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Cereal (1→3, 1→4)-β-D-glucans – functional properties and molecular interactions

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ABSTRACT

In the current study we provide applicable knowledge on the elemental food functional properties exhibited by barley (Glucagel™) and oat (PromOat™) β-glucan products used as additives in foods. Rheological properties of β-glucans and molecular interaction with aroma compounds have been investigated in order to exploit the matrix properties of β-glucan in foods. Additionally, we have determined purity, size, and the structural characteristics of the two β-glucan products.

INTRODUCTION

Health benefits and functional properties of dietary fibres like mixed linkage (1→3,1→4)-β-D-glucans from cereals make them interesting food constituents. Due to their viscosity enhancing effect and the fact that they are able to form gels, they are applicable as food hydrocolloids and have among others been suggested as ingredients in health promoting functional foods[(1-4)]. High levels (i.e. 3-10 %) of β-glucans are present in the aleuronic and starchy endosperm cell walls of barley and oat. The polymers consist of long, linear chains of glucosyl residues linked via (1→3)- and (1→4)-β-glucosidic linkages (Fig. 1). Structure, molecular weight and concentration of the β-glucan influence the functional effect in foods[(5, 6)].

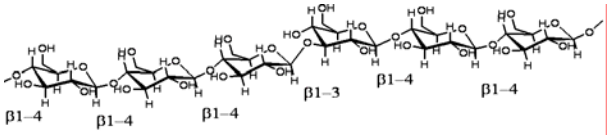

Figure 1. Primary structure of mixed linkage (1→3, 1→4)-β-D-glucan.

MATERIALS AND METHODS

Glucagel™ is a soluble barley β-glucan product supplied by GraceLinc Ltd. (Christchurch, New Zealand). PromOat™, supplied by Biovelop (Kimstad, Sweeden) is a soluble β-glucan product derived from oat bran.

Total β-glucan content was determined in triplicates using the Megazyme™ mixed linkage β-glucan assay kit (Megazyme International Ltd., Bray, Ireland).

Molecular weights were determined by gel permeation size exclusion chromatography (GPSEC) on a series of three columns; TSKgel GMPWxl (Tosoh Bioscience LLC, Montgomeryville, USA) Shodex B-806 HQ, and SB-806M HQ (Showa Denka K.K., Tokyo, Japan). The samples were calibrated against β-glucan standards (Megazyme International Ltd., Bray, Ireland) and Pullulans (Shodex

Standard P-82, Showa Denko K.K., Tokyo, Japan).

The ^{1}H NMR spectra were acquired on a Bruker Avance 400 (9.4 T) spectrometer (Bruker Biospin, Rheinstetten, Germany), operating at a Larmor frequency of 400.13 MHz for protons, using a High-Resolution Magic-Angle Spinning (HR-MAS) probe equipped with a 4 mm (o.d.) rotor. Experiments were performed at 75˚C using the *water* pulse sequence, a recycle delay of 4 s, 64 scans, a spin-rate of 7 kHz and a dwell time of 60.4 µs for acquisition of 32 k data points.

Viscosity measurements were conducted using a Stresstech rheometer (Reologica Instruments AB, Lund, Sweden), where temperature was regulated by a circulating water bath. Measurements were performed at shear rates between 1 and 30 s^{-1} for every 10˚C (10-80˚C). Activation energy was calculated from the Arrhenius equation.

Equilibrium dialysis was performed on mixtures of Glucagel™ (2.5% w/v), and various aroma compounds[a], that is vanillin derivatives (2 mM) (Sigma-Aldrich Co., Copenhagen, Denmark) using dialysis membranes passing 6-8 kDa globular molecules (Spectrum Laboratories Inc., Breda, The Netherlands) in 1 ml in-line equilibrium dialysis cells (Bel-Art products, Pequannock, U.S.A.). The dialysis cells were kept in a thermostat-regulated water bath (37 °C) from where sampling (10 µl) at times 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, and 300 min were performed. Absorbance of the dialysate samples was measured at 280 nm on a Spectra Max 190 microplate reader (Molecular Devices Corporation, Sunnyvale, U.S.A.). A curve derived from the exponential equation:

$$\text{Absorbance} = A\,(1-e^{-kt}) \qquad (1)$$

was fitted to data from each individual dialysis run, where A is the asymptotic or equilibrium value, t is the dialysis time and k is the dialysis rate constant. Asymptotic values were compared between dialyses of pure aroma compound and the mixture of β-glucan and aroma compound to quantitatively determine the level of dialysate retention (ΔA) by β-glucan. All samples were tested four times, that is, two replicates were dialysed 12 hours (day 1) after mixing and further two replicates were run after 36 hours (day 2).

