ELSEVIER

PII: S0144-8617(97)00134-3

*Carbohydrate Polymers* 34 (1997) 141–148
© 1997 Elsevier Science Ltd
All rights reserved. Printed in Great Britain
0144-8617/97/$17.00 + .00

# Physical and structural properties of barley (1→3),(1→4)-β-D-glucan—III. Formation of aggregates analysed through its viscoelastic and flow behaviour

C. Gómez[a]*, A. Navarro[a], C. Garnier[b], A. Horta[c] and J. V. Carbonell[d]

[a]*Asociación de Investigación de Cerveza y Malta (INVESCEMA). P.O. Box 73, 46100-Burjassot, Valencia, Spain*
[b]*Institut National de la Recherche Agronomique, LPCM, BP 71627, 44316 Nantes, Cedex 03, France*
[c]*Departamento de Ciencias y Técnicas Fisicoquímicas, Facultad de Ciencias, Universidad a Distancia (UNED), 28040 Madrid, Spain*
[d]*Instituto de Agroquímica y Tecnología de Alimentos (IATA, CSIC), P.O. Box 73, 46100-Burjassot, Valencia, Spain*

(Received 4 July 1997; accepted 16 July 1997)

The rheological properties (flow and viscoelastic) of barley β-glucan in aqueous solution have been studied. The results shed light on the association behaviour of these macromolecules and even on the formation of network-like structures under certain circumstances. The concentrations used for this study were both in the dilute and in the semidilute regime, close to the critical value where overlap of macromolecular coils sets in. However, the association behaviour detected in such a regime and together with the present experimental techniques, agrees with previous results obtained with other techniques such as capillary viscometry and light scattering, which operate in the dilute solution regime, where no overlapping of the chains is expected.

The influence of the molecular weight of the samples, the temperature and the ionic strength on the flow and oscillatory shear measurements has been studied. The preparation of samples and their thermal history have proved to be important factors in the development of associated structures, both affecting the results. © 1997 Elsevier Science Ltd

## INTRODUCTION

Barley (1→3),(1→4)-β-D-glucan, for short referred to as β-glucan, is the major component of the endosperm cell wall of this cereal. The viscosity of this polysaccharide in aqueous solutions can cause several problems in brewing (Bamforth, 1982), but it has been also suggested that the polymer could be used as a thickening agent in the food industry (McIntosh *et al.*, 1991; Autio *et al.*, 1992).

The viscosity of β-glucan solution depends on its concentration and molecular weight, and also on its capacity to form macromolecular aggregates. β-glucan is a linear chain of glucose residues, containing about 70% β-(1→4) and 30% β-(1→3) links (Wood *et al.*, 1994), in such a way that long blocks of up to 14 adjacent β-(1→4)-linkages exist, whereas the presence of contiguous β-(1→3)-linkages has not been detected by the latest studies (Izawa *et al.*, 1993). These long sequences of adjacent β-(1→4)-linkages have been described as potential foci for aggregation between chains by hydrogen bonds (Fincher & Stone, 1986).

Recent reports describe the tendency of the β-glucan molecules to associate, suggesting models and mechanisms for the formation of aggregates. Vårum *et al.* (1992) through results from assays of light scattering and HPSEC proposed that approx. 10% of the oat β-glucan in aqueous solution forms labile aggregates. Grimm *et al.* (1995) by light scattering and viscometry on solutions of β-glucan extracted from beer, proposed the formation of fringed micelles, with side-to-side aggregation of chains of molecular weight approx. 175 000. On the other hand, Doublier and

---

*To whom correspondence should be addressed.

Wood (1995) studied the viscoelastic behaviour of three samples of oat $\beta$-glucan having different molecular weights and indicated that only for low molecular weights did the system display gel-like properties, owing to self-association through cellulose-like sequences. Our team (Gómez *et al.*, 1997a) also found the presence of $\beta$-glucan aggregates in the analysis of barley $\beta$-glucan samples by light scattering in batch mode and in their detection by the fluorimetric Calcofluor-FIA method.

Results on aggregation of $\beta$-glucans in solution are not conclusive. Further studies must be carried out to elucidate the mechanism of aggregation of different $\beta$-glucan samples. Furthermore, no references in the literature have been found on the viscoelastic behaviour of barley $\beta$-glucan solutions. The aim of this work was thus to investigate the flow and viscoelastic properties of barley $\beta$-glucans, according to their molecular weights. The influence of temperature on the rheological properties was also investigated.

## MATERIALS AND METHODS

### Preparation of solutions

$\beta$-glucan samples used in this work were those named as A, G and N in a previous paper (Gómez *et al.*, 1997a). The physical characteristics of the samples are summarised in Table 1. Solutions were prepared by disolving the powdered $\beta$-glucan in boiling water (Milli-Q grade) with magnetic stirring. All $\beta$-glucan solutions were filtered through a 0.8 $\mu$m nylon filter before analysis. The concentrations investigated were 0.16%, 0.31% and 1.5% for samples A, G and N, respectively.

