Effect of long-term frozen storage on the rheological properties of pressurized glucomannan gels

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Abstract

Several weakly deacetylated glucomannan (GM) gels (pH= 9.1), at a concentration of 5 g/100 mL, were subjected to high hydrostatic pressure (HHP) at 0, 100, 200, 400 and 600 MPa. They were frozen and stored at -20 °C for two years to study the influence of long-term frozen storage on the rheological properties of pressurized samples (FP100, FP200, FP400, FP600) compared with a frozen unpressurized control (FP0) and an unfrozen unpressurized control (P0). In unpressurized gels, frozen storage reduced stress (σ_{max}) and strain (γ_{max}) amplitudes while forming a more solid-like network (FP0 vs P0). Gel FP0 maintained the rubber-like response from Temperature (T)>70 °C as in P0. HHP reduced loss of conformational stability and enhanced cohesiveness in FP100–FP600 vs FP0. Particularly, 400 MPa improved the degree of connectivity in the GM network producing a better thermoset response at T>70 °C (FP400).

Keywords: glucomannan, high hydrostatic pressure, frozen storage; weakly deacetylation

1. Introduction

Konjac glucomannan (KGM) is a neutral hydrocolloid from *Amorphophallus konjac* C. Koch which can form thermostable gels when deacetylated by adding an alkaline agent

(Nishinari, Williams, & Phillips, 1992). Herranz, Solo-de-Zaldívar & Borderías (2013) studied the possibility of taking advantage of the properties of glucomannan to make restructured seafood products by forming a gel network containing fish particles as filler. In recent years, high hydrostatic pressure (HHP) as an emerging technology was applied as an alternative to thermal processing to make foods with novel textures and better sensorial properties (Stolt, Oinonen, & Autio, 2001). Jimenez-Colmenero, Cofrades, Herrero, Solas, & Ruiz-Capillas (2013) reported that the freezing/thawing process affected texture and reduced the water binding capacity of GM gels at a concentration of 5%. The aim of this study was to analyse the influence of HHP processing on weakly deacetylated GM gels, for further use in making restructured seafood products which will be frozen stored.

2. Materials and methods

2.1. Sample preparation

Six lots of weakly deacetylated GM gels at 5 g/100 mL (w/v) were made from konjac glucomannan following the methodology described in previous works (Herranz, Tovar, Solo-de-Zaldívar & Borderías, 2013; Solo-de-Zaldívar, Tovar, Borderías & Herranz, 2014a). All GM gels (pH ~9.1) were then immediately vacuum packed and subjected to HHP at: 0, 100, 200, 400 and 600 MPa for 10 min at 10 °C and then were freezing and frozen stored at -20 °C in a freezer (Liebherr, Ochsenhausen, DE) for two years. The different frozen gels were named: frozen unpressurized control (FP0), and frozen pressurized (FP100, FP200, FP400 and FP600). The unfrozen unpressurized control lot was named P0. Analyses were conducted after 1 day of thawing in chilled storage in a fridge (Pershin, Barcelona, ES) at 5 °C.

2.2. Rheology tests

Small amplitude oscillatory strain (SAOS) tests were performed with a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ) using parallel-plate geometry (20 mm diameter and 1 mm gap). Samples were tempered at ambient temperature and were put on the lower plate of the rheometer, and then allowed to rest for 15 min before analyses (Solo-de-Zaldívar et al., 2014a). Temperature was controlled to within 0.1 °C using a Peltier element on the lower plate. All dynamic measurements were performed at least in quintuplicate.

To determine the linear viscoelastic (LVE) region, stress sweeps were run at 6.28 rad/s at 25 °C with shear stress (σ) of the input signal varying from 1 to 1000 Pa. 300 points on the continuous mode were recorded in all instances. To obtain mechanical spectra frequency sweeps were run from 10 to 0.01 Hz at fixed strain $\gamma = 1\%$ within the LVE range at 25 °C. Temperature sweep tests were performed for dynamic thermomechanical analysis (DTMA) from 20 °C to 90 °C at a linear heating rate (1 °C/min). Frequency (0.1 Hz) and strain $\gamma = 1\%$ were fixed.

2.3. Statistical analyses

Data are presented as the mean values of at least five independent measurements and were tested for each experiment, the expanded uncertainty limit (EUL) being the maximum and minimum deviation from the respective mean value. Trends were considered significant when the means of compared sets differed by $p < 0.05$ (Student's t-test).

3. Results

3.1. Stress sweeps

Stress sweeps were carried out to determine the influence of frozen storage and HHP on rheological response in terms of stress (σ_{max}) and strain (γ_{max}) amplitudes of the LVE range (Herranz, Tovar, Solo-de-Zaldívar, & Borderías, 2012). All frozen samples (FP0–FP600) maintained similar gel-like characteristics (*loss tangent* ~ 0.36). Frozen storage resulted in a marked decrease in σ_{max} (67%) and γ_{max} (66%) in FP0 vs P0 (Fig. 1a,b) giving rise to a more brittle (shear-sensitive) GM matrix compared with P0 (Solo-de-Zaldívar, Herranz, Borderías, & Tovar, 2014b). The structural benefit afforded by HHP was evident at 200 MPa, σ_{max} decreased (47%) as did γ_{max} (51%) compared with P0. This effect was more intense at 400–600 MPa where σ_{max} and γ_{max} decreased even less (22% and 40% respectively) compared with P0 (Fig. 1a,b). This would appear to indicate that HHP rendered samples more stable than FP0 after frozen storage.