RESULTS

Product characterisation

Total β-glucan content in Glucagel™ was 80.0 (±8.2) %, whereas the result for PromOat™ was 33.3 (±0.9) %. These figures approach the values stated by the suppliers, 75% and 30-40% respectively.

The molecular weight of β-glucan from Glucagel™ and PromOat™ determined as equivalent to pullulan standards were 0.191 × 10^{6} and 1.12 × 10^{6}, respectively. This gives a relative difference of 1:6.

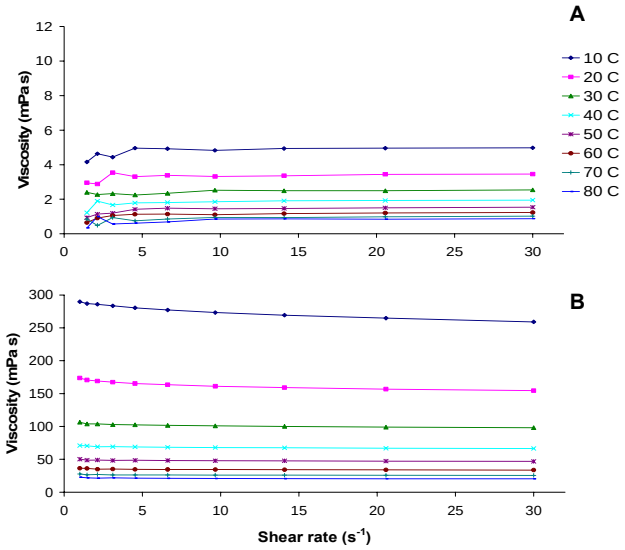

Figure 2. Viscosity of Glucagel™ solutions **A**: 1% and **B**: 5% (w/v) determined at 10-80˚C and shear rates of 1-30 s^{-1}. Values represent means of two replicates.

Proton nuclear magnetic resonance (^{1}H NMR) analysis showed significant differences in the ratio of β-(1→3) to β-(1→4) linkages between Glucagel™ and PromOat™, 41:59 (±1) % and 30:70 (±3) %, respectively. Furthermore, additional

carbohydrate residues were identified in both products (α- and β-glucose and α-dextrins), PromOat™ being the least purified product.

Rheological properties

The viscosity of Glucagel™ and PromOat™ at variable solution concentrations (1 and 5% w/v), temperatures (10-80˚C), and shear rates (1-30 s^{-1}) can be seen in Fig. 2 and 3. Viscosity decreased with temperature and increased with concentration for both β-glucan products.

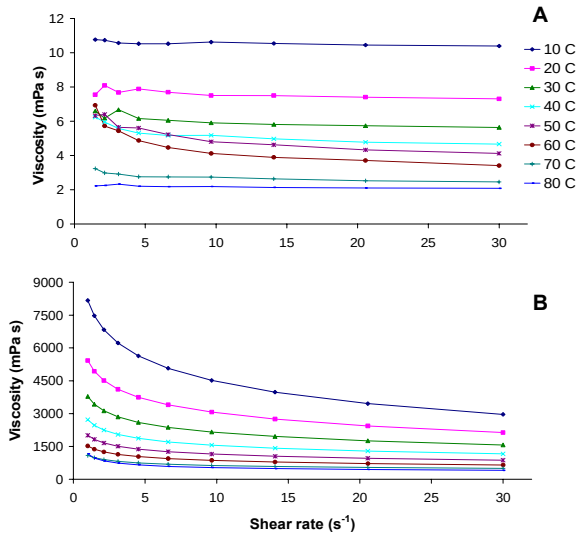

Figure 3. Viscosity of PromOat™ solutions **A**: 1% and **B**: 5% (w/v) determined at 10-80˚C and shear rates of 1-30 s^{-1}. Values represent means of two replicates.

