Comparative study of pH and high pressure treatment on the viscoelastic properties of glucomannan gels after long-term frozen storage.

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Abstract

Four lots of glucomannan (GM) gels (5 g/100 mL w/v): two lots of native GM gels (pH=6.6; lot N) and two lots of deacetylated GM gels (pH=11; lot D) were subjected to high hydrostatic pressure (HHP) at 0 (N0 and D0 respectively), and 400 MPa (N400 and D400 respectively). All samples were frozen and stored at -20 °C for two years to analyse the effect of long-term frozen storage on the viscoelastic parameters of pressurized samples according to pH (FN400, FD400) vs unpressurized samples (FN0, FD0), all in comparison with the respective unfrozen unpressurized controls (N0, D0). HHP improved the gel properties of frozen native gels as evidenced by significantly higher stress (σ_{max}) and strain (γ_{max}) amplitudes in FN400 vs FN0, albeit the effect was attenuated when GM was deacetylated (FD400 vs FD0). From $T > 65$ °C, HHP produced rubber-like elasticity in native GM gel (N400), and this thermal response was maintained in frozen native gel (FN400). Thermal profiles of deacetylated GM gels suggest that HHP reduced vitrification of physical networks in FD400 vs FD0.

Keywords: glucomannan; high hydrostatic pressure; frozen storage.

1. Introduction

Konjac glucomannan (KGM) is a neutral hydrocolloid from *Amorphophallus konjac* C. Koch which forms thermostable gels when deacetylated by adding an alkaline agent (Nishinari, Williams, & Phillips, 1992). KGM has been used in combination with minced fish to make gels so as to achieve restructured seafood products with an appropriate texture (Solo-de-Zaldívar, Herranz, Borderías, & Tovar, 2014a). Given the role of glucomannan (GM) gels in seafood products, time in frozen storage should be confined to the theoretical maximum shelf life of any frozen fish muscle. Teramoto & Fuchigami (2000) reported that the freezing/thawing process damaged the texture of GM gels. Herranz, Piñeiro, Borderías & Tovar (2017) studied frozen pressurized GM gels weakly deacetylated at fixed pH=9.1, showing that 400 MPa enhanced the thermostability of the GM network. The goal of the present study was to determine the effect of long-term frozen storage on the viscoelastic properties of pressurized (HHP=400 MPa) GM gels made at two pH levels—pH 6.6 (GM in native state) and pH=11 (GM deacetylated)—for purposes of further use in making restructured seafood analogues for frozen storage.

2. Materials and methods

2.1. Sample preparation

Four lots of GM gels (5 g/100 mL w/v) were made from konjac glucomannan following the methodology described in a previous work (Solo-de-Zaldívar, Tovar, Borderías, & Herranz, 2014b). Two lots of native GM gels (pH=6.6) were then immediately vacuum packed and subjected to HHP at 0 (N0) and 400 MPa (N400) for 10 min at 10 °C and then frozen and stored for two years at -20 °C in a blast freezer (Liebherr, Ochsenhausen, DE). The frozen gels were named: frozen unpressurized native GM gel (FN0) and frozen pressurized native GM gel (FN400). Another two lots of deacetylated GM gels (pH=11) were then immediately vacuum packed and subjected to HHP at 0 (D0) and 400 MPa (D400) for 10 min at 10 °C and then frozen and stored for two years at -20 °C in the same blast freezer. The frozen gels were named: frozen unpressurized deacetylated GM gel (FD0) and frozen pressurized deacetylated GM gel (FD400). Analyses were conducted after 1 day of thawing in chilled storage in a refrigerator at 5 °C (Pershin, Barcelona, ES).

2.2. Rheology tests

Small amplitude oscillatory strain (SAOS) tests were conducted using a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ) using parallel-plate geometry (20 mm diameter and 1mm gap). Samples were placed between parallel plates and

allowed to rest for 15 min before analyses (Herranz, Tovar, Solo-de-Zaldívar, & Borderías, 2013). 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 1 Hz and 25 °C with shear stress (σ) of the input signal varying from 1 to 1,500 Pa. Three hundred 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 $\gamma = 1\%$ in the LVE range at 25 °C. Temperature sweep tests were performed for dynamic mechanical thermal analysis (DMTA) from 20 °C to 90 °C at a constant heating rate (1 °C/min). Frequency (0.1 Hz) and strain $\gamma = 1\%$ were fixed (Mezger, 2006).

