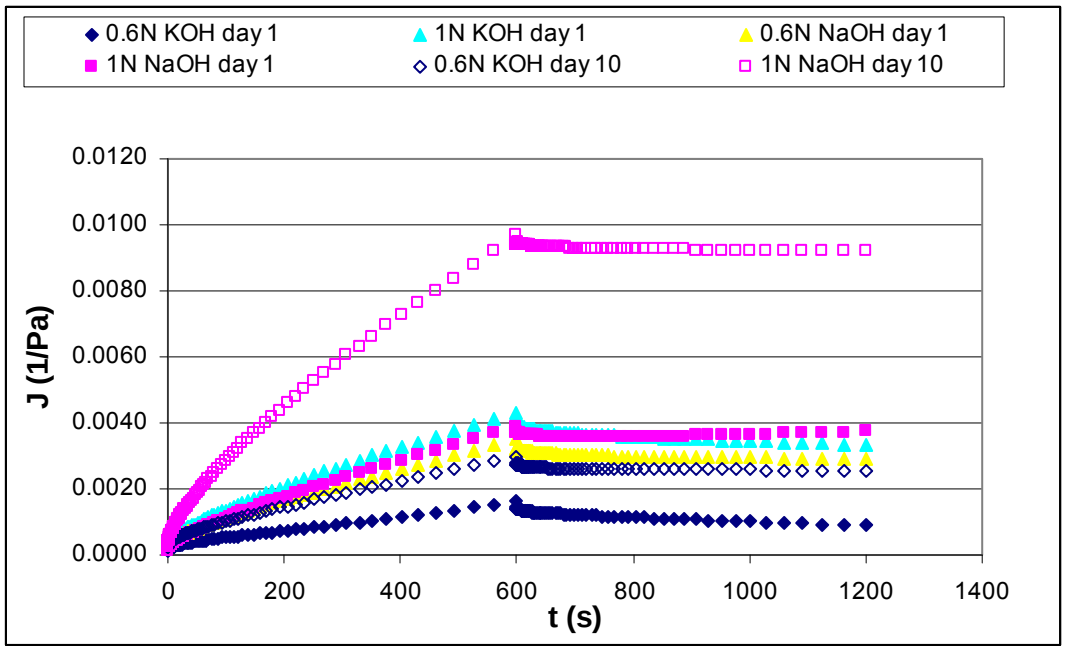
Graphical abstract



Breaking deformation in gels deacetylated with different alkalis at 0.6N and 1N after 1 and 10 days of refrigeration.



Influence of chilled storage time at 5 ºC on creep and recovery compliance data of glucomannan gels deacetylated with KOH 0.6N and NaOH 1N.

Abstract

Four dispersions of 3% glucomannan in water, deacetylated with 5% 0.6N and 1N KOH (lots L1 and L2) and 0.6N and 1N NaOH (lots L3 and L4) as gelling agents, were evaluated for use in raw restructured seafood products. Several properties (pH, moisture content, water binding capacity, cooking loss and lightness) together with puncture data (breaking force and breaking deformation) were determined after 1 and 10 days of chilled storage at 5°C. All these data were analysed together with different viscoelastic parameters obtained at small amplitude oscillatory strain (SAOS) after 1 day of chilled storage, showing that L1 and L4 samples were the most suitable gels for incorporation in raw restructured fish products. In both cases the highest stress (σ_{max}) and strain (γ_{max}) amplitude values were found in the linear viscoelastic (LVE) range; however, L1 showed both high strain amplitude and breaking deformation values. Moreover, creep and recovery (transient) data showed that L1 was the most-time stable gel with the highest elasticity and the lowest relaxation exponent (*n*). L4 gel showed strong rigidity, i.e. the highest values of breaking force and storage moduli (*G'*) and the highest *n* value, making it less gel-like. Both L1 and L4 gels became significantly less gel-like over 10 days of chilled storage due to the loss of gel strength (S) and a noticeable increase of *n*. These chilled storage effects were more intense in L4 than in L1.

Keywords: Konjac glucomannan; Breaking deformation; Restructured seafood product; Alkaline coagulant; Deacetylation