Glucagel™ exhibited Newtonian flow behaviour whereas PromOat™ exhibited shear thinning flow behaviour at concentrations >1% (w/v).

Activation energy (E_a) was calculated from the Arrhenius equation[7]. The activation energies of Glucagel™ solutions were only concentration dependent, whereas PromOat™ solutions showed to be both concentration and shear rate dependent. For the 1% PromOat™ solutions, which generally exhibited Newtonian flow behaviour, there was a tendency of shear-thinning behaviour at 50 and 60˚C (Fig. 3A). This behaviour was also observed as temperature dependent activation energies for the latter solutions around 50 to 60˚C.

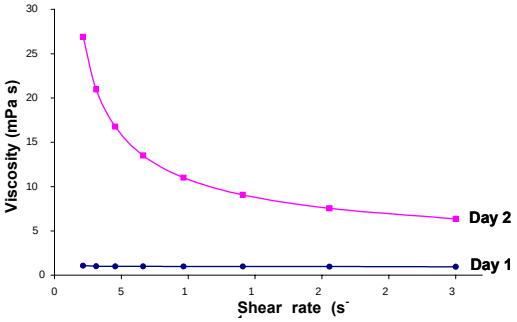

Figure 4. Viscosity of a 2.5% Glucagel™ solution at 37˚C mixed 1 and 2 days prior to measurements. Values represent means of two replicates.

As can be seen in Fig. 4, the viscosity of a 2.5% Glucagel™ solution at 37˚C increased significantly from day 1 to day 2. Hydrocolloids are expected to hydrate and increase solution viscosity upon mixing with water. In the 2.5% Glucagel™ solution we observed a 10-fold increase of viscosity at a shear rate of 30 s^{-1} within 24 hours. Additionally, the solution transformed from Newtonian to shear thinning flow behaviour.

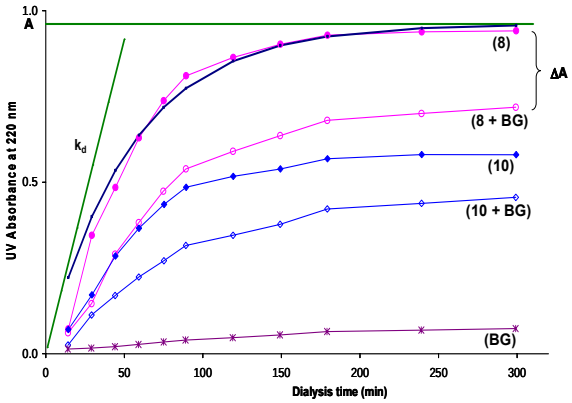

Figure 5. Dialysis curve of compound 8 and 10[a] in presence of 2.5% (w/v) Glucagel™ (BG). The green lines illustrate the curves for the dialysis rate (k) and the max absorbance (A) to be obtained at equilibrium of the dialysis of the exponential data curve (dark blue).

Equilibrium dialysis

The ability of the aroma compounds (1-10[a]) to bind to Glucagel™ was tested in the dialysis experiment (Fig 5). The β-glucan showed ability to bind all the aroma compounds tested as evidenced by a general retention of these in the β-glucan matrix (Fig 6). As can be seen in Fig. 6 the dialysate retention increased from day 1 to day 2. However, the different classes of chemical compounds (a total of 20 compounds were tested) such as ketones, aldehydes, phenols, esters, and acids did not differentiate significantly from each other and within the groups with respect to being retained by the β-glucan when data were analysed using molecular modelling and multivariate data analysis (results not shown).