2.3. Statistical analyses

Statistical analysis were carried out using Microsoft Excel software. Data are presented as the mean values of five independent measurements. Trends were considered significant when the maximum and minimum deviation from the mean value differed at $p < 0.05$ based on the expanded uncertainty limit (EUL), calculated by Student's t-test.

3. Results

Overview

To clarify the discussion, GM gels are considered separately according to pH. First, at pH=6.6 (native GM gels) the effect of frozen storage on the viscoelastic properties for unpressurized gels (FN0 vs N0) was analysed. Second, a similar analysis was conducted on frozen pressurized gels (FN400 vs N400). Finally, the role of high pressure processing at 400 MPa is discussed, comparing both frozen pressurized (FN400) and frozen unpressurized (FN0) gels with the unfrozen unpressurized control GM gel (N0). The same scheme was followed at pH=11 for deacetylated GM gels.

3.1. Linear viscoelastic (LVE) range

Stress sweeps were carried out to obtain the stress (σ_{max}) and strain (γ_{max}) amplitudes which characterize the LVE interval (Herranz et al., 2013). In the case of native GM gel, frozen storage promoted a marked decrease in both σ_{max} (66%) and γ_{max} (66%) in FN0 vs N0, with a slight increase of the loss factor ($\tan \delta$) (Table 1), which is consistent with weakly deacetylated GM gels (Herranz et al., 2016). This trend was maintained in frozen pressurized

native gel (FN400) compared with N400. HHP improved the gel properties of frozen gels, as evidenced by significantly higher values of σ_{max} and γ_{max} in FN400 than in FN0 (Table 1). As regards GM deacetylated sample, the effect of freezing and frozen storage on the viscoelastic parameters was different than in native gels. Thus, while σ_{max} increased slightly, γ_{max} decreased (44%) and so did $\tan \delta$ (61%) in FD0 vs D0 (Table 1). These results indicate that frozen storage reinforced the junction zones by hydrogen bonding (Moreno, Herranz, Borderías, & Tovar, 2016), increasing the degree of order in the FD0 network as compared with D0. This rheological response was improved in frozen pressurized gels, such that 400 MPa produced a significant increase of σ_{max} (64%) and a smaller reduction of γ_{max} (21%) comparing FD400 vs D400. A comparison of the two frozen gels shows that the frozen pressurized sample had better gel characteristics, as reflected by significantly higher σ_{max} and γ_{max} in FD400 vs FD0 (Table 1), indicating the positive effect of 400 MPa in frozen deacetylated GM gels.

3.2. Mechanical spectra

In all samples storage (G') was higher than loss (G'') modulus over the entire frequency range (Fig. 1), indicating solid-like behaviour. In unfrozen native GM gels (N0 and N400), G' increased continuously with increasing frequency while G'' increased from 0.06 to 1 rad/s, and exhibited a plateau zone between 1 and 60 rad/s (Fig. 1a). These results indicate that native GM samples are weak physical gels (Rao, 2007) with short-ranged repulsive forces created by steric hindrance (Larson, 1999), which form soft physical networks irrespective of HHP. Frozen storage induced a weak increase in both G' and G'' values at lower frequencies (Fig. 1a), maintaining the same rheological response as in unfrozen gels.

In the case of deacetylated GM gels, there was an increase in both G' and G'' values (Fig. 1b), particularly in the low frequency range (0.06–1 rad/s), as compared with native gels in the same frequency range. This behaviour indicates a natural increase in structural strength when GM is deacetylated (Solo-de-Zaldívar et al., 2014b). In this case, the effect of frozen storage was evidenced by the fact that G' increased in FD0 vs D0, and at the same time the frequency dependence decreased, as indicated by the shallower slope of the G' -plot in FD0 as compared with D0 (Fig. 1b). Moreover, G'' values decreased considerably with increasing angular frequency from 0.06 to 1 rad/s in FD0 (Fig. 1b). This specific trend of G'' at low frequency range suggests the formation of micro-crystalline zones in the physical network, produced by freezing dehydration of the GM matrix (Herranz et al., 2017). These structures seem to enhance inter-chain interactions and absorb mechanical energy during shear over

greater time scales (low frequencies), like intermolecular relaxations near glass transition (Larson, 1999).

