Effects of β-Glucans and Environmental Factors on the Viscosities of Wort and Beer

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ABSTRACT

J. Inst. Brew. 110(2), 104–116, 2004

This paper reports on the influence of molecular weight and concentration of barley β-glucans on the rheological properties of wort and beer. Environmental conditions such as pH, maltose level in wort, ethanol content of beer, shearing and shearing temperature were also examined for their effects on wort and beer viscosities. In the range of 50–1000 mg/L, β-glucans increased solution viscosity linearly with both molecular weights (MW) of 31, 137, 250, 327, and 443 kDa and concentration. The influence of MW on the intrinsic viscosity of β-glucans followed the Mark-Houwink relationship. Shearing wort and beer at approximately 13,000 s^{-1} for 35 s was found to increase the wort viscosity but reduce beer viscosity. Shearing wort at 20°C influenced β-glucan viscosity more than shearing at 48°C and 76°C whereas the shearing temperature (0, 5 and 10°C) did not effect the viscosity of beer. At lower pHs, shearing was found to reduce the viscosity caused by β-glucans in wort but had no effect in beer. Higher concentrations of maltose in wort and ethanol in beer also increased the viscosity of β-glucan polymers. It was found that β-glucans had higher intrinsic viscosities in beer than in wort (5°C), and lower critical overlap concentrations (C*) in beer than in wort.

Key words: Beer, beta-glucan, ethanol, maltose, shear, viscosity, wort.

INTRODUCTION

The linear polymeric β-(1→3)(1→4)-D-glucans are the major component of barley endosperm cell walls. These polymers are broken down to various degrees during malting and mashing. When high in MW β-glucans are solubilized from the cell walls, these polymers increase the viscosity of wort and the resulting fermented beer. The term viscosity can be defined as "the resistance to flow". The shear rate ($\dot{\gamma}$), shear stress (σ) and viscosity (η) can be defined by considering a fluid between two parallel planes of area (A) and separated by a distance (d). When a force (F) is applied to move one plane at a given speed (V), the following relationships govern the flow field:

$$\dot{\gamma} = \frac{V}{d} \tag{1}$$

$$\sigma = \frac{F}{A} \tag{2}$$

The apparent viscosity (η) as defined by Newton is the ratio of shear stress to shear rate:

$$\eta = \frac{\sigma}{\dot{\gamma}} \tag{3}$$

Fluids with viscosities independent of shear rates are termed "Newtonian fluids". Wort and beer viscosities normally have little, if any, shear dependence. In the examination of the viscous behaviour of polymers such as β-glucans in beer and wort, the analysis is aided by the calculation of a solution's relative viscosity:

$$\eta_{rel} = \frac{\eta_{solution}}{\eta_{solvent}} \tag{4}$$

where η_{rel} is termed relative viscosity when the viscosity caused by β-glucan is concerned. A second viscous property often employed is termed the specific viscosity (η_{sp}):

$$\eta_{sp} = \frac{\eta_{solution} - \eta_{solvent}}{\eta_{solvent}} = \eta_{rel} - 1 \tag{5a}$$

In the situation where one is examining the effects of β-glucans on either wort or beer, one can consider the solvent to be "β-glucan free" wort or beer. Thus, equation 5a can be rewritten as:

$$\eta_{sp} = \frac{\eta_{BG} - \eta_{NB}}{\eta_{NB}} \tag{5b}$$

where η_{BG} is the viscosity of a sample containing β-glucan and η_{NB} is the viscosity of a sample containing no β-glucan (i.e., the β-glucan-free wort/beer is treated as a solvent for β-glucan polymers). An additional parameter used in polymer rheology is the intrinsic viscosity. It is calculated by undertaking a linear regression of η_{sp}/C on concentration C (the Huggins equation) or regressing $\ln(\eta_{rel})/C$ on C (the Kraemer equation) where the intercept is defined as an intrinsic viscosity of the polymer[27]. The intrinsic viscosity was also termed "limiting viscosity number" by the International Union of Pure and Applied Chemistry (IUPAC) in 1952[22]. Only a few brewing re-

