Effect of thermal Processing and Freeze-thaw on the Molecular Integrity of high molecular weight oat beta-glucan

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Abstract

Beta-glucans of the 1-3, 1-4 type are structural polysaccharides in the cell walls of plants and are used a soluble dietary fibre and to help reduce cholesterol levels in humans. Their properties are strongly linked with molecular weight and viscosity. We explore the stability with respect to these parameters to autoclaving and freeze-thaw processing. Using a combination of size-exclusion chromatography coupled to multi-angle light scattering (SEC-MALS) with viscometry and quasi-elastic or dynamic light scattering (DLS) we show that these molecules largely retain their molecular integrity

Introduction

Cardiovascular disease affects 7 million people in the UK and is associated with 155,000 deaths per year. The condition places a large financial burden on the NHS with an estimated annual cost of over £26 billion. Cardiovascular disease describes all the disorders of the heart and the circulation such as coronary heart disease, peripheral vascular disease and stroke (British Heart Foundation, 2015). The incomplete or entire blockage of a single or multiple coronary arteries which deliver blood to the muscular walls of the heart (the myocardium) is a hallmark of coronary heart disease. The primary cause of this condition is a disorder called atherosclerosis, in which fatty deposits accumulate in the intima of the arterial walls. Atherosclerosis can develop in all of the major arteries in the body and may bring about a loss of blood supply to the limbs. However, sudden blockages in the brain or the heart may lead to mortality. The first suggestion that somebody is suffering from coronary heart disease is a condition called angina. This is described as dull discomfort in the chest and the symptoms usually appear on exertion, excitement or stress. Unfortunately, angina does not develop in all cases and the first suggestion that somebody is suffering from coronary heart disease is a myocardial infarction or best known as a heart attack. This is a severe clinical event with a high mortality rate.

One of the first events associated with atherosclerosis is the accumulation of cholesterol in the arterial wall. The relationship between the concentration of cholesterol in the blood and coronary heart disease was identified due to the higher incidence of the disease in some countries. The link became even more convincing when the effects of blood cholesterol-lowering drugs known as statins were observed. These agents were used in people with higher risk of coronary heart disease and led to considerably decreased mortality from coronary heart disease (Frayn, 2010). Therefore, reduction in the total serum cholesterol and LDL cholesterol are accepted as important mechanisms for minimising the risk of cardiovascular disease. In fact, identifying cholesterol-lowering agents in the diet and encouraging the population to consume them seems to be a cheaper solution than chemical agents (Kumar *et al.*, 2012).

The European Food Safety Authority (EFSA) supports the claim that oat beta-glucan can lower the blood cholesterol concentration and is effective in reducing the risk of cardiovascular disease. The statement refers to foods which contain at least 3 g of oat beta-glucan and contribute to a balanced diet on a daily basis. The approval covers oat beta-glucan which is typically found in

the bran of oats and those forms incorporated into foods (EFSA, 2010). However, the claim does not pertain to pure beta-glucans. Due to the fact that the precise mechanisms behind the cholesterol-lowering effects of these polysaccharides are still debated, more effective forms of dietary beta-glucans are not manufactured. Food processing and storage conditions were reported to reduce the beneficial health properties of beta-glucans. The most common hypothesis behind the cardioprotective effects of beta-glucans is the rise in lumen viscosity (Lazaridou and Biliaderis, 2007). Therefore, further research into the physicochemical properties of beta-glucans such as molecular weight and viscosity during food processing and under storage conditions can produce a better insight into their cholesterol-reducing effects and allow the incorporation into a variety of commonly-consumed foods.

Molecular structure and physical properties of beta-glucans. Glucans are polymers of glucose, which can be linked by either α or β bonds. Beta 1-3, 1-4 glucans are a form of non-starch polysaccharide, which monomeric unit is D-glucose with β-glycosidic type of linkage. β-chemical bonds are not susceptible to enzymatic breakdown in the small intestine of vertebrates and beta-glucans can thus be classified as dietary fibre (Kumar *et al.*, 2012). The macromolecular structure of these polysaccharides varies according to the source and the method of isolation (Khoury *et al.*, 2012). Beta-glucans are found in cereal grains such as oat, barley and wheat. Their content is reported to be in the range of 1.8 to 5.5% of the total dry weight and even around 7% for some cultivars (Miller *et al.*, 1993), (Decker, Rose and Stewart, 2014). The majority of oat beta-glucans are located in the thick inner layer of the cell walls of the starchy endosperm and in the aleurone and the subaleurone layers (Khoury *et al.*, 2012). Oat beta-glucan is a linear polymer of D-glucose units with β-(1→3) and β-(1→4) glycosidic bonds (See Figure 1) (Wang and Ellis, 2014). Most of the glucose residues form cellotriosyl and cellotetraosyl units with β-(1→3) linkages. The proportion of trisaccharides to tetrasaccharides in β-glucans varies according to the cereal crop. For example, the proportion in oats is 2:1, while in barley and wheat – 3:1 and 4:1 (Cui *et al.*, 2000).