### Rheological measurements

The rheological properties of $\beta$-glucan were analysed with a Rheometrics Fluid Spectrometer (RFS II, Rheometrics Inc., Piscataway, NJ) using a cone-plate device (cone angle 2°, diameter 5 cm). Measurements were carried out under steady shear and dynamic conditions at 25°C. Properties of samples G and N were also measured at 70°C. Viscoelastic measurements were performed in the frequency range $10^{-2}$ to $10^{2}$ rad/s. The amplitude of deformation was chosen to remain within the linearity limits of viscoelasticity. No thixotropy (time dependence) was detected during reverse measuring of the shear rates.

## RESULTS AND DISCUSSION

As can be seen from Table 1, all the samples used here had very different molecular weights, which explains that preparing all solutions at the same concentration was impossible. In order to take into account differences in the molecular weight of the different samples, directly related to their intrinsic viscosities $[\eta]$, and thus to compare their rheological properties, the concentrations have been chosen such that all samples were placed in equivalent conditions regarding macromolecular overlap, that is to say, fixing the same $c[\eta]$ for all the samples. The reduced polymer concentration $c[\eta]$ corresponds to the volume filled in solution by the polymer.

The overlap threshold concentration, $c^*$, corresponding to the crossover between dilute and semidilute solutions, is given by (de Gennes, 1979):

$$c^* \approx M/V$$

where $M$ is the molecular weight and $V$ is a measure of the macromolecular volume. Usually, V is replaced by the hydrodynamic volume, $M[\eta]$. This leads to:

$$c^* \approx [\eta]^{-1}$$

According to Graessley (1980), the overlap concentration is given by the relation $c^*[\eta]=0.77$. Two different $c[\eta]$ values have been chosen in this study ($c[\eta]\approx 0.84$ for samples A and G and 0.42 for sample N), the first value being at the beginning of the semidilute regime, and the last one being in the dilute regime.

### Flow behaviour

Figure 1 shows the flow curves at 25°C of a sample A solution at 0.16% and of a sample G solution at 0.31%. Both curves show typical shear-thinning behaviour. However, a newtonian regime was not

**Table 1. Characteristics of the barley $\beta$-glucan samples (Gómez *et al.*, 1997a) and concentrations used**

| Sample | Impurities (dry basis, %) | Mw ($\times 10^{-3}$) | Mw/Mn | Intrinsic viscosity $[\eta]$, (dl/g) | Concentration $c$, (g/100 ml) | Reduced concentration $c[\eta]$ |
|---|---|---|---|---|---|---|
| A | Protein, 1.50<br>Starch, 1.30 | 573 | 1.18 | 5.19 | 0.16 | 0.83 |
| G | Negligible | 231 | 1.41 | 2.76 | 0.31 | 0.85 |
| N | Negligible | 9.2 | 1.10 | 0.28 | 1.5 | 0.42 |