1. Introduction

Restructured seafood products are processed from minced and/or chopped muscle, usually with added ingredients, to make products with a new appearance and texture. The last 30 years have seen the development of a new generation of seafood products called analogues or substitutes, most of which mimic seafood or other high-value products and are formulated essentially from surimi, or in some cases mince. The processing is the result of thermal gelation or in some cases cold gelation with the help of ingredients like alginates or transglutaminase (Moreno, Carballo, & Borderias, 2008). However, if muscle protein functionality has been lost in processing, for example heating, gel formation to produce structures is not possible. One possibility in that case would be to find a substance, neutral in colour and flavour, that after being mixed with minced muscle could form a thermo-stable gel. Such a substance could be Konjac glucomannan (KGM) a neutral polysaccharide derived from the tuber of *Amorphophallus konjac* C. Koch (Nishinari, Williams, & Phillips, 1992) that has the property of making thermo-stable gels in certain conditions (Nishinari, 1987; Nishinari et al., 1992; Yoshimura & Nishinari, 1999; Zhang et al., 2001) and is generally recognized as safe (GRAS). It consists of a linear backbone of β-1—4-linked D-glucopyranose and D-mannopyranose sugars in a random order in a molecular ratio of 1:1.6 (Kato & Matsuda, 1969) and possesses between 5-10% of acetyl substituted residues (Dea et al., 1977; Maekaji, 1978a), which makes it soluble in aqueous solution and improves chain flexibility. Eliminating acetyl groups produces deacetylated konjac glucomannan, which is able to build junction zones through hydrogen bonding, Coulombic (between solvated (Na^+, K^+) cations and KGM deactetylated anions (R-CH_2O^-) being R the framework of carbohydrate, dipole-dipole (among water molecules and OH groups of deacetylated KGM), van der Waals, charge transfer and hydrophobic interactions (Lapasin & Pricl, 1999). However, it is not yet fully understood how this mechanism works under different conditions yet (Yin, Hongbin, Huang, & Nishinari, 2008; Yoshimura & Nishinari, 1999). The type of binding in the junction zone and the amount of molecules forming the junction zones are the most important factors determining the rheological and thermal properties of a gel (Williams & Phillips, 2004). Acidic deacetylation is possible, but alkaline reactions are preferred to limit hydrolysis (Imeson, 2010). The importance of KGM gels in rheological terms is firstly its capacity to extensively modify the rheology of aqueous media to which they are added, even at fairly low concentrations, and that is the basis of their functional properties as thickening and gelling agents. Secondly they are also involved in many other types of

applications, such as encapsulation, controlled release, etc. (Lefebvre & Doublier, 2005).

Therefore, small- and large-deformation mechanical tests need to be monitored in various different alkaline conditions to obtain information about the structure and mechanisms of gelation in different gels at a constant polysaccharide concentration.

The rate of gel formation is dependent on various factors: concentration and molecular weight of glucomannan, processing temperature, degree of acetylation, alkaline concentration (Nishinari & Zhang, 2004), and also pH (Huang, Takahashi, Kobayashi, Kawase, & Nishinari, 2002). In general, at a fixed alkaline concentration the gelation process accelerates with increasing molecular weight, KGM concentration or heating temperature (Yoshimura & Nishinari, 1999; Huang et al., 2002) but it is delayed with an increasing degree of acetylation (Huang et al., 2002). As regards pH, Kohyama and Nishinari (1990) reported that the gelation of KGM occurs from pH 11.3 to 12.6, suggesting that a change in molecular structure is necessary for gelation. However, Thomas (1997) indicated that the pH range necessary for gelation is 9 to 10. It seems that the gelation rate also depends on the alkaline concentration and is lower at higher concentrations, but there is a critical alkaline concentration below which gelation does not occur (Huang et al., 2002). In a previous work, Maekaji (1978a) suggested that the deacetylation produced by the addition of alkalis is governed by the concentration of hydroxide ion, irrespective of the kind of alkali, and the deacetylation ratio—i.e. acetyl groups removed/total acetyl groups—was practically independent of gelling temperature and increased with decreasing KGM concentration.

Very little scientific literature is available reporting studies of mixes of KGM and fish. Park (1996) reported that KGM has the ability to reinforce gel hardness 8-10 fold in both whiting and pollock surimi. Thomas (1997) also reported the use of KGM at a concentration of 1 % in surimi, but he did not elaborate. Iglesias-Otero, Borderias, & Tovar (2010) added glucomannan to reinforce squid surimi gelation.

Also a recent paper verified the good cryoprotective effect of KGM on protein from grass carp (*Ctnophryngodon idella)* during frozen storage (Xiong et al., 2009). The present authors have studied different KGM solubilization conditions, different KGM concentrations and types of alkali at different concentrations with a view to producing homogeneous gels with a texture similar to that of fish fillets. As regards the type of alkali, the literature reports the use of various different alkali coagulants such as phosphate buffers (Penroj, Mitchell, Hill, & Ganjanagunchorn, 2005), Na_2CO_3 (Hata, Ono, & Toda,1951; Huang et al., 2002; Huang & Lin, 2004), K_2CO_3 (Case, Knopp, Hamman, & Schwartz, 1992; $Ca(OH)_2$ (Hata et al.,1951; Jiménez-Colmenero et al., 2010; Na_3PO_4 (Nishinari et al., 1992), NaOH or KOH (Maekaji, 1978b). Using this

information, in preliminary studies for the work (not included in this paper), various types of alkaline solutions (KOH, NaOH, $Ca(OH)_2$ Na_2CO_3, K_2CO_3, Na_3PO_4 and K_3PO_4) at different concentrations (0.2, 0.6 and 1N) were added to make gels from KGM aqueous solutions. In these experiments gels firm enough to hold the mince as a filler and with a suitable colour only were obtained using KOH and NaOH, the alkaline agents used in the present work, at concentrations of 0.6 and 1N. These levels translate as 0.073- 0.196 g/kg, which is very little for standard food products, and do not pose problems as regards flavour or health properties.