<Figure 1 near here>

The molecular weight and the conformation of beta-glucans greatly influence other physical characteristics such as solubility and viscosity. The molecular weight is a measurement of the size of the molecule, while the conformation describes its shape. There is an agreement within the literature that in aqueous environments oat beta-glucans are in a random coil conformation This is certainly the case for oat beta-glucans as shown in a recent study (Channell et al, 2018). Similarly to all polysaccharides, beta-glucans are polydisperse. This means that they have a distribution of molecular weight (Wang and Ellis, 2014; Harding et al, 2015). Differences in the estimates may be influenced by the variety and the environmental conditions (Andersson and Börjesdotter, 2011; Ajithkumar, Andersson, and Aman, 2005). The action of endogenous hydrolytic enzymes during processing and storage is another determinant. Inactivation of these enzymes in order to avoid depolymerisation enables the extraction of high molecular-weight beta-glucans (Wood, 1986; Doehlert, Zhang and Moore, 1997). Consequently, the methods of isolation and purification can also affect the molecular weight (Wang and Ellis, 2014).

The solubility or the extractability of a solid polysaccharide relates to its property to disperse in a wet environment and produce a homogenous dispersion under particular conditions. Solubility shows how much of the fraction is dissolved compared to the entire amount of the macromolecule in the initial solid state. A reduction in the molecular weight usually leads to a higher degree of extractability of a polymer. The solubility of beta-glucans is affected by the action of digestive enzymes, the pH and the temperature. Some of the extraction methods for beta-glucans are employed under conditions which are similar to the human gut environment such as a temperature of 37°C. These techniques are suitable for investigating the physiological properties of beta-glucans. An alternative method involves hot-water extraction at a high temperature of 100°C. Few parallels have been drawn between the results obtained from various extraction techniques (Wang and Ellis, 2014). Due to the fact that enhancing the extreme temperature leads to a rise in the solubility of the polymer, the hot-water extraction method results in a larger amount of solubilised beta-glucans (Johansson *et al.*, 2007). The sources of beta-glucan also affect the solubility. Beer *et al.* (1997) emphasised that the percentage of soluble beta-glucan was larger in rolled oats than in bran samples. This can be explained by the fact that the thin layer of beta-glucan in the subaleurone layer is enclosed by a thick insoluble cellulosic and hemicellulosic outer layer, which makes it far less extractable (Miller and Fulcher, 2011; Wood and Fulcher, 1978). Food processing also influences the solubility of the beta-glucan.

Extrusion, cooking of oat flakes and fermentation were reported to improve the extractability of the beta-glucan (Johansson *et al.*, 2007; Zhang, Bai and Zhang, 2011; Degutyte-Fomins, Sontag-Strohm and Salovaara, 2002). On the other hand, Beer *et al.* (1997) observed a decrease in soluble beta-glucan in oat muffins after frozen storage. The authors associated the reduction in extractable beta-glucan with the stabilisation of its chains through hydrogen bonding, which probably caused a more ordered structure.

The viscosity of beta-glucans solutions is influenced by the shear rate and the temperature. When the cholesterol-lowering effects of this polysaccharide are of interest, the shear rate refers to the degree of mixing of fluid caused by peristalsis at a body temperature of 37°C. Wang and Ellis (2014) have reported that higher concentrations of beta-glucan lead to a shear-thinning behaviour. This means that the higher the shear rate, the lower values of viscosity are obtained. On the other hand, lower concentrations of beta-glucan were independent of the shear rate, similarly to a Newtonian fluid. However, it must be taken into account that a more complex behaviour can be observed in the human GI tract. For example, if the consumed food also consists of insoluble particles, the suspension can show a shear-thinning behaviour even at low shear rates and significant rises in viscosity may be observed (Rayment, Ross-Murphy and Ellis, 2000; Rayment, Ross-Murphy and Ellis, 1995). Therefore, the majority of the studies remove the insoluble particles by centrifugation before measuring the viscosity. In addition, the analysis of the viscosity is usually employed at a random shear rate. The reason for that is that the shear rates in the human GI tract are unknown and probably differ according to the specific region of the intestine and the postprandial times (Wang and Ellis, 2014). Lazaridou and Biliaderis (2007) suggested that the intrinsic viscosity [η] of cereal beta-glucans is in the range of 28 to 960 ml/g. However, the exact level of viscosity needed to achieve the cardioprotective effects of beta-glucans has not been thoroughly investigated to date.

Evidence of blood cholesterol-lowering effect of beta-glucans. A meta-analysis of 126 studies indicated that oat beta-glucans can significantly lower the LDL and the serum total cholesterol. A daily consumption of 3g of oat beta-glucan was associated with conferring this beneficial physiological effect (Tiwari and Cummins, 2011). Despite the fact that numerous studies have been done to investigate the cholesterol-lowering effects of oat beta-glucans, the results remain

inconclusive. For example, Lovegrove *et al.* (2000) concluded that there was no notable difference in total serum cholesterol and LDL cholesterol of healthy subjects who consumed either oat or wheat beta-glucan. Panahi *et al.* (1997) demonstrated that high-viscosity beta-glucan drinks produced a positive physiological effect in comparison to low-viscosity beta-glucan. Similarly, Kerchkhoffs *et al.* (2003) showed that a drink with beta-glucan was more effective in lowering the blood cholesterol levels than bread and cookies fortified with oat beta-glucan. The main explanation for these discrepancies may be that depolymerisation of oat beta-glucan occurs during food processing, which leads to a change in molecular weight and solubility and hence a reduction in viscosity.