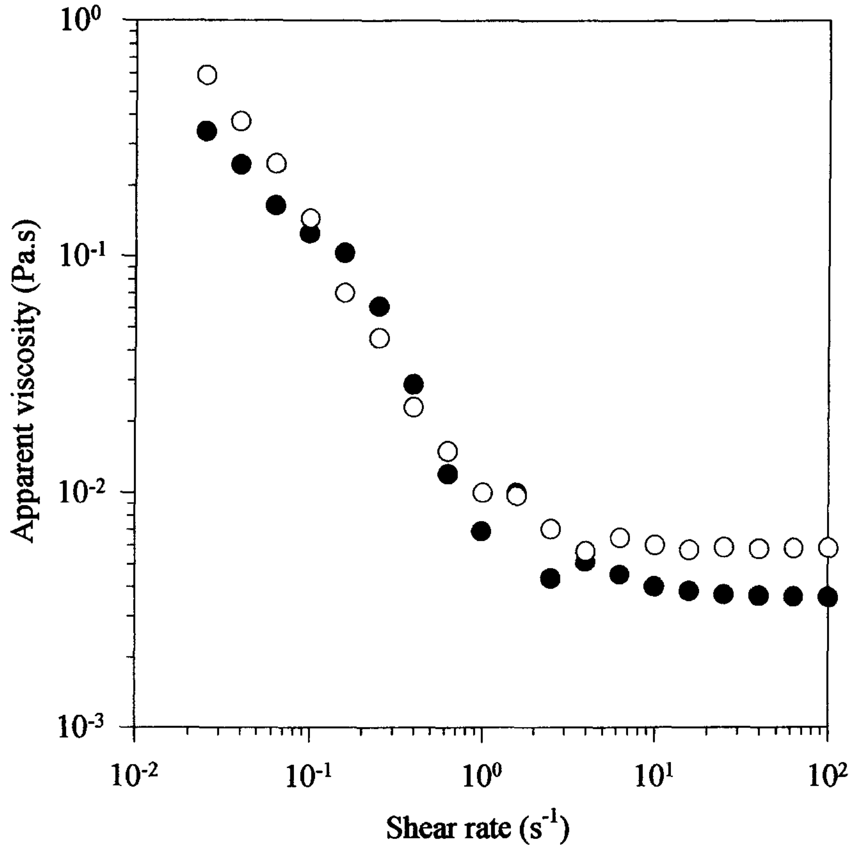

**Fig. 1.** Flow curves of barley β-glucan solutions of samples A (0.16%, black circles) and G (0.31%, white circles). Temperature: 25°C.

reached at lower shear rates, but instead a sharp increase in apparent viscosity occurred when the shear rate decreased. For the highest shear rates, the apparent viscosity reached a plateau. Thus, the value of apparent viscosity started at high values at low shear rates and dropped 100-fold down to the plateau value. It must be noticed that both curves were very close to each other: indeed, whereas the concentration of the solutions was different, their reduced concentration was the same (0.84). The same global trend was observed for the N sample solution at 1.5% and 25°C (Fig. 2) whereas in this case, the concentration was such that the measurement was made in the dilute regime. However, at low shear rate, the apparent viscosity value was very close to that obtained for the A and G samples.

The very high initial apparent viscosity and its drastic decrease with increasing shear rates can be ascribed to the existence of a structuration (presence of particles or network formation) in the solution. This structuration would be progressively disrupted by the increasing shearing forces, until a new state is reached in the plateau region. It must be pointed out that similar observations have been performed by Doublier and Wood (1995) on hydrolyzed oat gum rich in β-glucans with intrinsic viscosities in the same range as those of the samples used in this study. It is then possible to attribute the origin of the solution structuration to associations between β-glucan chains, an association which is labile and stress sensitive, which can be disrupted by mechanical shearing, the links created by the association being temporary. In the solution at rest, they could dissociate and reassociate freely giving an equilibrium structure, but in the highly sheared solution there is not enough time for the links to reform. Thus, there would be a transition from a solution with a developed structure to a solution with such structure relaxed on shearing.

The effect of the temperature on the flow curve has been determined for the lowest molecular weight β-glucan at the same concentration (sample N 1.5%, Fig. 2). The same global trends are obtained at 70°C when compared with the flow curve obtained at 25°C. At 70°C the apparent viscosity at low shear stress exhibited a higher value than that of the same sample at 25°C, which indicates that the association level is enhanced by an increase in the temperature. This result agrees with those obtained by light scattering of barley β-glucan in a previous paper (Gómez *et al.*, 1997a) and with the behaviour reported by Chambers *et al.* (1994), who described

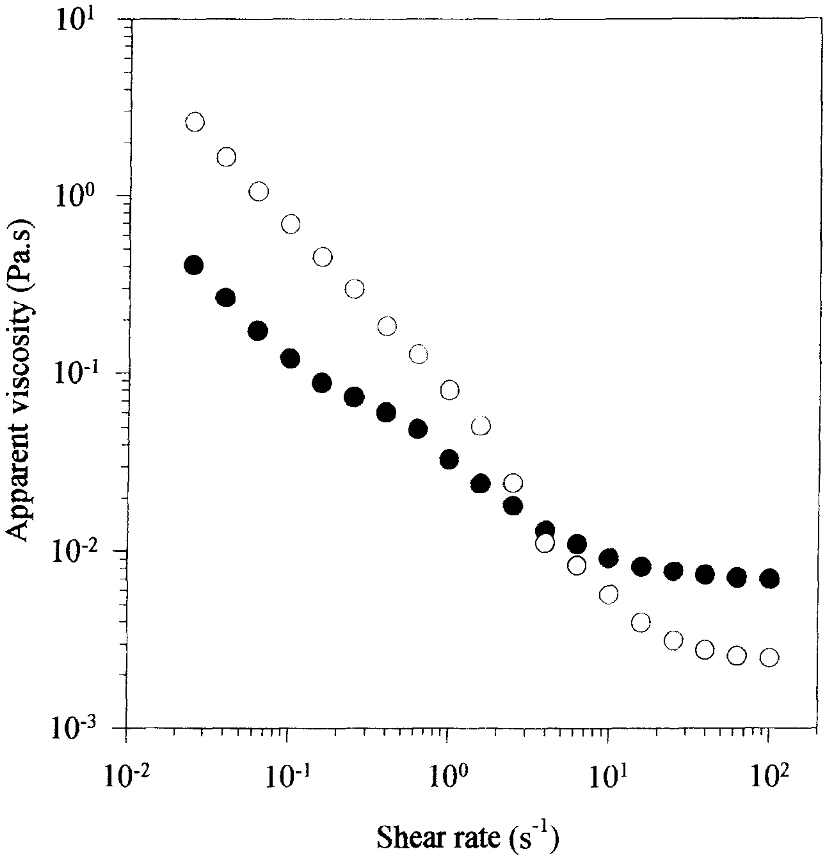

**Fig. 2.** Flow curves of barley $\beta$-glucan solutions (1.5%) of sample N at 25°C (black circles) and 70°C (white circles).