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Publication no. G-2004-0524-220

searchers have adopted these recommended terms in the past 50 years[24,32]. However, the term intrinsic viscosity has been recommended by the Society of Rheology[11] and is preferred by most authors[13,17,23,40,42,49,53]. The usage of intrinsic viscosity can also be found in many recent textbooks[15,27,48]. Therefore, the term of intrinsic viscosity will be used in this paper. The intrinsic viscosity of a polymer is dependent on the shape and deformability of the molecules and can be used to study polymer behaviour in solution. The intrinsic viscosity is also dependent on MW by the Mark-Houwink law[27]:

$$[\eta] = K\,MW^{\alpha} \qquad (6)$$

where $[\eta]$ is intrinsic viscosity, MW is molecular weight, K and α are polymer dependent constants related to the degree of molecular flexibility and polymer-solvent interactions. The Mark-Houwink exponent (α) of coil-like polymers varies from 0.5 to 0.8 in poor and good solvents, respectively. For rod like polymers, the α value is higher and can be as high as 1.8 according to Doublier and Cuvelier[12]. The intrinsic viscosity of a 327 kDa barley β-glucan has been reported to vary from 4.64–8.62 dL/g in model worts[42]. A 65°C water-soluble barley β-glucan has a reported intrinsic viscosity of 4.04 dL/g[53]. The intrinsic viscosity of barley β-glucan preparations decreased from 4.6–5.2 dL/g to 0.28–1.77 dL/g during enzymatic degradation[13] presumably due to the decrease in molecular size. The value of the exponent α in the Mark-Houwink equation (Eq. 6) for oat β-glucans has been determined by Vårum *et al.* as 0.75, suggesting a random coil conformation[49].

Intrinsic viscosities are also useful in deriving the overlap or entanglement concentration (C*) of a polymer in a given solvent system. Such a "critical concentration" indicates the transition of β-glucan solutions between dilute ($C < C^*$) and semi-dilute regimes as well as the onset of significant molecular overlap and interchain-penetration[36]. When the relationship between barley β-glucan concentration and the relative viscosity of its suspension was examined, an overlap concentration (C*) has been estimated to be 0.42 g/L[31]. Recently, the C* value of a 327 kDa barley β-glucan has been reported to be 2.1–6.5 g/L in various buffers[40,41].

The concentration at which $C[\eta] \cong 1$ has been taken as C* of a polymer[26]. Goodwin and Hughes[15] have reported the following theoretical relationship:

$$C^* = 1.08/[\eta]. \qquad (7)$$

The effect of temperature on a solution's viscosity can be described by the Arrhenius relationship[48]:

$$\eta = A_f e^{E_a/RT} \qquad (8)$$

where A_f is a frequency factor; E_a is the activation energy; R is the universal gas constant; and T is absolute temperature. A high E_a value indicates a more sensitive response of the solution viscosity to temperature. By measuring the apparent viscosities of an oat bran β-glucan solution (0.375% w/w) at 20, 40, 60 and 80°C, the Arrhenius relationship indicated activation energies of 42 and 17 kJ/mol sheared at 5.81 and 581 s^{-1}, respectively[51]. The finding suggests that the temperature sensitivity of β-glucan viscosity is lower at high shear rates.

The shear dependence of β-glucan viscosity has been reported in the literature[4,6,14,19,52]. Several factors may alter the flow behavior of β-glucan solutions (i.e., their deviation from Newtonian flow). The attachment of protein to β-glucan molecules causes more pronounced shear-thinning behavior (i.e., lower apparent viscosity at higher shear rates)[4,50]. Sucrose also increases the apparent viscosity of oat β-glucan solutions even at low concentrations and low shear rates[3] probably due to both the contribution of sucrose to the solution viscosity and its interactions with the β-glucan polymer. Ethanol, pH and maltose have also shown significant effects ($p < 0.05$) on the viscosity of a 0.5% w/w β-glucan suspension[43,44].

Mechanical shearing during brewing can influence the flow behavior and the precipitation of β-glucan polymers. A shear rate of 400 s^{-1} has been suggested as an "average" rate to be used to simulate that occurring in commercial beer production[40,43,44,47]. Because shearing enhances aggregation and precipitation of β-glucans[29,38,43,44], pumping of fermenting wort, centrifuging of beer, and cross-flow membrane filtration (that employs high rates of tangential flow) are believed to impair beer filtration rates particularly during membrane filtration.