This study seeks to provide precise characterisation of the physical attributes of oat beta-glucans which are associated with their cholesterol-lowering effect. In particular, the paper is focused on the hydrodynamic characterisation of these polysaccharides in a dilute solution environment. Therefore, the two experimental methods that were employed in this study were viscometry and Dynamic Light Scattering. The viscosity and more specifically the intrinsic viscosity of oat beta-glucans was measured in response to storage, freezing and autoclaving. Size measurements were performed to identify if a change in the viscosity is related to a change in size. The study aims to improve the understanding of the required quantities and forms of beta-glucans that should be incorporated in a range of commonly consumed foods in order to observe the reduction of blood cholesterol levels.

Materials and Methods

BG90 ® oat beta-glucan was obtained from Swedish Oat Fiber AB (Väröbacka, Sweden). The sample was dissolved in distilled water with stirring for 2 hours at 80°C. Later on, the beta-glucan solution was dialysed against phosphate buffered saline (Fisher Scientific, UK) at pH 7.0 and an ionic strength of 0.1 M. The concentration of the sample was measured with an ATAGO Differential Refractometer (DD7 – Jencons Scientific, UK). The refractive increment value of 0.151 ml/g was used (Theisen *et al.*, 1999). The beta-glucan solutions were labelled and split into 4 different parts: BG90, BG90_AUTOCLAVED and BG90_FREEZETHAW. BG90 represents the native beta-glucan and was analysed immediately. BG90_AUTOCLAVED was autoclaved

for 15 minutes at 112°C with the Phoenix Autoclave (Rodwell Scientific Instruments, Essex, UK). BG90_FREEZETHAW was left into frozen storage at -4°C for 1 week. Prior to analysis, all of the samples were diluted to 6 different concentrations: 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/ml. When necessary, more concentrations were analysed such as 0.8 and 1 mg/ml. It must be noted that these concentrations were chosen to be below the c* value for beta-glucans. The c* represents the concentration at which the chains of the molecule interpenetrate and a change in the rheological behaviour can be observed (Harding et al, 2017).

Viscometry. Beta-glucan solutions ranging in concentration from 0.1 to 0.6 mg/ml were analysed with an Ostwald U-tube capillary viscometer at a temperature of (20.00$\pm$0.01) °C. This apparatus estimates the viscosity with a large degree of accuracy at low shear rates (Gillis *et al.*, 2015). The flow times of the solvent (t_o) (sec) and of the solution (t_s) (sec) were obtained by 8 measurements for each of the different concentrations. The relative viscosity (η_r) was calculated by using the formula $\eta_r = (t_s/t_o)*(\rho/\rho_o)$, where t_s was the average value of the flow time of the beta-glucan solution and t_o was the average value of the flow time of the solvent. Due to the low concentrations of the solution, a correction for the solution density was not required and hence, t_s/t_o was considered as equal to ρ/ρ_o (see Harding, 1997). However, the relative viscosity at a specified temperature is influenced by the concentration of the polysaccharide, its shape and the volume it takes. When the shape and the volume of the polysaccharide are under investigation, the concentration impact needs to be excluded (Harding, 1997). In order to achieve that, the reduced viscosity (ml/g) was measured by the formula

$$\eta_{red} = (\eta_r - 1)/c \quad (1)$$

where c is the solution concentration (g/ml). The inherent viscosity (ml/g) was obtained by the equation

$$\eta_{inh} = (\ln \eta_r)/c \quad (2)$$

Similarly to other macromolecules, beta-glucans behave non-ideally in a solution through co-exclusion. In order to exclude these non-ideality effects, η_{red} and η_{inh} were obtained at different concentrations and extrapolated to zero concentration, yielding the value of the intrinsic viscosity [η]. The following equations were used

$$\eta_{red} = [\eta]*(1 + K_H[\eta]c) \quad (3)$$

and

$$\eta_{inh} = [\eta]*(1 - K_K[\eta]c) \quad (4)$$

in which K_H is the Huggins constant (Huggins, 1942) and K_K is the Kraemer constant (Kraemer, 1938). Apart from this, the intrinsic viscosity was also calculated with the Solomon and Ciuta equation (Solomon and Ciuta. 1962), where

$$[\eta] \simeq (1/c)*[2\eta_{sp} - 2\ln(\eta_r)]^{1/2} \quad (5)$$

where η_{sp} refers is the specific viscosity ($\eta_r - 1$) (Harding, 1997).

Dynamic light scattering. Beta-glucan solutions ranging from 0.1 to 0.6 mg/ml were analysed with the Zetasizser Nano-ZS dynamic light scattering apparatus (Malvern Instruments Ltd., Malvern, UK). Each sample was measured three times at a scattering angle of 173° at a controlled temperature of (20.0 $\pm$ 0.1) °C. A high scattering angle was chosen to minimize contributions from dust or other supramolecular contaminants with the assumption that because of the low asymmetry (random coil conformation see Channel et al, 2018), rotational diffusion effects are small. Data was automatically collected and analysed using the Zetasizer (Version 6.20) software. The beta-glucan solutions were filtered with a 0.45µm pore size filter (Whatman, UK) in order to decrease the supramolecular aggregates. The samples were injected into scrupulously clean cuvettes and any dust was removed with Ambersil air dusters.

The z-average hydrodynamic radius $r_{h,z}$ were evaluated from the z-average translational diffusion coefficient D_{trans} = by the Stokes-Einstein equation:

$$r_{h,z} = k_B T/\{6\pi\eta D_{trans}\} \quad (6)$$

where k_B is the Boltzmann constant, T is absolute temperature, η is the viscosity of the solution and r_H is the hydrodynamic radius. The following assumptions were made (1) the solutions were sufficiently dilute that non-ideality effects were not significant – i.e. an extrapolation to zero concentration was not necessary (2) the particles were not asymmetric so there was no angular dependence of the measured D_{trans} values on rotational diffusion effects – i.e. an extrapolation to zero angle was not necessary (Burchard, 1992). The software also measures the ' polydispersity

factor', PF, namely the z-averaged normalized variance of the distribution of the diffusion coefficients (Pusey, 1974).

