

# Comparative effect of high-pressure and pH on the viscoelastic characteristics of aqueous glucomannan dispersions

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## Introduction

Konjac glucomannan (KGM) has been reported as a novel alternative for making restructured fish products owing to its capacity to form thermoresistant gels when KGM is deacetylated by addition of an alkaline agent. Deacetylation produces a three-dimensional network in which fish particles with little or no protein functionality can be held as a filler. These networks are formed by locally ordered regions (junction zones) which are stabilized mainly by hydrogen bonds and other non-covalent interactions such as dipole–dipole and hydrophobic interactions. The number and size of these can fluctuate with time and temperature (1), so that the lifetime of these networks is finite (“transient networks”) (2). Previous papers reported that the stability of these networks was affected by temperature (3) and successive high pHs (increasing deacetylation) (4). The study of these parameters offered an idea of the optimal KGM gelation conditions for designing gels with adequate texture for use in technological treatments like cooking and pasteurization. Nowadays isostatic high pressure (HP) is widely used in the manufacture of surimi gels and restructured fish products (5, 6). Such HP can affect molecular interactions (hydrogen bonds, hydrophobic and electrostatic interactions) and protein conformation, leading to protein denaturation, aggregation and gelation (7). Hence, KGM network stability could also be seriously compromised by HP, necessitating a comprehensive analysis of network stability

before it can be used in the manufacture of restructured fish products.

This chapter is part of a study whose object is to determine the influence of HP on the structural characteristics of aqueous glucomannan dispersions (AGD) (3%) (w/v) at several pHs, in order to choose the most suitable HP conditions for the manufacture of restructured fish products. In this chapter, the effect of increasing HP on the viscoelastic properties of 3% AGD at two pHs close to the point of gelation (9.1 and 9.4) is examined to elucidate the differences produced by the alkalization level .

## Experimental Methods

Aqueous glucomannan dispersions (3%) (w/v) from konjac glucomannan (purity 100%, Guinama, Valencia, Spain) were prepared by continuous stirring for 30 min at low speed in a vacuum homogenizer (Stephan UM5, Stephan u. Söhne GmbH & Co., Hameln, Germany) at 60° C. Then KOH (Panreac Química, S. A., Barcelona, Spain) was added to increase the pH to 9.1 and 9.4, mixing for 1 minute at 50 rpm to induce gel formation. Cylindrical plastic containers were then filled with this mixture and left to set, for 1 hour at 30°C and then 4 hours at 5°C. After that they were placed in a 0.2 M citrate-phosphate buffer at pH=5 for 20 hours. Both samples, pH= 9.1 (lot A) and pH=9.4 (lot B), were then subjected to high pressure (HP) treatments (100, 200, 400 and 600 MPa) for 10 min. Samples A1 (100 MPa), A2 (200 MPa), A4 (400 MPa) and A6 (600 MPa) were prepared at pH=9.1 (lot A), and samples: B1 (100 Mpa), B2 (200 Mpa), B4 (400 Mpa) and B6 (600 Mpa) were prepared at pH=9.4 (lot B), all at 25°C.

Small amplitude oscillatory shear (SAOS) data were gathered using a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ) and a RS600 Haake rheometer (Thermo Electron Corporation Karlsruhe, Germany). For both rheometers the measurements were carried out using a parallel plate (20 mm in diameter and 1 mm gap). The temperature of the lower plate was kept at  $25.0 \pm 0.1$  °C. Stress sweeps were obtained from 1 to 800 Pa at frequency 1Hz. Frequency sweeps were performed over the range 0.01–10 Hz, keeping  $\gamma=1\%$  constant within the linear viscoelastic (LVE) region. Transient tests were carried out under constant stress ( $\sigma$ ) within LVE range for 600s, followed by a further 600s recovery time to obtain the reformation curve

## Results and Discussion

### Linear viscoelastic (LVE) range

Stress sweeps were used to determine the influence of HP on the limit parameters within the LVE range, such as strain amplitude ( $\gamma_{max}$ ) and rigidity ( $G^*$ ) of AGDs at pH= 9.1 and 9.4. The stress sweeps were recorded at 1 Hz, where the AGD behaves as a weak gel, close to the gel–sol transition phase (at lower frequencies) as indicated by mechanical spectra (next section). In A samples (pH=9.1) the effect of HP on  $\gamma_{max}$  was irregular and noticeably stronger than in B (pH=9.4) (Figure 1).

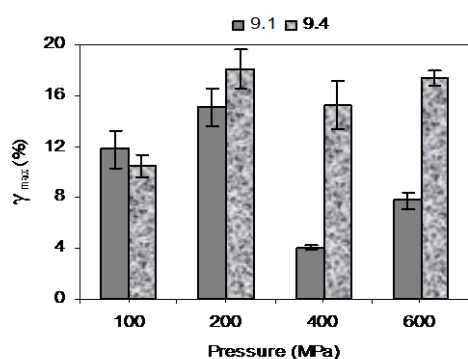


Figure 1. Influence of high pressure on strain amplitudes of the LVE range in AGD at two pH.  $T=25^\circ\text{C}$  and  $\nu=1\text{Hz}$ .