Viscosities of wort and beer influence the brewing process and beer quality in several aspects. For instance, beer viscosity positively contributes to its body[21]. As well, a high beer viscosity can retard the drainage of liquid from foam bubble walls and lead to better head retention of beer foams[33]. However, high viscosities of wort and beer can lower the efficiency of many unit operations including mixing and stirring of mashes, pumping, wort separation from spent grains, wort boiling, cooling of wort, "mixing" of beer by the convective current in fermenters, beer clarification (i.e., sedimentation of yeast cells and colloidal particles), as well as beer filtration. Wort viscosity has been reported to vary between 1.59–5.16 mPa · s for specific gravity (SG) 1.030–1.100 while beer viscosity can vary between 1.45–1.96 mPa · s at 20°C[20,40]. In practice, wort viscosity is proportional to its specific gravity[2,39]. Beta-glucan-free wort showed an exponential increase in viscosity at 20°C in the range of 2–24°P[25]. High concentrations of maltose (6–15% w/v) increased the viscosity of 0.1–0.5% barley β-glucans in artificial solvent systems[16,43]. Interestingly, the viscosity was reported to decrease when maltose increased from 2% to 5%[16]. The presence of maltose not only increases the solution viscosity, but also affects the dispersion/aggregation state of β-glucan polymers[16,28]. However, literature reports are contradictory as to how the aggregation of β-glucan polymers is affected by maltose level. The addition of 7–14% w/v maltose inhibited the precipitation of β-glucans by 20% v/v of ethanol although 3.3% w/v maltose did not[28]. Increasing maltose from 6% w/v to 10% w/v increased the intrinsic viscosity of β-glucan solutions as well as the apparent MW (i.e., the degree of aggregation) of β-glucans[16,17]. However, wort is usually processed at temperatures of 50 to 100°C whereas beer is processed at –1 to 4°C. Thus, the viscosity of *hot* wort is often lower than that of beer (although wort contains high levels of viscosifying fermentable sugars). Wort separation with lauter tuns or mash filters is the brewhouse unit operation which is most affected by high wort viscosities. Slow wort separation can result in longer equipment residence times making it difficult to complete scheduled brews on time. Downstream from fermentation, high beer viscosity values can cause serious filtration difficulties.

The purpose of this study was to determine how β-glucan molecular weight and concentration affect the viscosities of wort and beer, under various conditions of shearing, temperature, and wort and beer composition. The specific viscosity of β-glucans in wort and beer was examined to study the behaviour of these polymers. By determining the intrinsic viscosity of barley β-glucans in wort and beer, the critical overlap concentration of these polymers was also investigated.

MATERIALS AND METHODS

Barley β-glucans having weight average molecular weights of 31 kDa (Lot No. 41101), 137 kDa (Lot No. 90401), 250 kDa (Lot 60501) 327 kDa (Lot No. 40301) and 443 kDa (Lot No. 90501) were purchased from Megazyme International Ireland Ltd. (Bray, IRL). All β-glucan samples were dried at 50°C under a vacuum of 760 mm Hg for 6 h until constant weights were reached. Desiccated β-glucans were stored dry at 20°C. Purified lichenase (EC 3.2.1.73) from *Bacillus subtilis* was also purchased from Megazyme (Bray, IRL). Lichenase is a bacterial endo-(1→3)(1→4)-β-glucanase, which specifically cleaves the β-1,4-linkage of the O-3-substituted glucose units[34] and it has the identical specificity as barley malt (1→3)(1→4)-β-D-glucan 4-glucanohydrolase[37]. The name "lichenase" is used in this paper to distinguish this enzyme from other types and other sources of β-glucanases. A second commercial β-glucanase prepared from *B. subtilis,* Filtrase B™, was kindly provided by Gist-Brocades France S.A. (DSM Food Specialties, Seclin, FRA). A low β-glucan pale malt (*cultivar* Harrington, 1998 crop year, and 100 mg/L β-glucan in Congress wort[1]) was commercially prepared at Canada Malting Co. Ltd. (Calgary, AB). Malt samples were stored in airtight containers at 4°C until used (up to 12 months).

Preparation of β-glucan-free wort

The "low β-glucan" Harrington pale malt containing 100 mg/L β-glucan described above was used to prepare wort. An Osterizer 8 Food Processor (Sunbeam Corporation Canada Ltd., Mississauga, ON) was set at "Chop" and used to grind 125 g of malt for 20 s.

Mashing was carried out in a 20.5 L stainless steel container heated on an adjustable 1 kW hot plate. The mashing procedure was modified from the EBC Congress mashing method[1]. The mashing process started with mixing 3.0 kg of ground malt with 9 L of double distilled and de-ionized water (DDW) at 47°C leading to a mash temperature at 45°C. The initial pH value of the natural mash was 5.4. The mash was kept at 45 ± 1°C for 30 min followed by heating at 1 C°/min for 25 min up to 70°C. The mash was then held at 70 ± 1°C for 60 min for saccharification. The mash was gently stirred manually with a 6 × 9 cm paddle, constantly during heating and for 10 s every 5 min while the temperature maintained at 45°C and 70°C. Finally, the mash temperature was brought to 76°C to separate wort from the spent grains by lautering. The small-scale lautering was performed using a ceramic flat-bottomed Büchner filter (24 cm diameter and 10 cm height) using one layer of Whatman No. 2 filter paper. Lautering was accelerated by application of 500 mm Hg of vacuum. The resulting clarified wort was collected and termed the "first wort" (15°P). Two sparges (distilled water at 76°C, 3 L and 2 L, respectively) were used to collect two aliquots, termed second and third worts (of 7°P and 3.5°P, respectively). Pooled wort was boiled for 1 h allowing 25 mL/100 mL of evaporation. The concentrated hot wort was filtered through Whatman No. 1 filter paper to remove the hot trub. Approximately 9 L of high gravity (16.9°P) wort was thus collected.