Results

Intrinsic viscosity

The intrinsic viscosities [η] of the three different beta-glucan solutions were measured following the Huggins, Kraemer and Solomon-Cuita relations and Figure 2 shows the values that were obtained. The intrinsic viscosities of BG90, BG90h and BG90f were found to be: (600± 30) ml/g, (550 ± 20) ml/g and (510±20) ml/g respectively. The values that were obtained from the autoclaved and the frozen storage samples were lower than the intrinsic viscosity of the native beta-glucan, with the autoclaving having by far the most severe effect.

<Figure 2 near here>

Dynamic light scattering (DLS)

Figure 3 shows the size and volume distribution of all beta-glucan solutions at a concentration of 0.6 mg/ml at 173° light scattering angle. It shows that the values of the hydrodynamic size were relatively in the same range.

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The mean hydrodynamic radius $r_{H,Z}$, which is the z-average of the measured size distribution, and the polydispersity factor PF (Pusey, 1974) are compared in Figure 3. All beta-glucan solutions at a concentration of 0.6 mg/ml were analysed. The z-average values that were obtained from the 173° light scattering angle were in the same range between 33 and 37 nm. No difference was observed between the samples that were autoclaved (BG90h) and those that were put into frozen

storage (BG90f). In terms of the polydispersity factor, there seemed to be no large difference between the native beta-glucan solutions and the treated samples at 173° light scattering angle. Nevertheless, the figure suggested that there was a change in the polydispersity factor of the autoclaved beta-glucan solutions in comparison to the samples that were kept in frozen and room temperature storage.

<Figure 3 here>

<to add: SEC-MALS data> [Guy Channell]

Discussion

In general, viscosity is a critical factor behind the texture of foods. However, this hydrodynamic feature is also associated with some positive health effects of oat beta-glucans such as the reduction of plasma cholesterol (Kivelä *et al.*, 2012). As postulated in the literature, beta-glucans have the potential to lower the absorption and reabsorption of cholesterol, bile acids and other lipid metabolites through raising the lumen viscosity and postponing the emptying of the GI tract. In addition, these polysaccharides delay the absorption of other biological molecules such as carbohydrates and decrease the level of glucose in the blood as well as the insulin secretion (Lazaridou and Biliaderis, 2007). The rise in viscosity may also lead to the reduction of emulsification of lipids in the stomach (Pasquier *et al.*, 1996). As a result, the precise determination of the viscosity of oat beta-glucans is extremely important when the cholesterol-lowering effects are under investigation. The first major step in this process is the measurement of the intrinsic viscosity [η] of the beta-glucan solution. This hydrodynamic characteristic provides valuable information on the size and the shape of the macromolecule (Harding, 1997). We find that BG90 has an intrinsic viscosity [η] of ~ (600 $\pm$ 20) ml/g. Our values are in accordance with the previous findings of Lazaridou and Biliaderis (2007) who pointed out that the intrinsic viscosity [η] of cereal beta-glucans is in the range of 28 to 960 ml/g. The values indicate that there was a relatively small reduction in intrinsic viscosity following autoclaving and frozen storage. The intrinsic viscosities that were obtained from these samples were within the same range: [η] = (550$\pm$30) ml/g for BG90h and [η] = (510$\pm$20) ml/g for BG90f, suggesting

that autoclaving and frozen storage of oat beta-glucans can lead to a similar hydrodynamic effects.

Due to their application in the food industry, oat beta-glucans are often sterilised through autoclaving. As a result, the impact of this heat treatment on the hydrodynamic characteristics of this polysaccharide has to be carefully explored (Wang *et al.*, 2001). The results of this study show that autoclaving of the beta-glucan samples led to a slight reduction in the value of the intrinsic viscosity [η]. This is in close agreement with the available literature (Wang *et al.*, 2001). The small decrease in intrinsic viscosity corresponds to the fact that unlike other water-soluble polysaccharides such as guar galactomannan and κ-carragenan, beta-glucans are much more resistant to heat treatment (Kök, Hill and Mitchell, 1999; Lai *et al.*, 2000). In general, the plausible reason behind the decrease in intrinsic viscosity may be a partial depolymerisation of the beta-glucan molecule and a reduction in the molecular weight. Several studies have demonstrated that autoclaving leads to molecular degradation of beta-glucans. For example, Wang and his colleagues (2001) have reported a decrease in the intrinsic viscosities [η] of oat beta-glucan samples following autoclaving due to polymer degradation. Dongowski *et al.* (2005) also demonstrated that this heat treatment can reduce the intrinsic viscosity and the molecular weight of beta-glucans that were extracted from flour. However, the reduction in intrinsic viscosity can also be attributed to the occurrence of oxidative reactions. There is evidence that autoclaving can cause the creation of oxidised functional groups such as carbonyl groups together with cleavage of the backbone (Kivelä *et al.*, 2012).