$\gamma_{max}$  was highest in A2 (200MPa) and lowest in A4 (400 MPa) (Figure 1).  $G^*$  was higher in A4 (400MPa) (Figure 2) than in all the other AGDs

(lot A) under different HP. This fact suggests that in A4, HP increased the number and size of the junctions in the AGD matrix, since the progressive development of a physical structure is reflected by a substantial narrowing of the  $\gamma_{max}$  value (1) (Figure 1), indicating a more rigid and less flexible structure (8). However, in samples at pH=9.4 (lot B) there was a significant increase in  $\gamma_{max}$  only between 100 (B1) and 200 MPa (B2). Beyond this pressure, from HP=200 (B2) to 600 MPa (B6),  $\gamma_{max}$  values remained practically constant with little dependence on HP (Figure 1). Note also that in lot B (pH=9.4) HP did not significantly affect the  $G^*$  data (Figure 2).

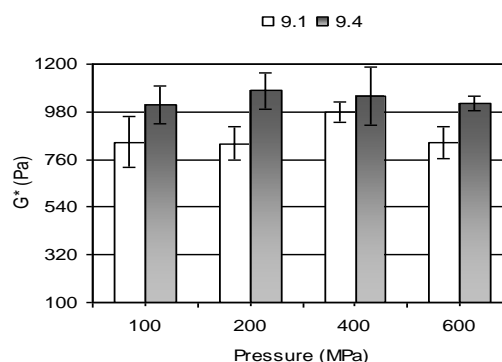


Figure 2. Influence of high pressure on the limit complex modulus in the LVE range in AGD at two pH.  $T=25^\circ\text{C}$  and  $\nu=1\text{Hz}$ .

These results indicate that the viscoelastic response in the LVE range under the same HP was very much dependent on the alkalinity of the AGDs and consequently also on the deacetylation ratio (4). Thus, at pH 9.1 there were more acetyl groups in KGM chains (less polymer-polymer association), resulting in more free volume within the AGD matrix. The resulting structures were consequently more sensitive to pressure-treatment, as shown in Figures 1 and 2.

Increasing HP in the lower range (100–200MPa) caused a similar rheological response irrespective of pH. Thus,  $\gamma_{max}$  significantly increased in both A1–A2 and B1–B2 (Figure 1), and in both cases  $G^*$  did not change (Figure 2). This suggests that between 100 and 200MPa, stability and molecular flexibility were enhanced in AGDs without this affecting the overall rigidity of the matrix. However, if we compare the effect of pH on  $G^*$  in

the same HP range (100–200MPa), we find that  $G^*$  was greater in  $B1$ – $B2$  (pH=9.4) than in  $A1$ – $A2$  (pH=9.1), indicating that the degree of cross-linking was greater at the higher alkalinity than at the lower. Thus, when the deacetylation ratio increased, both the number of chain segments acting cooperatively (junction thickness) and the extent of the KGM associations (junction length) increased, reinforcing the final structure in the AGD matrix.

### Mechanical spectra

Figure 3 shows mechanical spectra of AGDs from lot A (pH=9.1). The corresponding results at pH=9.4 were practically indistinguishable from these, and are not shown in Figure 3 for the sake of clarity.

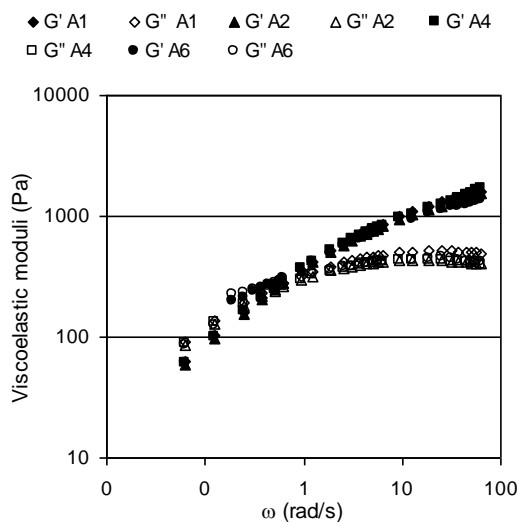


Figure 3. Influence of high pressure on mechanical spectra in AGD at pH=9.1 (A1-A6).  $T=25^\circ\text{C}$ .

From 10 to 0.20 Hz,  $G'$  was somewhat higher and more frequency-dependent than  $G''$ , indicating weak solid-like behaviour. The crossover interval ( $G' \approx G''$ ) was located between 0.10 and 0.06 Hz, corresponding to the gel-sol phase transition. At frequencies lower than 0.06 Hz,  $G''$  was slightly higher than  $G'$  (Figure 3), indicating the predominance of liquid-like behaviour in AGD samples. HP did not significantly influence mechanical spectra at either pH.