To remove any β-glucan from the high gravity wort, a β-glucanase preparation (Filtrase B, containing at least 180 U/g, with maltodextrin as carrier, DSM Food Specialties, Seclin, FRA) was added at 0.1 g/L wort to hydrolyze β-glucan in the pooled, collected wort (pH 5.4) for 3 h at 48°C. Beta-glucan in the treated wort was undetectable by Congo red assay[25,30]. This β-glucan-free wort was dispersed into 1000 mL Erlenmeyer flasks, covered with four layers of aluminum foil, and autoclaved at 121°C for 15 min to ensure inactivation of any remaining β-glucanase. The wort was then filtered through a "M" Kimax Büchner funnel (Fisher Scientific Co. Ltd., Nepean, ON) with 1.0 g/100 mL of diatomaceous earth (Sigma-Aldrich Canada Ltd., Oakville, ON) as a filter aid to remove coagulated and suspended particles. The original wort gravity was 16.9°P at 20°C. The clarified wort was preserved with 100 mg/L of NaN_3 and stored at 20°C until used (up to 16 weeks). This wort was found to be stable (i.e., no evidence of microbial growth or haze development) during the storage.

Preparation of wort samples at various β-glucan levels

Commercial "Megazyme" barley β-glucans of various MWs (i.e., 31, 137, 250, 327 and 443 kDa) were dissolved in DDW with 100 mg/L of NaN_3 to make 0.500 g/100 mL stock solutions. The pH value of the high SG wort was 5.28 and was adjusted to pH 5.40 by adding 0.5 mL of 1 *N* NaOH per liter of wort. An adequate amount of β-glucan stock solution was mixed with the pH 5.4, β-glucan-free wort (16.9°P), and DDW (pH adjusted to 5.4) to prepare β-glucan solutions of 0, 50, 100, 200, 400, 600, 800 and 1000 mg/L at 12.0°P and pH 5.4. Duplicate wort samples (at five MWs and seven concentrations) were sheared as described later. Sheared and unsheared wort samples were then examined for their viscosities.

To investigate the effect of β-glucans at various pH values and maltose levels, the 16.9°P, β-glucan-free wort and a 443 kDa β-glucan solution (0.500 g/100 mL) were used to prepare 8°P wort containing 600 mg/L of β-glucan. Maltose (Sigma Chemical Co.) was incorporated into the 8°P wort to prepare 12°P and 18°P worts, which differed only in maltose content from the 8°P wort. The maltose content of the 8°P wort was 6.1% w/w as determined by Fehling's assay[1]. Therefore, the 8°P, 12°P and 18°P worts contained 6.1%, 10.1% and 16.1% w/w of maltose, respectively. Each wort sample was divided into three equal portions and the pH value was adjusted from 4.95 to 4.0 (with 5.40 mL of 1 *N* HCl per liter of wort), 5.4 (with 0.16 mL of 1 *N* NaOH per liter of wort) and 6.8 (with 9.60 mL of 1 *N* NaOH per liter of wort), respectively. The ionic strengths of worts differed between 4.2–9.4 m*M*, due to pH adjustment. This minor difference in ionic strength was assumed not to affect experimental results. These worts were then examined with a three factor, three level experimental design to investigate the effects of pH, maltose content and shearing temperature. A 1.0 *M* sodium azide solution was added to the worts to adjust the final concentration of NaN_3 to 100 mg/L. Measurements for each sample were completed within 5 days after preparation although no microbial growth was observed for at least 6 months.

Preparation of β-glucan-free beer

A commercial lager beer (ethanol content 5% v/v, Oland Brewery Ltd., Halifax, NS) was purchased locally and used as the beer base in which existing β-glucan was hydrolyzed. The real extract was determined to be 3.3% with the ASBC method[1]. Beer was first degassed by filtering through Whatman No. 1 filter paper under a vacuum of 20 mm Hg. The degassed beer (25 L) was then boiled for 2 h to remove ethanol and other volatile components (which resulted in 55 g of wet precipitate). The concentrated beer was cooled and held at 50°C while lichenase at 0.1 U/mL (Megazyme, Bray, IRL) was added to hydrolyze β-glucan for 30 min until the residual β-glucans were undetectable by Congo red dye. One lichenase activity unit (U) is defined as the amount of enzyme required to release 1 μmol of glucose per min from a barley β-glucan substrate at 10 mg/mL[35]. The enzyme was then inactivated by autoclaving in Erlenmeyer flasks covered with 4 layers of aluminum foil at 121°C for 15 min. The concentrate had an extract content of 7.14°P and was kept at 20°C until used (up to 2 months). There were no stability problems observed during the storage.