It is largely accepted that freezing is an effective preservation technique for many food products and thus its effects on oat beta-glucan solutions should be investigated more thoroughly. For instance, frozen storage can slow down the staling of bread because of decreased movement of the molecules and the retained moisture content (Stear, 1990). The analysis of the results of this study showed a slightly more pronounced decrease in intrinsic viscosity of oat beta-glucan solutions which were kept in frozen storage for 1 week. This is consistent with the findings of previous research (Moriartey, Temelli and Vasanthan, 2010). It must be noted that the samples were instantly put into frozen storage at a temperature of -4° C, which may have led to the rapid formation of small ice crystals (Lazaridou and Biliaderis, 2004). These structures may have aided in bringing the molecular bonds apart and reducing the molecular weight of the beta-glucan

solutions. In addition, the reduction of intrinsic viscosity of these samples can be related to the loss of beta-glucan solubility (Moriartey, Temelli and Vasanthan, 2010).

The intrinsic viscosity value of the samples stored at room temperature for two weeks was much higher than the value of the native beta-glucan solutions and this is in disagreement with the available literature (Papageorgiou *et al.*, 2005). Even though the intrinsic viscosity value doesn't disclose a lot of information on its own, higher intrinsic viscosities are associated with bigger molecules and/or a conformation which is more extended (Gillis *et al.*, 2015). However, this discrepancy may be due to a variety of reasons. For example, the containers in which the samples were stored were not sterilised. This may have promoted growth of various bacteria and hence a large increase in intrinsic viscosity. Apart from this, there might have been a problem with the U-tube viscometer, resulting in the miscalculation of the flow that time of the solution. The samples might have also been diluted down to the wrong concentration. Nevertheless, beta-glucans are also susceptible to the formation of aggregates. Sometimes a small change in the concentration or in the macromolecular size can cause substantial increase in the intrinsic viscosity (Lazaridou, Biliaderis and Izydorczyk, 2003). It has been suggested that the reason for that is the probably the fact that the backbone of the low molecular size solutions diffuses more freely. As a result, there is a higher chance of producing aggregates through chain associations (Doublier and Wood, 1995).

The analysis of the results from the dynamic light scattering shows that all of the beta-glucan solutions with a concentration of 0.6 mg/ml yielded different size distributions with at 173° light scattering angle. The biggest hydrodynamic size was observed in the non-treated sample. This corresponds to the higher intrinsic viscosity that was measured for the native samples in comparison to the autoclaved and the frozen storage samples. However, although the room temperature storage samples had the highest intrinsic viscosity, they didn't produce the largest hydrodynamic size. When the z-average values were explored, the mean hydrodynamic radius $r_{H,Z}$ of all of the beta-glucan solutions with a concentration of 0.6 mg/ml at 173° light scattering angle appeared to be within the same range. The lower scattering angle of 12.8° revealed that the mean hydrodynamic radius of the native beta-glucan solution is the smallest one. This is not consistent with the value of the intrinsic viscosity that was obtained. On the other hand, the mean hydrodynamic radii of the autoclaved and the frozen storage samples were the largest and closely

resembled each other. This similarity is analogous to the similar range of the intrinsic viscosities obtained from these samples.

Concluding Remarks

In conclusion, different storage and processing conditions definitely have an impact on the hydrodynamic characteristics of oat beta-glucan solutions. Both autoclaving and frozen storage reduce the intrinsic viscosity of the oat beta-glucan solutions and hence the cholesterol-lowering effect of this polysaccharide. The room temperature storage led to an increase in intrinsic viscosity, which is not in accordance with the literature. As a result, future experiments should repeat this method and compare the data. In addition, more hydrodynamic techniques can be employed to further characterise the beneficial health effects of oat beta-glucans. Future studies can also investigate other food processes such as extrusion.

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Legends to Figures

Figure 1. Part of the 1-3, 1-4 β-glucan molecule.

https://commons.wikimedia.org/wiki/File:Beta-1,3-1,4-glucan.png

Figure 2. Viscosity plots for (a) native BG90, autoclaved BG90h and freeze-thawed BG90f. Huggins (black line), Kraemer (red line) and Solomon-Ciuta (green line). Solvent; PBS buffer, pH6.8, I=0.10M

Figure 3. Dynamic Light Scattering of native and processed BG90. (a) z-averaged hydrodynamic radius (b) polydispersity factor (PF). Beta-glucan solutions at a concentration of 0.6 mg/ml. PBS buffer, pH6.8, I=0.10M. Scattering angle = 173^{o}. Rotational diffusion and non-ideality effects assumed to be negligible.