This work is the first step of a study whose objective is to produce restructured seafood products in which KGM aqueous solutions and non-functional minced fish muscle are mixed, in such a way that the glucomannan forms a gel structure and the mince acts as a filler, with a view to forming structures of various kinds fibres, myotomes, etc.). The aim of this paper is to study the influence of the type and concentration of the alkali on the viscoelastic, mechanical and water retention properties of KGM gels in order to choose the most suitable thermo-stable gel for making restructured seafood products with different textures.

2. Materials and methods

2.1. Preparation of glucomannan gels

Aqueous solutions (3% (w/v) of glucomannan from konjac (glucomannan purity 100%, Guinama, Valencia, Spain) were prepared by continuous stirring for 30 min at low speed in a homogenizer (Stephan UM5, Stephan u. Söhne GmbH & Co., Hameln, Germany) at 60º C. Then 5% of NaOH or KOH (Panreac Química, S. A., Barcelona, Spain) was added at a concentration of 0.6 or 1N of alkali coagulant, mixing for 1 minute at 50 rpm to induce gel formation. After that, Petri dishes were filled with these mixtures and immediately vacuum packed into plastic bags (Barrier bag®, Cryovac Air Corporation, Barcelona, Spain) to compact the samples. Subsequently the samples were set by heating at 30ºC for 1 hour and then at 5ºC for 4 hours to obtain heat-stable gels. The last step was to reduce the high pH values by taking the gels out of the Petri dishes and placing them in a 0.2M citrate-phosphate buffer at pH 5 (gel:buffer proportion 1:10). After 20 hours at 5ºC, the pH of thermostable gels changed to neutral. They were then kept refrigerated (5º±1ºC) for 10 days. Analyses were performed at day 1 after gel preparation and after 10 days of chilled storage.

The different lots were named L1, L2, for gels made with 0.6N and 1N of KOH respectively, and L3 and L4 for the gels made with 0.6N and 1N of NaOH respectively.

2.2. Analyses

111 2.2.1. Physicochemical analysis

112 The pH was measured using a model 9165BNWP pH probe (Analítica
113 Instrumental, S.A., Barcelona) inserted in the gel. The pHmeter was an Orion model
114 720A (Analítica Instrumental, S.A., Barcelona).

115 Water content was determined by drying the sample to constant weight at 110ºC
116 and the results are expressed as a percentage (AOAC, 2000). Samples were analysed
117 in triplicate on days 1 and 10 after gel preparation.

118 The pH was measured in triplicate on days 0, 1, 5 and 10 of chilled storage; day 0
119 was the day immediately prior to putting the samples into the pH 5 buffer.

120

121 2.2.2. Water binding capacity (WBC)

122 Gels were cut into small pieces (2 g) and placed in a centrifuge tube (diameter 10
123 mm) with enough filter paper (2 filters Whatman nº 1, diameter 90 mm). Then the
124 samples were centrifuged in a Jouan MR1812 centrifuge (Saint Nazaire, France) for 10
125 min at 3000g at room temperature. WBC was expressed as per cent water retained per
126 100 g water present in the sample prior to centrifuging. Measurements were carried out
127 in triplicate on days 1 and 10 of refrigeration storage.

128

129 2.2.3. Cooking loss determination

130 A sample (40 g) was cut into small pieces and placed in a plastic bag where small
131 holes had been made to drain the drip. Then, this bag with the sample inside was put
132 inside another bag, hung with the holes at the bottom and cooked in that position in an
133 oven (Rational Combi-Master CM6) for 20 min at 100ºC. The sample was then cooled
134 and weighed. Cooking loss was expressed as g/100 g by weight difference between
135 uncooked and cooked samples.

136

137 2.2.4. Colour measurements

138 Lightness (L*), was analysed using a CIELab scale. Measurement was analysed
139 using a colorimeter (Minolta Chroma Meter Cr-200, Japan). The colour coordinates
140 were measured five times on the surface of the gel at three different analysis times (on
141 days 0, 1 and 10 of chilled storage). Before use, the colorimeter was standardized
142 using a white calibration plate.

143

144 2.2.5. Puncture tests

145 Cylindrical samples (diameter 3 cm x height 3.5 cm) were filled after gel
146 preparation. After neutralization in the buffer probes they were removed from the
147 cylindrical cells. Before the analyses, probes were tempered at room temperature

(25ºC). They were then taken out of the bags and analysed. Gels 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) and the depth of depression (as deformation) when the gel sample lost its strength and ruptured were recorded. All determinations were carried out at least in sextuplicate.

2.2.6. Dynamic rheometry measurements

Small deformation shear oscillatory testing was performed using a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ). The measurements were carried out using parallel-plate geometry (20 mm in diameter and 1 mm gap). Definitive gels were cut into disk-shaped slices 20 mm in diameter and 1 mm thick on a 570 S.T.E slicer (Germany). Any excess sample protruding beyond the upper plate was carefully removed. Samples were allowed to rest for 15 min before analysis 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 limit evaporation. No evidence of specimen slippage at the bottom plate was detected in any case (disk-shaped slices remained intact at the same initial position). The temperature was controlled to within 0.1°C by a Peltier element in the lower plate and was kept at 25.0 ºC.