the formation of aggregates on heating a $\kappa$-carrageenan solution. On the other hand, the plateau obtained at 70°C at high shear rates was at lower viscosity values than at 25°C, which indicates that the association formed at high temperatures are more stress sensitive than those formed at 25°C. If the associating structure of $\beta$-glucan solutions is relaxed by shearing (here, at the high shear rates corresponding to the plateau region), then the same can be said when the solutions are studied to determined their intrisic viscosity. In the capillary viscometer used for such a purpose (Gómez *et al.*, 1997b), the shear rates are even higher (of the order of $10^3\,s^{-1}$) so that the argument of a shear-induced relaxation of associating structures can be used also to explain why a good correlation between intrinsic viscosities and molecular weight could be obtained in the cited work.

## Viscoelastic behaviour

Figure 3 shows the mechanical spectrum of a sample A solution at 0.16% and 25°C. Storage modulus ($G'$) was over loss modulus ($G''$) over the whole frequency range and both moduli presented a slight dependence upon the frequency ($\omega$), more important for $G''$ than for $G'$. Furthermore, at low frequency, $G'$ reached a constant value of approx. 0.02 Pa. This indicates that the system has a solid-like behaviour, the structure resulting from intermolecular interactions. From the very low $G'$ value obtained, it can be said that the gel formed has a very tenuous structure. This behaviour is consistent with the shape of the flow curve obtained for this sample under the same conditions. A plateau in $G'$ was also observed in mechanical spectra of oat $\beta$-glucans by Wikström *et al.* (1994) and in mechanical spectra of hydrolysed oat gum of lower intrinsic viscosities by Doublier and Wood (1995). In addition, they observed a crossover between $G'$ and $G''$ at high frequency.

Figure 4 shows the viscoelastic spectra of sample N at 1.5% at 25 and 70°C. At 25°C, the spectrum was different from that observed for sample A. Indeed, $G'$ and $G''$ were both highly dependent on the frequency ($\omega$). At high frequency, $G'$ was higher than $G''$; a crossover between both moduli occurred at a certain frequency value and at lower frequency $G''$ was higher than $G'$, with a frequency dependence of $G'$ as $\omega^2$ and of $G''$ as $\omega$. This behaviour is typical of that of a macromolecular solution without any structuration and has also been observed by Autio (1988) and by Doublier and Wood (1995) for unhydrolysed oat gum. This typical solution behaviour appears in contradiction with