<SEC-MALS-viscostar results to be added>

Fig 1

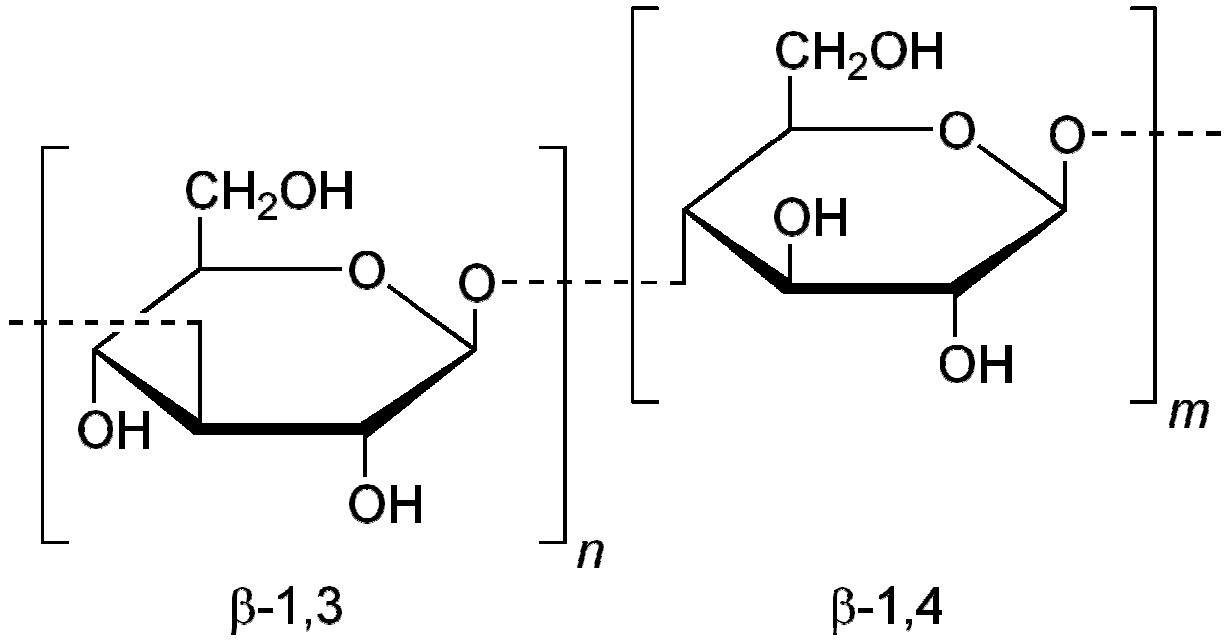


Fig 2a

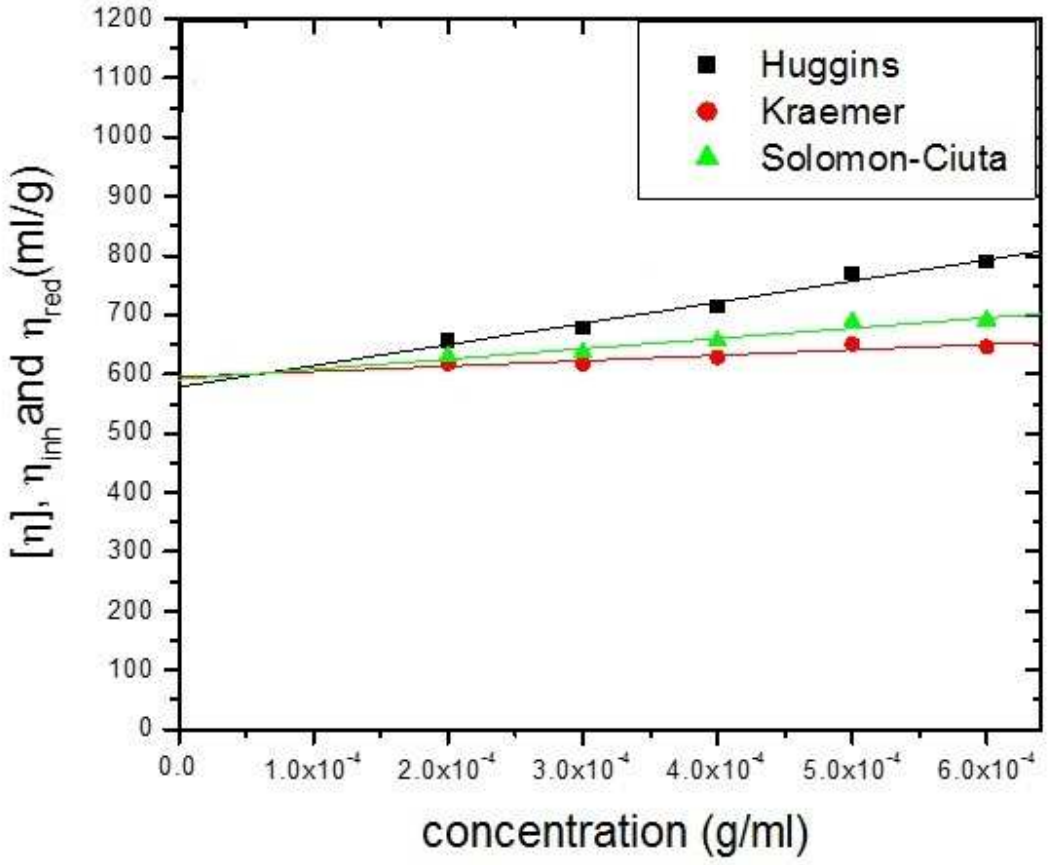

Huggins
Kraemer
Solomon-Ciuta
[η], η_inh and η_red(ml/g)
concentration (g/ml)
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
0.0
1.0x10⁻⁴
2.0x10⁻⁴
3.0x10⁻⁴
4.0x10⁻⁴
5.0x10⁻⁴
6.0x10⁻⁴