Stress sweep tests

To determine the linear viscoelastic (LVE) region, stress amplitude sweeps were run at 6.28 rad/s, and 25 °C. The amplitude sweeps were conducted by varying the shear stress (σ) of the input signal from 0.24 to 1000 Pa. 300 points in the continuous mode were used in all instances. Changes in storage modulus (*G'*), loss modulus (*G''*) and complex modulus (*G**) were recorded. The critical (maximum) values of the amplitude sweeps—shear stress (σ_{max}) and shear strain (γ_{max}) at which the *G** values are just beginning to show a noticeable deviation from the previously constant values—were determined from these data. The range of tolerable deviation (± 10%) was corroborated using creep and recovery tests (Mezger, 2006).

Creep and recovery tests

An instantaneous stress σ_0 (30 Pa) was applied for 600 s to each sample in the creep tests and the resulting change in strain over time was monitored. When the stress was released, some recovery was also observed for 600s. Creep measurements were made over the linear viscoelastic range on each sample (σ_0 corresponding to 0.5% shear strain). The creep and recovery results are 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).

From *J(t)* data we obtained the relaxation modulus *G(t), which was used* to find the *gel strength* (*S*) and relaxation exponent (*n*) (Ferry, 1980).

All measurements were made at 25 °C.

Mechanical spectra

Samples were subjected to stress that varied harmonically with time at a variable frequency. The shear strain amplitude was fixed at 0.5 %; oscillatory frequency sweeps were run from 10 to 0.1 Hz, and measurements were made at 25 ºC. The complex modulus (*G**), storage modulus (*G'*), loss modulus (*G''*), and loss factor, tan *δ*, were determined as functions of frequency. Data were obtained in such a way as to ensure that the resulting *σ* in the sample would always fall within the linear viscoelastic range.

2.2.7. Statistical analyses

At least five independent batches were tested for each experiment and data are presented as averages. Statistical analysis was carried out using Microsoft Excel software. Trends were considered significant when means of compared sets differed at $p < 0.05$ (Student's t-test).

Statistical correlations between the textural and viscoelastic parameters were determined by multiple regression with confidence intervals of 95% ($p < 0.05$) using the SPSS Statistics 17.0 software**.**

3. Results and discussion

3.1. Evolution of pH

At Table 1 shows, the evolution of pH was the same in all the gels. The values were around 12 just after addition of 5% alkali and gel setting (day 0). These values dropped drastically to around 6-7 after the samples had been kept for 20 hours in citrate-phosphate buffer 0.2M pH 5 and remained constant over 1, 5 and 10 days of chilled storage. There were no significant differences ($p < 0.05$) in the average values in the different lots.

3.2. Moisture content, Water Binding Capacity (WBC) and cooking loss

Table 2 shows the moisture content, water binding capacity (WBC) and cooking loss of samples L1- L4.

All the samples showed very high moisture content (96-97%). There were no significant differences ($p < 0.05$) among the different lots at days 1 and 10. Teramoto & Fuchigami (2000) reported that konjac glucomannan gel as a food had high water content (approximately 97%) but he did not indicate the percentage of glucomannan and the type of alkali (sodium carbonate or calcium hydroxide) used in the formation of these gels. Some authors (Kök, Abdelhameed, Ang, Morris, & Harding, 2009; Shinzato, Broussalis, & Ferraro,,1996) have reported that KGM forms highly viscous solutions when dissolved in water, suggesting that KGM has the highest viscosity at lowest concentration of any known dietary fibre (Shinzato et al., 1996; Yassen, Herald, Aramouni, & Alavi, 2005) so that it can take up to 200 times its weight in water.

WBC values were between 64.8%-78.5%. At day 1 values seemed to tend to be higher in samples made with NaOH (L3 and L4), although none of the samples showed significant differences ($p < 0.05$). These WBC values were stable after 10 days of chilled storage. L3 (NaOH 0.6N) showed the highest value ($p < 0.05$) but was only significant respect L2 (KOH 1N). Extraordinarily high water binding capacity has been reported in KGM although no data were given (Kök et al., 2009). The values for cooking loss were slightly but significantly ($p < 0.05$) higher in samples treated with KOH (L1 and L2) than in samples with NaOH added (L3 and L4), both at day 1, just after making the gel, and after 10 days. Cooking loss values were higher ($p < 0.05$) in the sample with the lower alkaline concentration (0.6N KOH) (L1) than in the one with the higher concentration (1N KOH) L2. Water retention values of about 86% have been reported in restructured fish muscle products made with minced fresh horse mackerel muscle, and about 75% in frozen hake muscle (Sánchez-Alonso, Haji-Maleki, & Borderias, 2007).