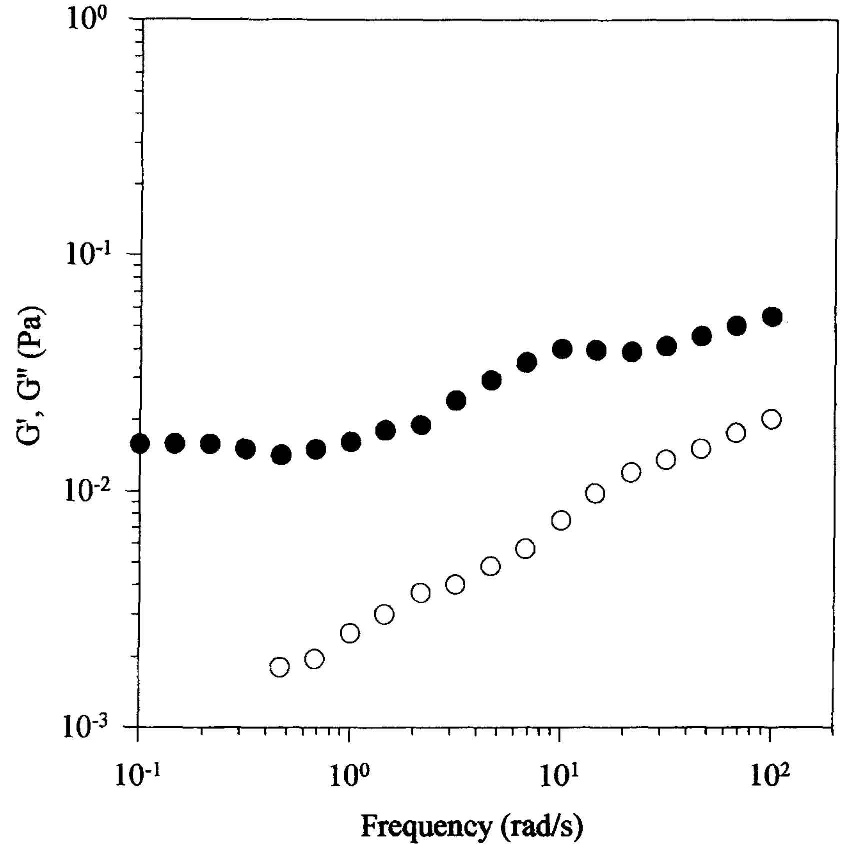

**Fig. 3.** Storage modulus (black circles) and loss modulus (white circles) as a function of frequency of a 0.16% barley β-glucan solution of sample A. Temperature: 25°C.

the result obtained by flow experiments on the same sample. An increase in the apparent viscosity at low shear rate was observed, whereas for macromolecular solutions, a constant value of the apparent viscosity is normally reached in the low shear rate range, corresponding to the newtonian viscosity.

An increase in temperature produced dramatic changes on the viscoelastic properties of the system. Indeed, at 70°C, the behaviour is close to that observed for sample A at 25°C, with $G'$ over $G''$ at low frequency and $G'$ reaching a constant value in this zone; it means that a weak gel structure has been formed by increasing the temperature and this was also observed for sample G, having a molecular weight substantially higher (Fig. 5). This observation is consistent with the results obtained by flow experiments, where a higher apparent viscosity value was reached at low shear rates at 70°C than at 25°C. It must be noticed that the $G'$ value obtained at the plateau in the low frequency range is approx. 0.3 Pa, higher than the value obtained for sample A, whereas the concentration of sample N is in the dilute regime and not in the semi-dilute one as for sample A. Furthermore, it seems that a crossover between $G'$ and $G''$ could occur at a frequency higher than $10^2$ rad/s for sample N at 70°C.

It appears then from these results that samples of different molecular weights can form weak gel structure in different temperature conditions. Association between chains and the eventual development of a tenuous gel can occur by physical links between adequate fragments of the chains. These fragments are probably short sequences of units, so that it is possible to find several sequences in any chain, even the shortest ones. The associating capability would thus be the same regardless of chain length, since every chain would have along its backbone enough such fragments to form several links. Even the participation of two consecutive fragments of a given chain in the formation of two links would be easier for the shorter chains because, once a link is formed, the rearrangement of the remaining chain to form another link would be easier for a short chain than for a long one. This would serve also as an explanation for the higher tendency to association which has been attributed in the literature to the higher mobility of the chains of lower molecular weight of β-glucan, as reported by Doublier and Wood (1995) in oat gum solutions.

The exact mechanism of interaction which governs the formation of links between chain fragments is an open question. The fragments could consist on (1→4) sequences, cellulose-like structures which are prone to crystallisation (Fincher & Stone, 1986). But, if this were the mechanism, then raising the temperature would lead