Preparation of beer samples with various β-glucan levels

Beta-glucans (31, 137, 250, 327 and 443 kDa) were dissolved in DDW at a concentration of 0.300 g/100mL. Degassed "beer" samples were prepared by mixing adequate amounts of the β-glucan stock, beer concentrate, anhydrous ethanol and DDW. To investigate the effect of MW and concentration of β-glucans on beer viscosity, β-glucans (MWs at 31, 137, 250, 327 and 443 kDa) were included at concentrations of 0, 50, 100, 200, 400, 600, 800 and 1000 mg/L in beer, which contained 5.0% v/v of ethanol and 3.3% w/w of real extract at pH 4.2. Beer samples were also subjected to shearing and their viscometric properties examined.

Beer samples containing 600 mg/L of 443 kDa β-glucan were prepared to investigate the effect of pH (3.8, 4.2 and 5.4) and ethanol (0, 5 and 10% v/v) on beer viscosity and other properties discussed in the following sections. These samples were also subjected to shearing at different temperatures (0, 5 and 10°C). Fresh beer samples (sodium azide was not used as a preservative) were stored at 5°C and analyzed within 3 days (samples were stable until tested).

Shearing test of wort and beer samples

A Lourdes blender (Model MM-1B, Lourdes Instrument Corp., Brooklyn, NJ) was employed to shear wort and beer samples. A custom-made flat paddle (13 × 30 mm in breadth × height) rotated inside a 25 mL "cloverleaf shaped" cup. The cup fit the blending paddle device tightly limiting the contact of samples with air during shearing. To minimize the interference of wort/beer oxidation, the headspace of the cup was flushed with compressed N_2 for 10 s prior to shearing. Samples after shearing were transferred into a 50 mL plastic centrifuge tube and flushed with N_2 for 10 s followed by sealing with caps. In a preliminary shearing experiment, 10.0 mL of a 1000 mg/L solution of 443 kDa β-glucan dissolved in 100 m*M* acetate buffer (pH 4.0, containing 5.0% v/v of ethanol) was sheared at speed settings of "30", "60" and "90" for 10, 35 and 60 s. As the highest turbidity was observed when the samples were sheared at a speed setting of "60" for 35 seconds[25], shearing tests thereafter were carried out under these conditions.

Under the above shearing conditions, the power consumption of water (10 mL) was measured with a transducer. An average shear rate during the shearing process was calculated from the power dissipation of the liquid[9,43]. The shear rate was found to average $1.3 \pm 0.2 \times 10^4$ s^{-1} ($n = 3$). This value should be considered a first approximation of the shear rate since the volume of sample was very small and accurate power consumption measurement was difficult.

Determination of wort and beer viscosities

Viscosities of wort and beer were measured with a Bohlin VOR controlled-rate rheometer (Bohlin Instruments Inc., Cranbury, NJ) by using a C-14 coaxial cylinder geometry and a 4.00 g·cm torsion bar. The rheometer was calibrated with double distilled and deionized water. Other conditions were set as follows: shear rates of 91.9, 184, 367, 581, 731 and 921 s^{-1}; an initial equilibrium time of 30 s; autozero "On" and continuous rate option "On"; an autozero delay time of 3 s; a delay time of 10 s and an integration time of 10 s. Measurement temperature was 20°C for wort and 5°C for beer unless otherwise specified. Shear stress values were measured and the viscosity was obtained from linear regressions of the dependence of shear stress on shear rate ($r^2 \geq 0.99$; $n = 3$). Previously, Oonsivilai[40] had reported possible shear thickening in commercial worts at shear rates below 100 s^{-1}. However, the wort and beer samples were found to be Newtonian at the higher range of shear rates (91.9–921 s^{-1}) employed in this study. The specific viscosity was then calculated using Eq. 5b. Also, a term "viscosity relative to water" (VRW) was defined as the viscosity of wort and beer caused by certain components (e.g., β-glucans) divided by the viscosity of water at the same temperature. For example, the VRW of β-glucan was ($\eta_{BG} - \eta_{NB}$) divided by water viscosity (η_{H2O}). Similarly, the VRW of wort and beer compo-