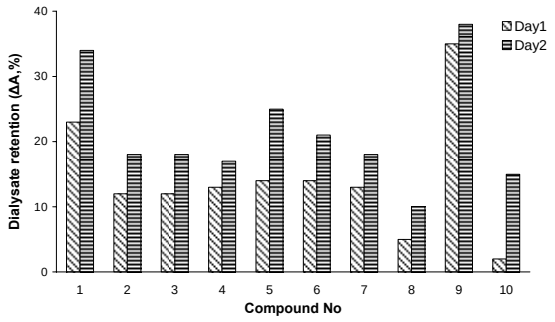

Figure 6. Percentage dialysate retention from mixed solutions of 2.5% (w/v) Glucagel™ and 10 different compounds[a]. Values represent means of two replicates.

DISCUSSION

Product characterisation

The molecular weight values reported in the present study agree with those generally reported for barley and oat β-glucans, 0.126-2.5 × 10^6 and 0.044-3 × 10^6, respectively[5, 6]. The ^{1}H NMR spectra revealed significant differences in the β-(1→3) to β-(1→4) linkage ratio between Glucagel™ and PromOat™. Proton NMR determinations are likely to give uncertain values for high molecular weight polymers[8] and this probably explains a part of the deviation seen between ratios obtained in the present study and values generally reported for cereal β-glucans elsewhere in the literature (30 and 70%). To verify a possible trend of barley β-glucans having a species specific β-(1→3) to β-(1→4) ratio further investigations are needed.

Rheological properties

The overall rheological features of Glucagel™ and PromOat™ corresponded to the low and high viscosity β-glucans examined by Burkus and Temelli[9], respectively. In the present study, no direct relationship between the increase in dry matter concentration and viscosity was found for neither of the products (Fig. 2 and 3). This suggests that the influence of product impurities on rheological properties needs to be further examined.

The method of fitting the Arrhenius equation to the viscosity data proved to be beneficial for the identification of deviating behaviour among the β-glucan solutions. It is likely that the relative large dextrin to β-glucan proportion in PromOat™ had an unexpected impact on product stability at low solution concentrations and around the starch gelatinisation temperature of 60˚C.

Equilibrium dialysis

The effect of hydrocolloids on aroma release from food may be affected due to two mechanisms. One is the physical entrapment of aroma within the food matrix[10]. Another mechanism involves interaction between the aroma compound and the hydrocolloid components, e.g. β-glucan[11]. In the present study the interaction could not be explained by simple correlation to the chemical groups made up by 20 tested compounds and the results

[a] **1:** 4'-Hydroxy-3'-methoxyacetophenone, **2:** 2,6-Di-tert-butyl-4-methylphenol, **3:** 4-Hydroxy-3-methoxybenzyl alcohol, **4:** 3-Hydroxy-4-methoxybenzyl alcohol, **5:** 3-Ethoxy-4-hydroxybenzaldehyde, **6:** 3,5-Dimethoxyphenol, **7:** 2,5-Dimethylphenol, **8:** Ethyl 4-ethoxy-2-hydroxybenzoate, **9:** 3,5-Dimethoxy-4-hydroxybenzaldehyde, **10:** 4-Hydroxy-3-metoxybenzoic acid

could not confirm or disprove the previous described hydrophobic binding or micelle capture of small molecules to β-glucan[10]. However, the time dependent increase in aroma compound retention by β-glucan indicates that tertiary and hygroscopic properties exhibited by the β-glucan matrix are important features (Fig. 4 and 6). More *in vitro* studies on the interaction between β-glucan and small molecules such as aroma compounds are warranted in order to establish the nature of the molecular interactions, e.g. involved in aroma retention and release from food matrices.

CONCLUSION

The two commercial β-glucan products, Glucagel™ and PromOat™ showed large differences in composition, molecular weight, structural characteristics, and rheological properties. Glucagel™ was determined as a low viscosity β-glucan product with Newtonian flow behaviour and PromOat™ was determined as a high viscosity β-glucan product with shear thinning flow behaviour at >0.1% (w/v) concentrations.

From the equilibrium dialysis experiment it was found that binding of the small molecules to β-glucans must depend on multiple characteristics that are not captured by a single molecular descriptor. However, aroma retention in the Glucagel™ matrixes increased with incubation time suggesting the network formation of the β-glucan polymers to be a crucial factor in aroma encapsulation. Based on the above, we have now begun developing a culinary attractive fibre enriched functional food product for testing in a human intervention study.

ACKNOWLEDGMENTS

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