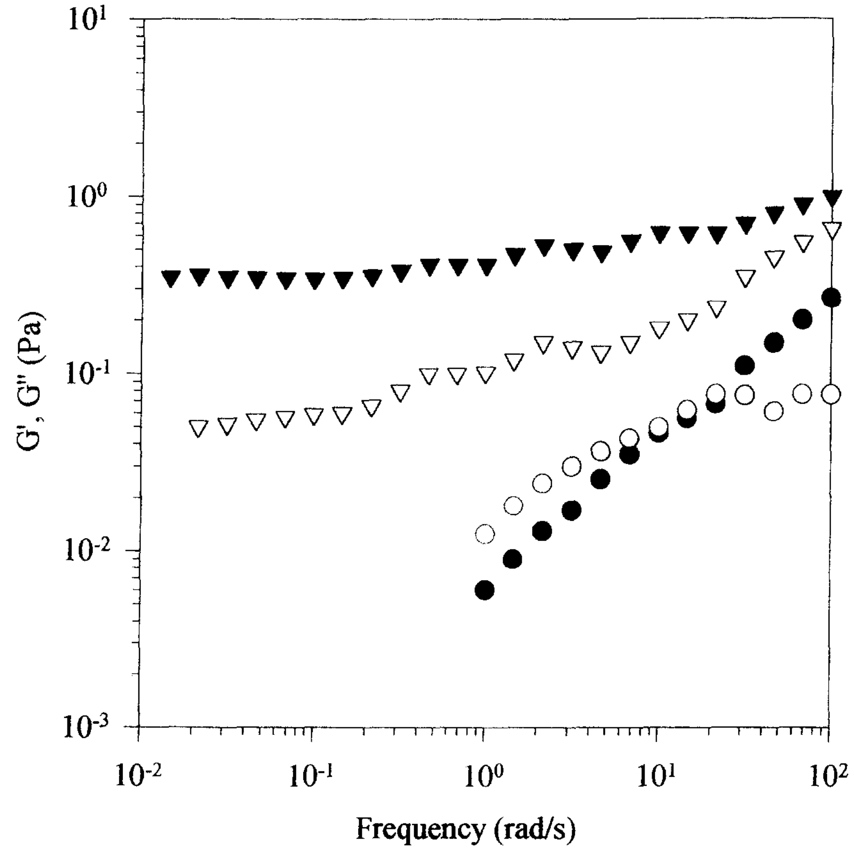

**Fig. 4.** Storage moduli (black symbols) and loss moduli (white symbols) as a function of frequency of 1.5% barley β-glucan solutions of sample N at 25°C (circles) and 70°C (triangles).

to the 'melting' of such crystallizable sequences and thus to a lower level of association. An opposite phenomenon was observed here. Our observation agrees with the results of light scattering experiments performed on dilute solution. First, let us explain that light scattering results can be disturbed by association, as has been reported by several authors (Vårum *et al.*, 1992; Berth *et al.*, 1994; Lang & Burchard, 1993), or can be free of such disturbance, depending on the operational mode of the technique. When light scattering was used as detector in a chromatographic line (that is, with the solution flowing inside a capillary tube), no disturbance due to association was detected. Namely, the angular dependence of scattered light was moderate, and the results regarding molecular weight and radius of gyration were reproducible. However, when light scattering was used in batch mode (static solutions held in vials) the angular dependence was steep with curvatures leading to extremely high and non-reproducible molecular weights, as well as to negative second virial coefficients (Gómez *et al.*, 1997a). These same solutions, contained in vials, when studied at higher temperature (70°C) gave much larger intensities of scattered light at all angles than at 25°C, thus indicating the existence of much larger species at high temperatures.

Another possible mechanism of interaction responsible for the aggregation could be hydrogen bonding between β-glucan fragments. It is known that increasing the temperature destroys hydrogen bonds. However, in the solution most of the hydrogen bonds present are water–water, next come the water–β-glucan hydrogen bonds, and finally the tiny number of hydrogen bonds between β-glucan chains (which are scarce and, at most, represent only a few percent of the total mass). Thus, the overall number of hydrogen bonds diminishes on increasing temperature, but it is perfectly possible that the detailed balance could lead to an increase of those hydrogen bonds between β-glucan fragments while the other types decrease.

To try to elucidate the aggregation phenomenon, the effect of salt addition to β-glucan solution was investigated. On Fig. 6 are shown the mechanical spectra of a solution of sample N at 1.5% in water and in 1.5% NaCl at 25°C. The presence of NaCl induced a transition of the system from a system with gel-like properties to a macromolecular solution. The change obtained could be related to the second mechanism described previously. According to this mechanism the aggregation would be produced by the formation of hydrogen links between β-glucan chains. In this case, the salt would weaken the hydrogen bonds, decreasing