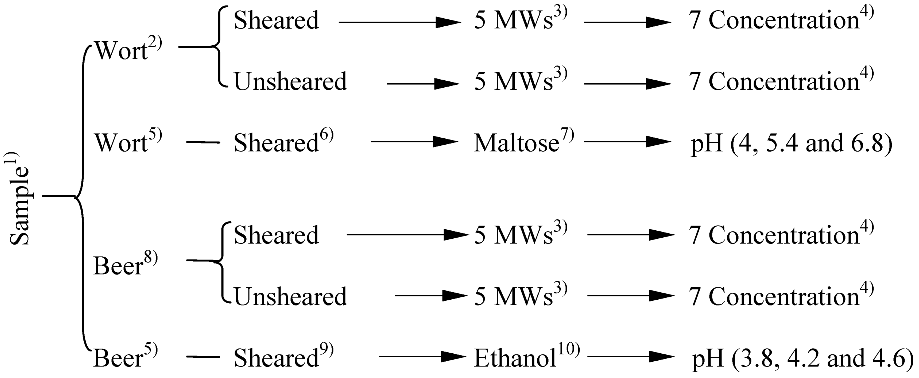

Fig. 1. Experimental design.

1) Experiments were carried out in duplicate.
2) Tested at pH = 5.4, SG = 12.0°P and T = 5.0, 20.0, 48.0 and 76.0°C; sheared at 20.0°C.
3) 31, 137, 250, 327 and 443 kDa.
4) 0, 50, 100, 200, 400, 600, 800, 1000 mg/L.
5) Containing 443 kDa β-glucan at 600 mg/L.
6) Sheared at 20.0, 48.0 and 76.0°C.
7) Worts (12°P and 18°P) were prepared from 8°P wort (containing 6.1% w/w of maltose) by supplementing maltose to 10.1 and 16.1% w/w, respectively.
8) Samples were tested at pH = 4.2, ethanol = 5.0% v/v and T = 5.0°C; sheared at 5°C.
9) Sheared at 0, 5.0 and 10.0°C.
10) Ethanol concentrations at 0, 5.0 and 10.0% v/v.

nents rather than β-glucan is ($\eta_{NB} - \eta_{H2O}$) divided by η_{H2O} (numerically equivalent to specific viscosity of β-glucan-free samples).

Experimental design

Worts (12°P, pH 5.4) containing β-glucans of different MWs (31–443 kDa) at various concentrations (0–1000 mg/L) were examined for their viscosities at 5, 20, 48, and 76°C. The above temperature range was selected to reflect the temperatures encountered during mashing, wort separation, fermentation and maturation. These worts were also sheared at 1.3×10^4 s^{-1} (20°C) to simulate the shear effect they may experience during brewing; and their viscosities at 20°C ($\eta_{20°C}$) measured. A second series of worts were prepared to investigate the effect of β-glucan (443 kDa at 600 mg/L), shearing temperature (20, 48 and 76°C), wort pH (4.0, 5.4 and 6.8), and maltose content (6.1, 10.1 and 16.1% w/w) on wort viscosity ($\eta_{20°C}$). Viscosities of sheared and unsheared beers containing 31–443 kDa β-glucans at 0–1000 mg/L were determined at 5°C. In contrast to β-glucan-free beers (containing 0, 5, and 10% v/v of ethanol at pH 3.8, 4.2 and 4.6), beer samples containing 600 mg/L of 443 kDa β-glucan were sheared at 0, 5, and 10°C and their viscosities were measured at 5°C (to compare the effect of shearing temperature). Duplicate experiments were carried out and results were reported as mean value ± one standard deviation. In such a design, the amount of replication must be at least two to give ten or more degrees of freedom[8]. However, if there is a large number of treatments, the amount of replication can be a minimum of two[8]. Experimental results were analyzed with SYSTAT version 5.05 for Windows (SPSS Inc., Chicago, IL) to test the analysis of variance (ANOVA) and multiple linear regression. The General Linear Model was used to perform forward stepwise regression with α-to-enter = 0.15 and α-to-remove = 0.15. Each of the terms in a model had $p \leq 0.05$. The overall p-values of the models are reported. Models with and without a constant were compared and the better fit with a higher determination coefficient (i.e., adjusted R^2) was chosen to be reported. The factors and levels examined in this study are illustrated in Fig. 1.

RESULTS AND DISCUSSION

Effect of MW and concentration of β-glucans and temperature on wort viscosity

The base wort used in this study was 12°P unhopped wort containing no β-glucan. The viscosity of this wort at 20°C was 1.521 mPa · s. Barley β-glucans (31–443 kDa) added to this wort up to 1000 mg/L increased its viscosity to 1.555–2.185 mPa · s as shown in Fig. 2a. Wort viscosity increased with MW and concentration with the following relationship (R^2 – adj = 0.782; $n = 80$; $p < 0.001$):

$$\eta = 1.443 + 3.999 \times 10^{-4}\,\mathrm{MW} + 3.622 \times 10^{-4}\,\mathrm{C} \quad (9)$$

where MW is the molecular weight (kDa) and C is the β-glucan concentration (mg/L). However, the low MW β-glucan (31 kDa) showed a limited viscosity increase up to 1000 mg/L (Fig. 2a). When the data for individual β-glucans of 137 to 443 kDa were examined with linear regression, the wort viscosity increased with concentration linearly ($r^2 > 0.99$). When the lowest MW viscosity values at 31 kDa were excluded and the remaining viscosity values were analyzed by multiple regression, a better correlation existed (R^2 – adj = 0.907; $n = 64$; $p < 0.001$):

$$\eta = 1.445 + 2.738 \times 10^{-4}\,\mathrm{MW} + 4.411 \times 10^{-4}\,\mathrm{C}. \quad (10)$$