In general, all these data seem to indicate that glucomannan could reinforce the ability of the final product to capture moisture during cooking and retain its texture. There also seem to be some small and non significant differences depending on the alkali used for WBC and slightly higher for cooking drip retention when Na^+ ions are added as NaOH than when K^+ ions are added as KOH. One possible explanation is the difference in the radius of Na^+ and K^+ ions, which would contribute to the degree of hydration of the structures. Large ions, as in the case of K^+, possess a lower hydration number (N_w), i.e. they are surrounded by a larger number of water molecules (not cation-linked), which retain their translational degrees of freedom. K^+ ions show a smaller average hydration number (N_w= 2) compared to Na^+ ions (N_w= 3). The later one possess a higher change density since the same positive charge is located on the

255 smaller Na^+ ion. Therefore it polarizes the negative electronic clouds of water
256 molecules more effectively (Moore, 1978). Hence NaOH can bind the water more
257 easily (Fennema, 1976). Moreover, Kragh (1977) reported that in the ordered
258 Lipotropic Series of Hofmeister, a cation series from higher to lower hydratation ability,
259 the Na^+ is classified with more hydration ability. Kragh (1977) reported that the
260 mechanism in this series is not clear but is related to the polarity and size of the ion. As
261 noted earlier, these could be the reasons why in general WBC and cooking drip
262 retention are higher when Na^+ ions are added, since this causes greater hydratation.
263
264 *3.3. Colour*
265 All the gels obtained were translucent, showing high values of lightness (L*). At
266 day 1, L* was higher in gels made with NaOH than those made with KOH ($p < 0.05$)
267 (Table 3), but after 10 days of chilled storage, L* did not differ significantly in any of the
268 samples except L2 (KOH 1N). The other two colour parameters (a* and b*) were not
269 affected by the type and/or concentration of alkali (data not shown). The lightness of
270 whiting and pollock surimi with added glucomannan was reported by Park (1996), who
271 found that L* was increased by the addition of glucomannan-rich konjac flour. We have
272 found no more colour data for konjac gels in the literature.
273 In general, when gelation is performed at low ionic strength and in acidic or
274 alkaline conditions, it produces fine-stranded gels which are translucent and have high
275 water binding capacity (Rao, 2007). For that reason, in the light of their colour and the
276 WBC data (Table 2) the four samples may be classified among the fine-stranded type
277 physical gel networks.
278
279 *3.4. Puncture test*
280 Figure 1 shows the Breaking force (1a) and Breaking deformation (1b) of samples
281 L1-L4 at 0 and 10 days of chilled storage. There are significant differences ($p < 0.05$) in
282 breaking force (1a) between L4 (1N NaOH) and the other samples. After 10 days, L4
283 still had the highest values for strength and L1 the lowest.
284 Although there was an increase in breaking force between day 1 and day 10 in all
285 samples, this was much smaller and not significant in L1 than in the other samples,
286 which could mean that the gel was more stable over chilled storage.
287 On the other hand, breaking deformation was very similar in all samples. The
288 higher values were found in L1 and L4 at day 1, and L1 differed significantly ($p < 0.05$)
289 from L2 and L3 (Figure 1b). As in the case of breaking force, L1 showed practically no
290 change between day 1 and day 10. L4 likewise showed no significant differences ($p <$
291 0.05) over the storage time, although these differences were slightly greater than in L1.

Therefore again it seems that the L1 network was the most time-stable of all the samples.

In short, these large-deformation rheological measurements made it possible to distinguish L1 and L4 samples, since when NaOH 1N was used as an alkaline agent, the resulting gel possessed similar breaking deformation but significantly higher breaking force, making the gel significantly more rigid. This mechanical behaviour is consistent with the fact that viscoelastic moduli values from mechanical spectra were higher for the respective samples, as will be discussed in the next paragraph. The trend was sustained after 10 days of chilled storage (Figures 1a and 1b).

Although large-strain deformation mechanical tests, provide information that correlates more with the sensory perception and handling properties of food products (Bollaín, Angioloni, & Collar, 2005; van Vliet, 1995), small deformation oscillatory measurements have been considered a necessary and useful tool to study the network structure of foods, especially in food gels that present complex viscoelastic behaviour (Romero et al., 2009). Therefore, we considered it necessary to conduct a thorough rheological study to identify the properties of these gel networks.

3.5. Rheological measurements at small deformation. Influence of chilled storage

3.5.1. Overview of the small amplitude oscillatory (SAOS) results

The viscoelastic behaviour of the four samples was characterized at initial time in terms of several critical parameters which determine the linear viscoelatic range (LVE) , together with their mechanical spectra. The information from the oscillatory tests was then supplemented by the results of transient tests, also at initial time, to discriminate which samples had better gel properties. Thereafter, we examined the influence of 10 days of chilled storage on the viscoelastic magnitudes, but only in the samples with better gel characteristics (higher stress and strain amplitudes from stress sweeps, low frequency-dependence on G', and lower viscous moduli from mechanical spectra).