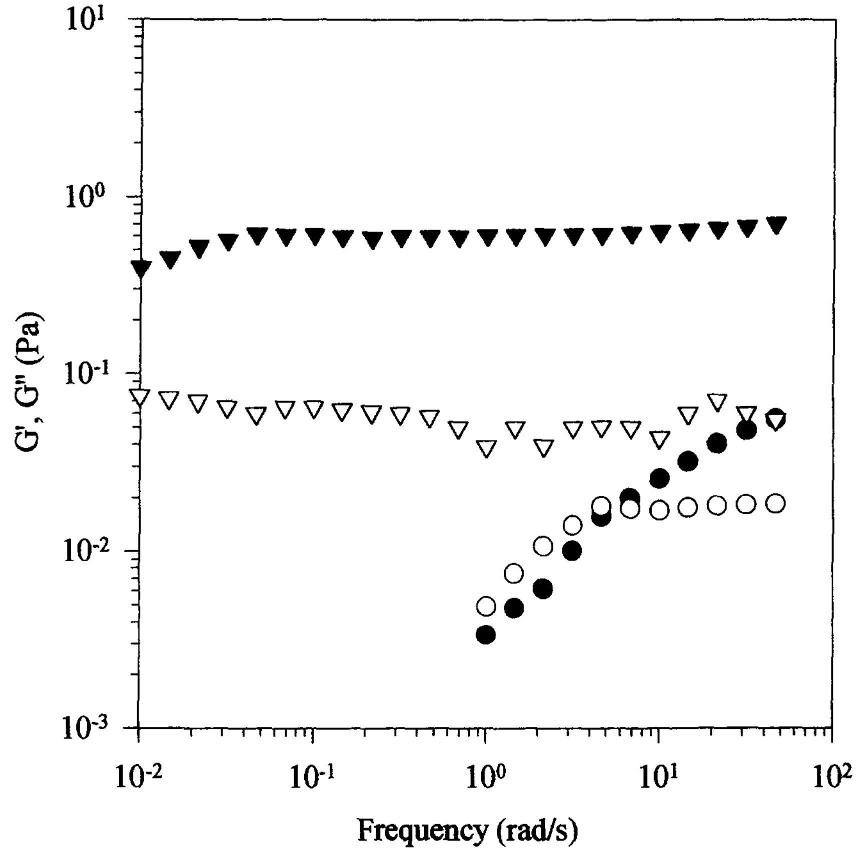

**Fig. 5.** Storage moduli (black symbols) and loss moduli (white symbols) as a function of frequency of 0.31% barley $\beta$-glucan solutions of sample G at 25°C (circles) and 70°C (triangles).

the structuration degree in the solution so that we obtain a spectrum typical of a macromolecular solution. Wikström *et al.* (1994) also observed strong changes in the viscoelastic properties of the oat $\beta$-glucan system in the presence of salt.

By comparing Fig. 4 and Fig. 6, it can be seen that the behaviour of the same sample in the same ionic and temperature conditions can be very different from one experiment to another (macromolecular solution or weak gel behaviour). After having carried out many series of experiences with different samples of $\beta$-glucans, we have detected considerable deficiencies in reproducibility of the mechanical spectra. We have reported here the more consistent results. The problems of reproducibility seem to be related to the mechanical and thermal history of the product (storage, waiting time before analysis,...). Indeed, sample N stored at 4°C and analysed at 25°C leads to the curves shown in Fig. 6, quite different from that shown in Fig. 4, for which the sample was stored at 20°C.

As a conclusion, barley $\beta$-glucan in water appears to form structured solutions owing to some aggregation mechanism involving temporary links between short chain fragments. This structuration has the following features: (a) it occurs both in the low and high molecular weight samples; (b) it is stress sensitive and relaxes upon shearing; (c) it can give rise to a tenuous gel-like behaviour in dynamic experiments; (d) this tenuous gel-like behaviour can be destroyed by added salt; (e) upon heating, the structuration appears to be more developed at high temperatures than at room temperature. However, the reproducibility of features (c) and (d) is very much affected by the sample history, which suggests the need for further study.

## ACKNOWLEDGEMENTS

This work was supported by Cerveceros de España and the Comisión Interministerial de Ciencia y Tecnología (CICyT), Project ALI 93-0067.

## REFERENCES

Autio, K. (1988) Rheological properties of solutions of oat $\beta$-glucans. In *Gums and stabilisers for the food industry*, ed. G. O. Phillips, P. A. Williams and D.J. de Wedlock, Vol. 4, pp.483–488. IRL Press, Oxford.

Autio, K., Myllymaki, O., Suortti, T., Saastamoinen, M. and Poutanen, K. (1992) Physical properties of (1→3),(1→4)-$\beta$-D-glucan preparates isolated from Finnish oat varieties. *Food Hydrocolloids* **5**, 513-522.