In the case of native and deacetylated (frozen/unfrozen) gels, mechanical spectra were practically independent of HHP. This fact shows the main effect of frozen storage *vs* pressure treatment, enhancing the intermolecular packing of deacetylated GM network, which could reflect the dehydration process associated to frozen storage resulting in a more concentrated GM matrix. The same preponderant effect of frozen storage on the greater G' and G'' values was also observed from stress sweeps (data no shown).

3.3. Dynamic mechanical thermal analysis

Frozen and unfrozen unpressurized native gels (FN0 and N0) exhibited similar G' -curve thermal patterns, with three regions for thermo-viscoelastic behaviour: 1) a plateau G' -zone up to ~ 50 °C; 2) a G' -minimum peak at 70 °C for N0 which shifted to a higher temperature in FN0 (~ 80 °C); and 3) a further slight increase of G' with increasing temperature up to 90°C, reaching G' values similar to those at 25 °C (Fig. 2a). In the case of the N0 gel, the G'' -curve diminished slowly up to ~ 60 °C, and something more between 60 and 75 °C (Fig. 2a). In frozen gels (FN0), the temperature dependence of G'' was qualitatively similar to N0, but was structurally more favourable given that the G'' values were lower up to 60 °C (Fig. 2a), whereas in most other frozen GM gels G'' tended to increase (Jiménez-Colmenero, Cofrades, Herrero, Solas, & Ruiz-Capillas, 2013). Frozen pressurized native gel (FN400) presented a qualitatively similar thermal profile to unfrozen pressurized gel (N400), but with higher G' values in two characteristic regions: 1) a quasi plateau zone up to ~ 60 °C; 2) from $T > 65$ °C, G' increased steadily as did G'' modulus from $T > 75$ °C (Fig. 2a), reflecting the rubber-like elasticity produced by hydrophobic interactions, which enhance the degree of connectivity in gel networks at high temperatures (Herranz et al., 2017). Hence, HHP treatment promoted a hydrophobically-driven effect that could be explained considering that pressure reduced the free volume, increasing consequently the excluded-volume forces between GM chains, these repulsive forces have mainly entropic nature (Larson, 1999).

In the case of deacetylated samples (Fig. 2b), thermal profiles of frozen unpressurized gels (FD0) were qualitatively similar to those of unfrozen unpressurized (D0) gels, with G' and G'' significantly higher than in D0. Moreover, in the case of FD0 the higher G' -plateau zone extended up to 85 °C (Fig. 2b). This result indicates a more closely cross-linked network in FD0 *vs* D0, resulting in a thermoset system, which is also reflected in the fact that the G'' modulus increased steadily up to 90 °C (FD0), while in D0 sample G'' increased up to 65 °C

(Fig. 2b). This means that when GM is deacetylated, dehydration of the GM matrix associated with frozen storage causes the formation of larger microcrystalline junctions, similar to sheet-like structures, which produce strong “*vitrification*” (Larson, 1999). These microstructures are able to absorb thermal and mechanical energy due to vitreous phase transitions (Moreno et al., 2016), which is consistent with the increase of G'' observed at low frequency range in the mechanical spectrum analysed earlier. This specific behaviour is associated with a higher glass transition temperature (T_g), which lies outside the experimental temperature range of this study (FD0). Frozen pressurized gel (FD400) maintained the same thermal profile as FD0, but dehydration by frozen storage produced less structural impact, as evidenced in the lower degree of vitrification and hence the lower T_g , indicated by the G'' -peak (Sperling, 2001) at ~ 78 °C for FD400 (Fig. 2b). This result is consistent with the fact that conformational stability and flexibility were significantly higher in FD400 than in FD0, as noted above.

4. Conclusions

Pressurization at 400 MPa improved the structural stability and conformational flexibility of long-term frozen-stored GM gels at low (6.6) and high (11) pH. However, at 25 °C when GM was in native state (pH=6.6), the stiffness of frozen pressurized gel (FN400) was similar to that of frozen unpressurized gel (FN0). The thermal profile of frozen pressurized native GM gel from $T > 65$ °C indicated rubber-like elasticity in contrast with frozen unpressurized native GM gel, whose G' values were similar at $T > 80$ °C and at 25 °C. At pH=11, when GM is deacetylated, frozen pressurized (FD400) and unpressurized (FD0) gels were stiffer than corresponding unfrozen gels, but the degree of network vitrification in frozen pressurized gels was lower than in frozen unpressurized gel, and consequently glass transition occurred at a lower temperature. These results suggest that restructured seafood products can be developed with different textures for retail marketing as frozen product.

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