3.5.2. Stress sweeps at initial time

The first step was to investigate the LVE range for all the polysaccharide gels when a different kind and concentration of alkali was used. Within this range the viscoelastic moduli are independent of the stress. As the applied stress increases, the bonds holding the network together begin to rupture. At critical values of stress (σ_{max}) and strain (γ_{max}), the network structure breaks down leading to a sharp decrease in the

moduli. These critical values, which may serve as a measure of the stability of viscoelastic materials, were obtained from an automatic analysis (Campo-Deaño, Tovar, Pombo, Solas, & Borderias, 2009) and were corroborated using creep and recovery tests, since compliance curves generated at different stress levels overlap when data are collected in the LVE range (Steffe, 1996).

Table 4 shows the more representative magnitudes which characterize the amplitude of this LVE region. *tanδ=G''/G*' values were similar (≈0.2), and significantly lower than 0.5 in the four samples, meaning that samples behave like a viscoelastic gel since *G'* is larger than *G''* indicating the presence of a network structure (Mezger, 2006).

There was no statistically appreciable difference in the overall rigidity of their networks (*G**) (Table 4), however L1 and L4 samples showed higher stress and strain amplitudes than L2 and L3, which is consistent with the fact that they showed the highest breaking force and breaking deformation at initial time, as confirmed by large deformation measurements (Figure 1a and b).

3.5.3. Frequency sweeps at initial time

The frequency dependence of *G'* and *G''* can provide valuable information about the structure of a gel. Mechanical spectra for selected alkali/concentration values of glucomannan gels are presented in Figure 2. The mechanical spectra show little variation of *G'* and *G''* over the entire frequency range studied. The *G'* values can be fitted to the power law (equation 1), but the *G''* moduli remain practically constant from high to low frequencies.

$$G' = G_0' \cdot \omega^{n'} \quad (1)$$

Table 5 shows the power law parameters for G_0' and n' together with the mean viscous modulus between 0.63 and 63 rad/s. In L1-L4 samples, *G'* is practically frequency-independent over this time scale (n'<0.1). In addition $G_0' >> G''$ so samples, can be classified as true gel systems (Kaur, Singh, Singh, & McCarthy, 2008). There was significant ($p < 0.01$) negative correlation of *n' (-0.998)* and *tanδ* (-0.970) with *WBC*, confirming that when a network gel approximates to a true gel (low *n'* and *tanδ*), the gel functionality is better and hence water binding capacity noticeably increases.

This rheological behaviour is also quantifiable in terms of quality factor *Q* (Campo-Deaño, Tovar, & Borderias, 2010), a dimensionless quantity which represents the degree of damping of an oscillator. The *Q* factor is 2π times the ratio between the energy stored and the average energy loss per period (Arya, 1990). On the basis of the oscillatory character of frequency sweeps and the peculiar mechanical spectra data

(Figure 2 and Table 5), *Q* can be calculated from *G'* (eq. 1) and the mean viscous modulus in a sinusoidal strain (Table 5):

$$Q = 2\pi \frac{G_0^{'}}{G^{''}} \omega^{n'} \quad (2)$$

Note that except for L2 gel, all the rest possess high *Q* values, which is a measure of structural stability in their networks on short time scales. The fact that the mean viscous moduli are lowest in L1 gel in particular indicates that the relevant polar interactions to cross-links are junction zones with finite energy, which act more cooperatively to ensure gel stability at short times. A more permanent three-dimensional network was formed, so that although the gels contained around 97% solvent (Table 2), they are macroscopically connective, generating a superstructure which can store more energy. Thus, a minimum amount of energy is required for the internal relative motion between molecular segments (low molecular friction) and hence *Q* increases, indicating higher viscoelastic stability in physical gels over short experimental times.

3.5.4. Creep and recovery tests at initial time

Creep analysis is a transient test which was done at constant stress within the LVE range. The results of these experiments were used to compare the different structural responses of the four gels. This type of analysis produces data on creep compliance, *J(t)*, the ratio of strain to stress over time. Thus, the time dependent properties connected with the viscoelastic characteristics of physical gels can be studied on longer time scales than those associated with oscillatory tests (Mezger, 2006). The rheological characterization was completed with transient experiments, which provide a means of trying regimes of t> 100s and can help to distinguish among noncovalent crosslinked gels. Long-term behaviour may be associated with the re-orientation of chain segments, and probably with the movement of whole molecules relative to one another, causing relatively strong crosslinks to break (Lapasin and Pricl, 1999). Transient tests, then, can cause the breakage of short-range interactions. Therefore information can be obtained about the relative long-range properties of these physical networks (Steffe, 1996). From J_{max} and J_{min} on the creep and recovery curves respectively, it is possible to quantify the percentage of elasticity in the networks according to equation 3:

$$Elasticity(\%) = \left(\frac{J_{\max} - J_{\min}}{J_{\max}}\right) \cdot 100 \quad (3)$$

Figure 3 shows creep-recovery compliances for glucomannan gels. L1 sample presents the lowest compliance data over the entire time interval. When the load was applied at time t=0, there was an instantaneous deformation, from which this sample showed the most complete recovery upon removal of the load, indicating that it was the most elastic (Table 6). Moreover, the fact that it presented the least permanent deformation indicated that for longer loading times there was less structural collapse and less irreversible breakage of interactions (Deman & Beers, 1987).