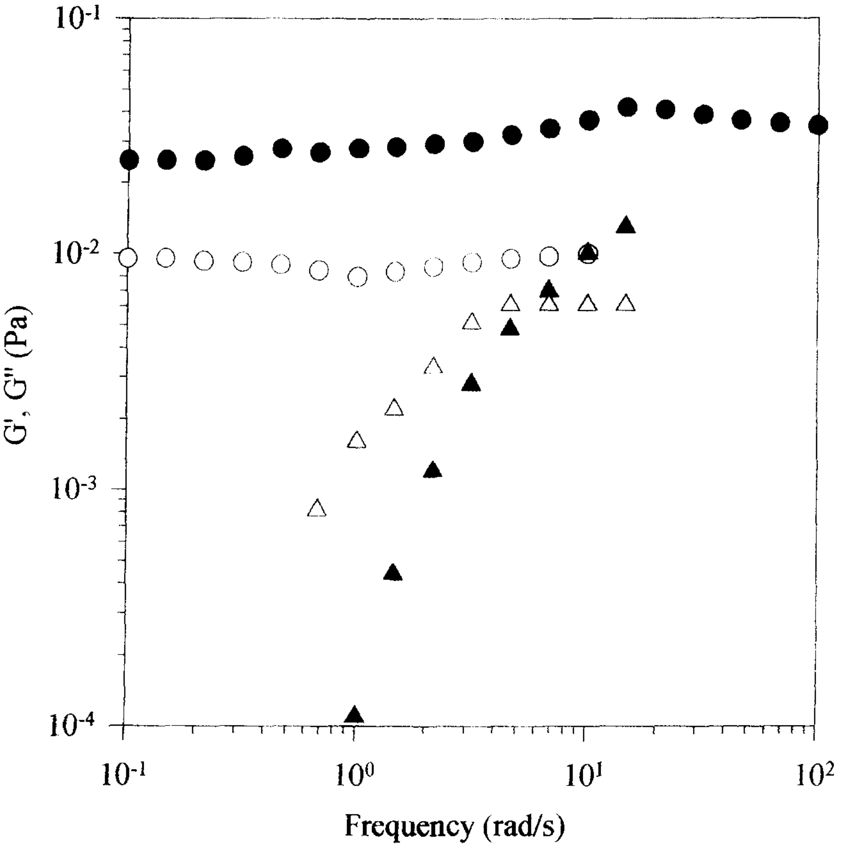

**Fig. 6.** Storage moduli (black symbols) and loss moduli (white symbols) as a function of frequency of 0.31% barley β-glucan solutions of sample N at 25°C, with 1.5% NaCl (triangles) and without NaCl (circles).

Bamforth, C.W. (1982) Barley β-glucans. Their role in malting and brewing. *Brewers Digest* **35,** 22–27.

Berth, G., Dautzenberg, H. and Rother, G. (1994) Static light scattering technique applied to pectin in dilute solution. Part I: Reliability of experiments and model calculations. *Carbohydrate Polymers* **25,** 177–185.

Chambers, S. J., Langdon, M. J., Williams, P. A. and White, R. J. (1994) Combined differential light scattering with size exclusion chromatography techniques for monitoring conformational changes in κ-carrageenan. In *Gums and Stabilisers for the Food Industry*, ed. G. O. Phillips, P. A. Williams and D. J. De Wedlock, Vol. 7, pp. 37–53. IRL Press, Oxford.

Doublier, J. L. and Wood, P. J. (1995) Rheological properties of aqueous solutions of (1→3),(1→4)-β-D-glucan from oats (*Avena sativa* L.). *Cereal Chemistry* **72**(4), 335–340.

Fincher, G. B. and Stone, B. A. (1986). Cell walls and their components in cereal grain technology. In *Advances in Cereal Science and Technology*, ed. Y. Pomeraz, Vol. VIII, pp. 207–295. American Association of Cereal Chemists, St. Paul, Minnesota, USA.

de Gennes, P. G. (1979) *Scaling Concepts in Polymer Physics*. Cornell University Press, Ithaca, New York.

Gómez, C., Navarro, A., Manzanares, P., Horta, A. and Carbonell, J. V. (1997a) Physical and structural properties of barley (1→3),(1→4)-β-D-glucan. I. Determination of molecular weight and macromolecular radius by light scattering. *Carbohydrate Polymers* **32,** 7–15.

Gómez, C., Navarro, A., Manzanares, P., Horta, A. and Carbonell, J. V. (1997b) Physical and structural properties of barley (1→3),(1→4)-β-D-glucan. II. Viscosity, chain stiffness and macromolecular dimensions. *Carbohydrate Polymers* **32,** 17–22.

Graessley, W. W. (1980) Polymer chain dimensions and the dependence of viscoelastic properties on concentration, molecular weight and solvent power. *Polymer* **21,** 258–262.

Grimm, A., Krüger, E. and Burchard, W. (1995) Solution properties of β-D-(1,3)(1,4)-glucan isolated from beer. *Carbohydrate Polymers* **27**(3), 205–214.

Izawa, M., Kano, Y. and Koshino, S. (1993) Relationship between structure and solubility of (1→3),(1→4)-β-D-glucan from barley. *Journal of the American Society for Brewing Chemistry* **51,** 123–127.

Lang, P. and Burchard, W. (1993) Structure and aggregation behavior of tamarind seed polisaccharide in aqueous solution. *Makromolekulare Chemie* **194,** 3157–3166.

McIntosh, G. H., Whyte, J., McArthur, R. and Nestel, P. J. (1991) Barley and wheat foods influence on plasma cholesterol concentrations in hypercholesterolemic men. *American Journal of Clinical Nutrition* **53,** 1205–1209.

Vårum, K. M., Smidsrød, O. and Brant, D. A. (1992) Light scattering reveals micelle-like aggregation in the (1→3),(1→4)-β-D-glucans from oat aleurone. *Food Hydrocolloids* **5,** 497–511.

Wikström, K., Lindahl, L., Andersson, R. and Westerlund, E. (1994) Rheological studies of water-soluble (1-3),(1-4)-β-D-glucans from milling fractions of oat. *Journal of Food Science* **59**(5), 1077–1080.

Wood, P. J., Weisz, J. and Blackwell, B. A. (1994) Structural studies of (1→3),(1→4)-β-D-glucans by $^{13}$C-nuclear magnetic resonance spectroscopy and by rapid analysis of cellulose-like regions using high-performance anion-exchange chromatography of oligosaccharides released by lichenase. *Cereal Chemistry* **71,** 301–307.