### Creep and Recovery tests

Comparative effects of HP on creep and recovery compliances  $J(t)$  of AGDs at both pH=9.1 and 9.4

are shown in Figure 4.

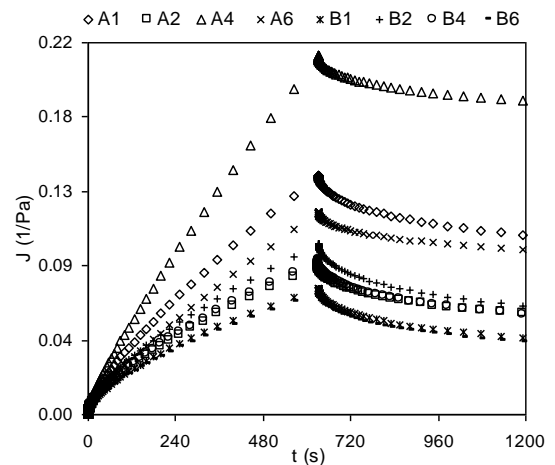


Figure 4. Influence of high pressure and pH on creep and recovery compliances in the LVE range of AGD at pH=9.1 (A1-A6) and pH=9.4 (B1-B6)  $T=25^\circ\text{C}$ .

An irregular (non-linear) trend was identified between  $J(t)$  data and HP. This is a consequence of the weak and random nature of the physical cross-links that form the junction zone, producing transient, heterogeneous aggregates which form a network of super-strands in polysaccharide gels (1). There were no pH-dependent differences in compliance curves during either creep or recovery steps. Thus, while at the higher pH (9.4; lot B) the results were practically independent of HP, at the lower pH (9.1; lot A) there were significant differences depending on the HP applied (Figure 4). For example, A4 (400 MPa) showed the highest values of both creep and recovery compliances, and conversely A2 (200 MPa) presented significantly smaller  $J(t)$  during both loading and recovery processes; these last values were practically indistinguishable from those in B2. Where the increase of  $J(t)$  was small, as in samples B1–B6, A2 and A6, there was likewise little breakage of cross-links in the AGD matrix, suggesting that the size (length and thickness) of the junction zone in AGDs is homogeneous, and hence suitable for the formation of more flexible and cohesive networks. Thus, the degree of molecular stabilization in A2 and A6 (with more acetyl groups in KGM chains) was comparable to that in more deacetylated dispersions like B1–B6 pH (9.4), irrespective of HP. In short, for less

alkalinized AGDs, 200MPa is enough pressure to improve time-stabilization to levels similar to those found in more deacetylated networks (B1–B6).

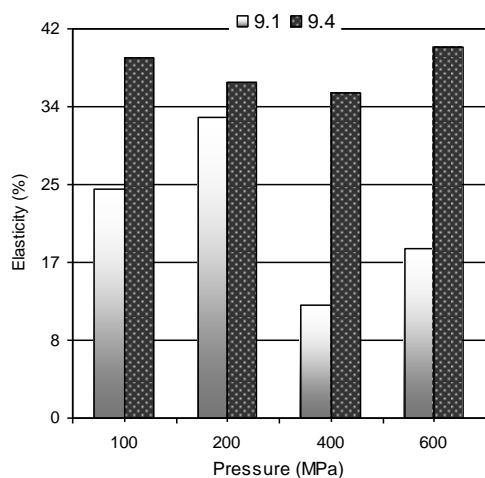


Figure 5. Influence of high pressure on elasticity from creep and recovery tests of the AGD at pH=9.1 and 9.4 T=25°C.

These results were corroborated by the elasticity values calculated, as reported by Herranz (9). The more deacetylated AGDs (lot B) were generally more elastic than the less deacetylated AGDs (lot A) (Figure 5). However, when HP=200MPa in A2, there was a considerable increase in elasticity, only slightly less than in B2 (Figure 5). This indicates that 200 MPa is a suitable pressure for improving the internal structure of less deacetylated samples. This makes for more stable networks with an optimum junction size, providing flexible, elastic structures similar to B samples. Conversely, 400 MPa (A4) was too high a pressure for AGDs at lower pH, making for more compact and less elastic networks as reflected in the minimum values of both  $\gamma_{max}$  (Figure 1) and elasticity (Figure 5).

### Conclusions

Small differences in alkalization levels of 3% aqueous glucomannan dispersions (AGD) produce strong rheological responses in terms of the effect of HP on their viscoelastic characteristics. In the case of less deacetylated AGDs (pH=9.1), the effect of HP was greater than in more deacetylated samples (pH=9.4). Creep and dynamic tests at 25°C converged, leading to the same conclusion: namely, that HP=200MPa is

an optimal value for HP treatment to induce mechanical and viscoelastic stabilization of 3% AGDs at low levels of alkalization.

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