As mentioned earlier, higher viscosities are detrimental for many unit operations such as pumping and filtration. Thus, brewers desire a minimal level of β-glucans, and corresponding lower wort and beer viscosities. Although other components such as protein and maltose in wort

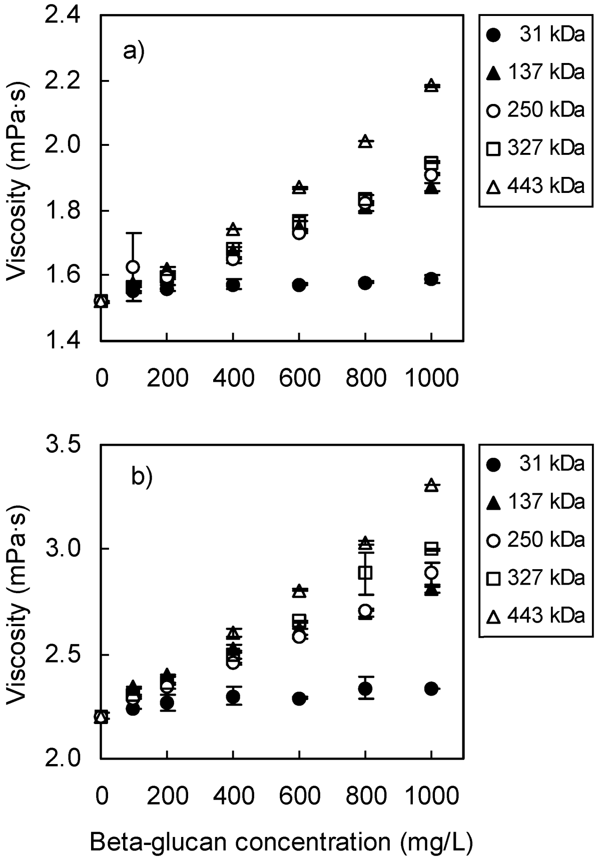

Fig. 2. Effects of molecular weight and concentration of β-glucans on the viscosity of 12°P wort at (a) 20°C and (b) 5°C.

contribute to viscosity, such components cannot be simply reduced as their concentrations have influence on the physical and sensory properties of beer. Thus, a most effective way to obtain low viscosity wort is to reduce the level of β-glucans.

The influence of β-glucan molecular weight (at 1000 mg/L and 20°C) on wort viscosity can be described by a linear correlation between MW and wort viscosity (r^2 = 0.903; $n = 10$; $p < 0.001$):

$$\eta = 1.6025 + 1.3 \times 10^{-3}\ \mathrm{MW}. \qquad (11)$$

The low MW (31 kDa) β-glucan had less impact on the wort viscosity at 1000 mg/L (1.589 mPa · s compared to 1.521 mPa · s of the same wort containing no β-glucans). The viscosity of worts containing high MW (137, 250, 327 and 443 kDa) β-glucans at 1000 mg/L (20°C) increased in an exponential relationship with MW (R^2 = 0.965; $n = 8$; $p < 0.001$):

$$\eta = 1.1885\ \mathrm{MW}^{0.0872}. \qquad (12)$$

This relationship is similar to the Mark-Houwink equation, which describes the relationship between intrinsic viscosity and molecular weight of polymers (Eq. 6).

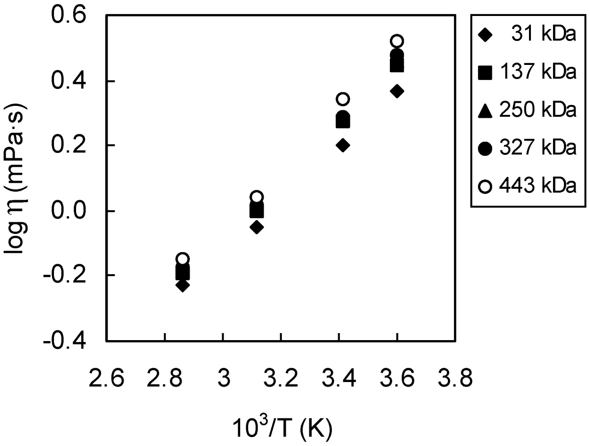

Fig. 3. Arrhenius plots of β-glucans (1000 mg/L) in 12°P wort.