3.2. Frequency sweeps

Mechanical spectra are expressed in terms of frequency dependence of storage modulus (G') (Fig. 2a) and loss factor (L_f) ($\tan \delta = G''/G'$) (Fig. 2b). All samples behaved as gel-like materials ($\tan \delta < 1$) in the full range of frequency (Fig. 2b). However, for the unfrozen unpressurized control (P0) the effect of acetyl groups preventing inter-chain

associations was evident in terms of lower G' (Fig. 2a) and higher loss factor (L_f) values at the low-frequency range (Fig. 2b). In order to quantify these trends, both G' and L_f were fitted to the power law with angular frequency (ω) according to equations 1 and 2:

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

$$L_f = L_{f0} \cdot \omega^n \quad (2)$$

Exponents n' and n provide network time-stability (Lapasin & Prici, 1999). Specifically, the negative sign of the n exponent directly quantifies the loss of the gel-like character due to shearing at high oscillation time (low frequencies) resulting from relaxation processes at junctions (Rao, 2007). G_0' and L_{f0} are the elastic modulus and loss factor at 1 rad/s respectively. As regards the effect of frozen on lot control, G_0' and L_{f0} significantly increased and decreased respectively in FP0 vs P0 (Table 1). Ice formation during freezing removes water from the GM matrix thus disrupting GM-water hydrogen bonding enhancing it in GM-GM interactions (Hui et al., 2006) resulting in an ordered packing effect on the GM network with greater elasticity (higher G_0'), and solid-like nature (lower L_{f0}) (Table 1). A higher degree of intermolecular order is also reflected in the significantly lower n' and n (absolute value) exponents in FP0 vs P0 (Table 1) due to the longer life-time of junctions and, consequently, the permanent stability of the physical network (Lapasin & Prici, 1999).

The structural improvement of frozen pressurized gels was evidenced in higher G_0' , lower L_{f0} parameters and in the lower n exponents in FP100–FP600 samples vs FP0, specifically FP400 exhibiting the highest G_0' and the lowest L_{f0} and n' exponent (Table 1). Therefore, 400 MPa noticeably increased the degree of connectivity in the GM network after frozen storage promoting larger junctions which slowed internal relaxation processes, similar in some sense to glasses (Larson, 1999).

3.3. Dynamic thermo-mechanical analysis

From 25 °C to 60 °C all thermal profiles exhibited a plateau zone of G' up to ~60 °C (Fig. 3a), maintaining the gel-like nature ($\tan \delta < 1$) (Fig. 3b). The effect of frozen storage up to 60 °C was evidenced in a reduction in loss moduli (G'') thus producing a noticeable decrease in $\tan \delta$ in FP0 vs P0 (Fig. 3b). This indicates greater conformational order in the GM matrix (FP0) in contrast with the most common increase of G'' related to frozen storage (Jiménez-Colmenero et al., 2013). In general for pressurized samples (FP100–FP600), G' and $\tan \delta$ values were higher and lower,

respectively than those for FP0 at the plateau zone (Fig. 3a,b). Thus, HHP enhanced structural strength producing a more densely packed GM matrix, especially at 400 MPa (FP400).

Important differences were detected from $T > 60$ °C. Both controls (FP0 and P0) exhibited a similar rubber-like pattern after a weak G' -minimum ~ 70 °C for the P0 sample absent in FP0 (Fig. 3a). This rubber-like elasticity from 70 to 90 °C results from an increase in network connectivity caused by hydrophobic interactions (entropic control) (Lapasin & Priel, 1999), resulting in a new gelation process in P0 and FP0 as evidenced in the progressive decrease of $\tan \delta$ at high temperatures (Fig. 3b). Regarding HHP, FP200 and FP600 followed the same pattern as controls (FP0 and P0). However, G' moduli were practically temperature independent up to 90 °C in FP100 and even more so in FP400 (Fig. 3a). That means that 100 and 400 MPa could produce some crystalline zones analogues to those of deacetylated GM gels (Moreno, Herranz, Borderías, & Tovar, 2016), giving a degree of thermo-stability to gel networks. Moreover, FP400 exhibited a $\tan \delta$ peak at ~ 80 °C, indicating possible changes of physical state in crystalline regions (Sperling, 2001).

4. Conclusions

Long-term frozen storage of weakly-deacetylated GM gel damaged the conformational stability of the gel network FP0, although its network was orderly packed and more solid and time-stable. Gel FP0 maintained the rubber-like response from $T > 70$ °C as in P0. HHP (200, 400 and 600 MPa) reduced the conformational stability loss resulting from frozen storage. Specifically, 400 MPa improved the degree of connectivity in the GM network promoting larger junctions and a thermoset response at $T > 70$ °C in gel FP400.

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