Fig 2b

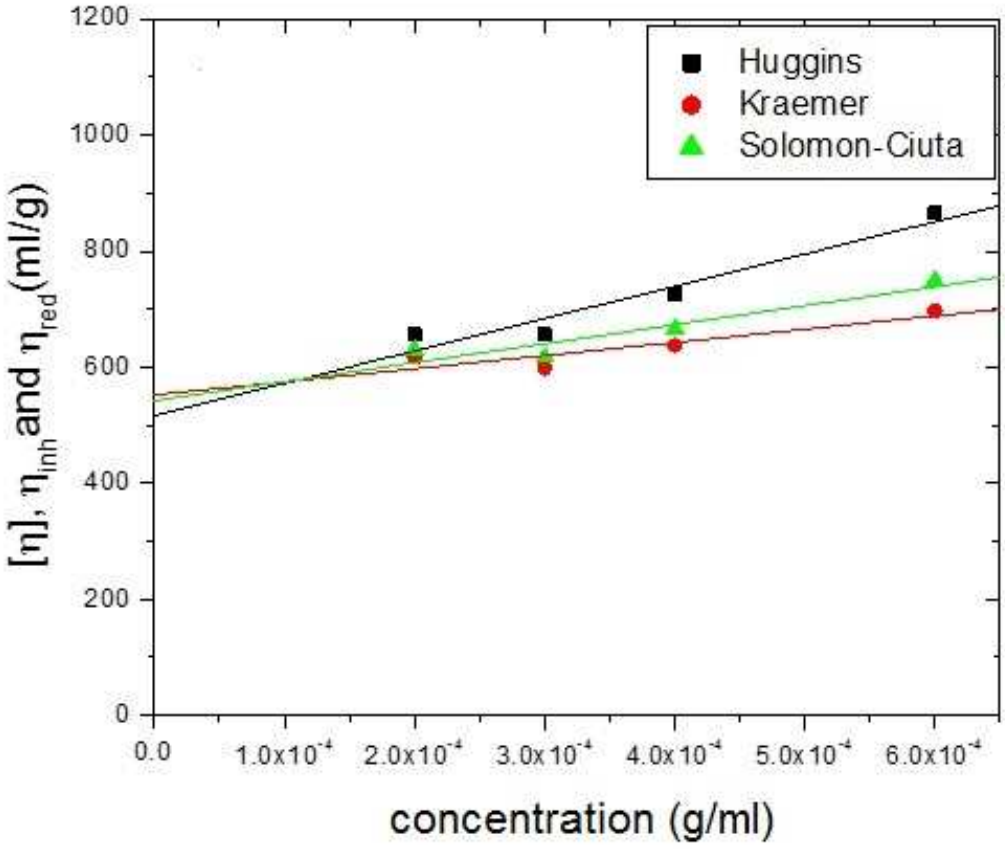

Huggins
Kraemer
Solomon-Ciuta
[η], η_inh and η_red(ml/g)
concentration (g/ml)
0
200
400
600
800
1000
1200
0.0
1.0x10^-4
2.0x10^-4
3.0x10^-4
4.0x10^-4
5.0x10^-4
6.0x10^-4

Fig 2c

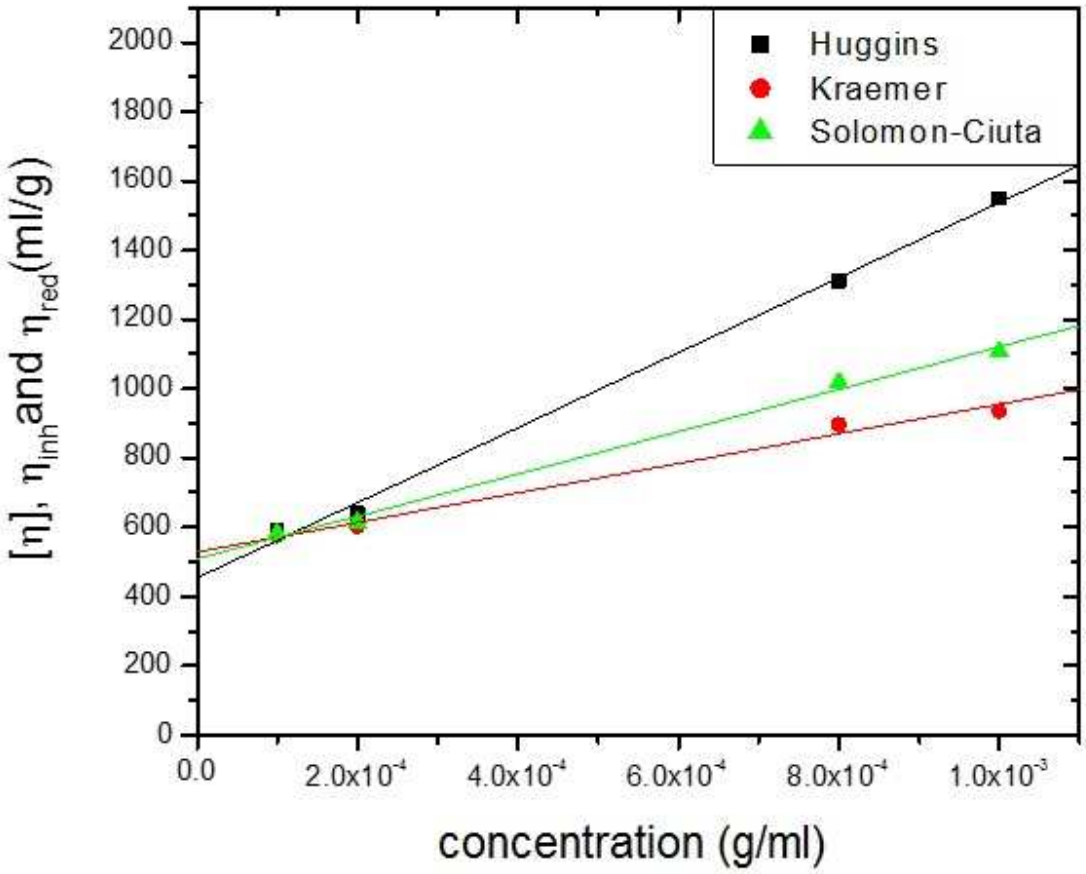

Huggins
Kraemer
Solomon-Ciuta
[η], η_inh and η_red(ml/g)
concentration (g/ml)
0
200
400
600
800
1000
1200
1400
1600
1800
2000
0.0
2.0x10^-4
4.0x10^-4
6.0x10^-4
8.0x10^-4
1.0x10^-3