By means of an experiment based on a different physical principle, we can corroborate the viscoelastic stability of the physical network of L1 sample. This stability effect was deduced from stress sweeps (higher strain amplitude) and from mechanical spectra (lowest loss modulus) (Tables 4 and 5 respectively). We should note that the evaluation of the viscous component of gels from frequency sweeps presented the same trend as the irreversible compliance data obtained from the residual strain at the end of the recovery process. We can therefore state that the polymer network in L1 sample possesses similar connectivity to the covalently crosslinked networks, as was also reported by Case et al. (1992) for konjac gels.

For their part, the L2-L4 gels exhibited higher creep and recovery compliance values, with a significantly low degree of elasticity (Table 6). During creep time, break a lot of hydrogen bonds which origined certain degree of the irreversibly structural damage, thereafter when the load was removed the higher proportion of the structure collapsed gave *J(t)* on the recovery increased and elasticity diminished. This result is consistent with the higher viscous modulus values of L2-L4 samples (Table 5); therefore, if residual strain increases at the end of the recovery period, as in L2-L4 gels, can be attributed to rearrangement of the gel network (Williams & Phillips, 2004), then there would be more broken interactions, which would explain their higher G'' values.

L4 gel was made with the alkali with the lowest cation diameter (Na^+) at maximum concentration, so that there were more Na^+ ions to link a larger number of water molecules, producing more layers of associated water molecules and hence a larger number of ion-dipole interactions. This could originate a *physical principle* based on the predominance of strong cation-dipole interaction, added to the corresponding anion-dipole (deacetylated KGM-water), which is less important since the anion is larger. This causes first an average preferential direction among molecular domains, which may generate a particular dipolar chain orientation (local order), and second a noticeable decrease of the available water, which cannot act as a lubricant between anionic polymer chains; this could explain the fact that the sample's physical network was more

brittle (high breaking force and low breaking deformation) than L1 (Figure 1a and b), as discussed in section 3.4.

Moreover, the *J(t)* from the creep values gives us the relaxation modulus *G(t),* since if we plot log *J(t)* versus log *t* over the entire time interval, the slope of the function m<<1 (data not shown here), *G(t)* becomes the reciprocal of *J(t)* (Ferry, 1980). Thus, the equation of Winter and Chambon (te Nijenhuis, 1997) can be used to calculate other parameters related to *gel strength (S)*, and also to the *relaxation exponent (n)*, by:

$$G(t) = S \cdot t^{-n} \quad (4)$$

Where *S* ($Pa \cdot s^n$) depends on the strength of the zone junctions between molecular domains and *n* is related to the density of these zone junctions, i.e. the degree of connectivity in the gel (Gabriele, de Cindio, & D'Antona, 2001). Table 6 shows that L1 gel presented the highest *S* and the lowest *n* parameters. The lower the *n* values, the higher is the density of physical crosslinks, which increases the extension of the junction zones in noncovalently crosslinked networks (Lapasin & Pricl, 1999). L4 gel possessed a similar *S* value to L1, but *n* was the highest as a result of less connectivity and may reflect more levels of heterogeneity than in the other samples. Also, there was a significant ($p < 0.01$) positive correlation between *S* and the critical stress ($r^2 = 0.997$) and strain ($r^2 = 0.996$) (from stress sweeps). This is because strong gels may remain in the LVE range at greater strains level, reflecting higher gel strength values (Steffe, 1996).

To summarize, from large and SAOS measurements we can deduce that sample L1, followed by L4, was the most stable, with the most ordered three dimensional network. Both had the highest maximum stress (σ_{max}) and strain (γ_{max}) values, as reflected by their high values of breaking force and breaking deformation respectively. However, L1 was the most elastic, presenting the lowest viscous moduli (*G"*), and recovery compliance (*J*) together with the lowest *relaxation exponent (n*), indicating a better-organized and better-ordered network as explained above. In addition, L1 showed the least variation in breaking force and breaking deformation over 10 days, which means that this gel was very stable, an important characteristic in a restructured fish product intended for chilled storage. All this means that L1 (0.6N KOH) offers the best properties for use as a gelling agent in restructured fish products. L4 (1N NaOH) could also be used although its network is less well- ordered and time-stable than L1's. It was therefore decided to study the influence of 10 days of chilled storage on viscoelastic properties of L1 an L4 gels.

The last step of processing is neutralization with a citrate-phosphate buffer. Although this should theoretically stimulate hydrogen bonding, the gel texture remains

unchanged as unpublished previous analyses showed. As most of the bonds probably might already be formed at the time of deacetylation and therefore contribute to a certain thermodynamical stability rearragements of bonds should be limited.

3.5.5. Effect of chilled storage time on the LVE interval and mechanical spectra

First, stress sweeps were used to evaluate the influence of 10 days of chilled storage on the σ_{max} and γ_{max} values in both L1 and L4 samples. There were practically no differences with respect to the corresponding values at initial time (Table 4): σ_{max} values after 10 days were: 90±9 and 70±7 Pa for L1 and L4 respectively. Although γ_{max}. values increased after the 10 days, 2.19 ± 0.59 and 1.62 ± 0.33 for L1 and L4 respectively, there were no significant differences ($p < 0.05$) with respect to the corresponding γ_{max}.values at day 1 (Table 4).

However, the frequency sweeps showed considerable changes in the viscoelastic properties when compared to day 1. There was thus a general decrease in the gel-like character of both samples after 10 days: on the one hand the G_0' parameter (equation 1) decreased significantly in both samples, more so in L4 (29 % with respect to initial value) than in L1 (18%) (Fig. 4a). There was also a greater increase in frequency-dependence, similar in L1 and L4 (Fig. 4c), while G'' increased considerably in L1 (40% with respect to initial value) and decreased in L4 (Figure 4b), making for a smaller gap between G' and G''. This meant a sharp decline in the elasticity of the networks.

These results show that in this kind of transient networks, chilled storage breaks the junction zones stabilized by thermolabile polar interactions such as ion-dipole and hydrogen bonds between polymer chains and with water. This effect was more pronounced in L1 sample given that the quality factor Q decreased by 39% while in L4 it decreased by 10% with respect to the corresponding values at day 1 (Table 5). This trend reflects a higher degree of connectivity by means of physical interactions in L4 sample, since Na^+ has more hydration ability (Kragh, 1977), and hence the loss of solidity was smaller than in L1 on short time scales

3.5.6. Effect of chilled storage time on the creep and recovery data

Figure 5 shows the influence of 10 days of chilled storage on the gel strength and relaxation exponent parameters from equation 4. In both L1 and L4 samples, these transient data confirm that chilled storage time caused some structural damage. This effect can be seen in the noticeable loss of the gel strength (Figure 5a) and the significant increase of the relaxation exponent (Figure 5b), which is associated with a decrease of polymer molecular weight due to the rupturing of a sequence of physical crosslinks (Lapasin & Pricl, 1999). After 10 days this damage was somewhat greater in

L4, which presented a slightly greater decrease in extent (17%) and strength of connectivity (45%) than L1 (12 % and 39% respectively). In general, the effect of chilled storage was slightly greater in gels made with NaOH than those made with KOH; in the former the number and distribution of anionic sites of KGM is altered by higher number of big hydrated ions that could generate, more dipolar fluctuations within the network (number and position of all these noncovalent cross-links), and hence the effect of experimental and storage time will be greater. On the other hand, as noted earlier, L1 (with minor quantity of small hydrated ions) was more time-stable in terms of breaking force and breaking deformation (Fig. 1), with hardly any difference between day 1 and day 10 for either parameter.

4. Conclusions

In this study various physicochemical measurements and various rheological techniques based on different physical principles such as large and SAOS deformations on different time scales (oscillatory and transient tests), were used to determine the influence of several alkalis at different concentrations on deacetylated glucomannan gels. Of the samples analyzed aqueous dispersions of 3% glucomannan deacetylated with 0.6N KOH and with 1N NaOH seem to possess well-structured gel properties and to be moderately stable over time, making them suitable for use in raw restructured seafood products that have to be stored chilled.

Of the samples assayed, the one with 0.6 N KOH performed best in terms of elasticity and time- and structural stability. Tests done over longer time scales, such as creep-recovery experiments, show low residual strain values and greater connectivity, which is consistent with results from experiments performed over shorter times, such as low viscous moduli values from mechanical spectra and high breaking deformation (from large deformations).

In order to be able to use them as gelling agents in restructured fish products, a thermal rheological study of these two gels (0.6N KOH and 1N NaOH) is required to demonstrate their thermo-stability.

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688 **Captions for Figures**
689
690 **Figure 1.** The change of the breaking force (a) and breaking deformation (b) of
691 glucomannan gels deacetylated with different alkali at 0.6N and 1N after 1 and 10 days
692 of chilled storage.
693
694 **Figure 2.** Mechanical spectra data of glucomannan gels deacetylated with different
695 alkali at 0.6N and 1N after 1 day of chilled storage. Closed symbols *G'*, open symbols
696 *G''*. T= 25°C
697
698 **Figure 3.** Creep and recovery compliance J(t) data of glucomannan gels deacetylated
699 with different alkali at 0.6N and 1N at day 1 of elaboration. T=25°C.
700
701 **Figure 4**. Effect of refrigerated time at 5°C on power law parameters from equation 1
702 G_0', n', and viscous modulus (G") from frequency sweeps of L1 and L4 . T= 25ºC.
703
704 **Figure 5**. Influence of refrigerated time at 5°C of L1 and L4 gels, on gel strength (*S*)
705 and relaxation exponent (*n*) from equation 4. T= 25ºC.
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