Fig 3a

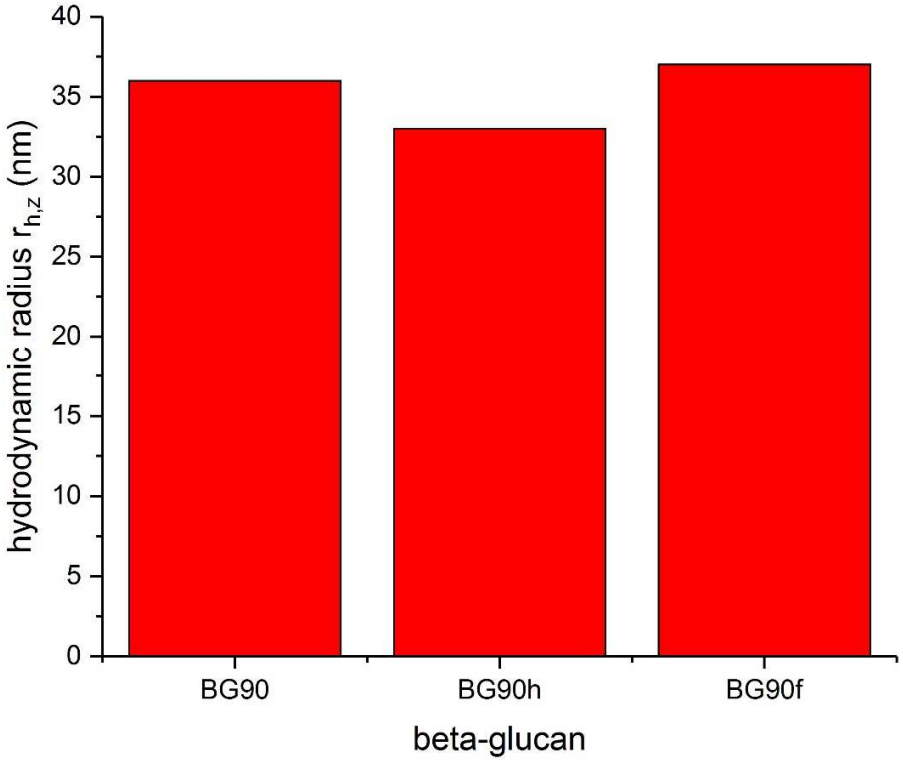
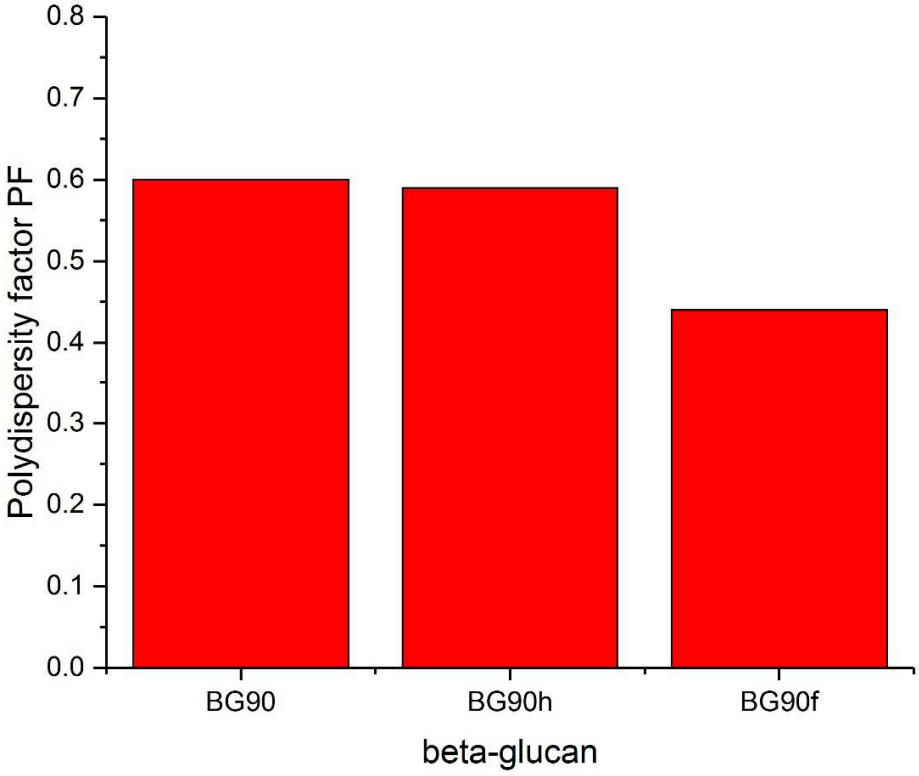

hydrodynamic radius $r_{h,z}$ (nm)
0
5
10
15
20
25
30
35
40
BG90
BG90h
BG90f
beta-glucan

Fig 3b


Polydispersity factor PF
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
BG90
BG90h
BG90f
beta-glucan