As expected, wort viscosity decreased at 20°C vs. 5°C (Fig. 2a and b) with the β-glucan MWs and concentrations studied. This finding agrees with the work of Barrett and co-workers[5]. In this study, the temperatures were set at 48°C to reflect the mashing-in temperature; 76°C to reflect the lautering (wort filtration) temperature; 20°C for that of ale fermentation and 5°C to compare wort viscosity with that of beer at 5°C. In this temperature range, the effects of MW and concentration of β-glucans on wort viscosity were temperature-dependent. A multiple linear regression was used to model the changes in wort viscosity (R^2 – adj = 0.981; $n = 320$; $p < 0.001$):

$$\begin{aligned}\eta = {} & 3.050 + 3.3 \times 10^{-4}\ \mathrm{MW} + 1.3 \times 10^{-4}\ \mathrm{C} - 0.666\ \mathrm{T} \\ & + 1.12 \times 10^{-6}\ \mathrm{MW} \times \mathrm{C} + 4.7 \times 10^{-4}\ \mathrm{T}^2 \\ & - 1.0 \times 10^{-5}\ \mathrm{MW} \times \mathrm{T} \end{aligned} \qquad (13)$$

where T is temperature (°C). It is noteworthy that Eq. 13 is only a first approximation as the effects of MW and temperature on viscosity could possibly be non-linear at higher values. The Arrhenius model (Eq. 8) was fit to the viscosity data for β-glucans at 1000 mg/L (Fig. 3). The coefficient of determination of this fit for all β-glucans (31–443 kDa) was >0.99. The E_a value was affected by β-glucan MW ($p < 0.001$). Beta-glucans having MWs of 137, 250 and 327 kDa had similar activation energies 4.04–4.09 kcal/mol, whereas the 443 kDa β-glucan had a slightly higher E_a (4.22 kcal/mol). The 31 kDa β-glucan had a lower activation energy at 3.75 kcal/mol. This suggests that viscosity of the β-glucan solutions with a higher molecular weight is more sensitive to temperature. Reported E_a values[3] for 0.6–1.0% oat β-glucans of 2.4–4.6 kcal/mol were in general agreement with the data in Fig. 3.

The above models of wort viscosity are influenced by solvent conditions (i.e., wort composition). To compare the contributions of MW and concentration of β-glucans to wort viscosity, specific viscosity (η_{sp}) terms were used to cancel the solvent. These specific viscosities of β-glucans are shown in Fig. 4. The η_{sp} of β-glucans of 31–443 kDa at 1000 mg/L in the range of 5–76°C varied from 0.0448 to 0.502 suggesting that β-glucan polymers are responsible for 4.5% to 50% of the wort viscosity. Similarly, low β-glucan levels (200 mg/L) at molecular weights 31–443 kDa accounted for an increase of 3–12% in the viscosity of the 12°P wort studied. The value of η_{sp} increased with MW and concentration, although these effects were lower at higher temperatures ($p < 0.001$). The following expression can be used to describe the changes in β-glucan η_{sp} with concentration, molecular weight and temperature (R^2 – adj = 0.758; $n = 320$; $p < 0.001$):

$$\eta_{sp} = 2.142 \times 10^{-4}\ C + 1.893 \times 10^{-4}\ MW - 2.317 \times 10^{-4}\ T. \tag{14}$$

The relationship between η_{sp} and the concentration of each β-glucan was essentially linear up to 1000 mg/L (Fig. 4). It has been reported that at higher concentrations (0.4–2.4% w/v) crude barley β-glucans showed non-linear increases in their η_{sp}[7,45]. The effect of MW on β-glucan η_{sp} was similar to its effect on wort viscosity discussed above, (i.e., higher MWs of β-glucans led to greater specific viscosities, $p < 0.001$). When temperature was lowered from 76°C to 5°C, β-glucan η_{sp} increased ($p < 0.001$) up to 2 fold (Fig. 4) whereas the wort viscosity increased 4 to 5-fold. This finding implied that other contributors to wort viscosity (e.g., water and protein) were also temperature sensitive.

To compare the contributions of β-glucan and other wort components to wort viscosity, the viscosity caused by β-glucans was calculated from the difference between viscosities of β-glucan worts and their controls. The value of such a "differential viscosity" relative to viscosity of water (VRW) was further calculated (Fig. 5). The data agree with the trend shown in Fig. 4 (i.e., the viscosities caused by β-glucans increased at higher MWs and concentrations, but decreased with increasing temperatures). By using water as a reference solvent, the β-glucans (31–443 kDa) at 200 mg/L only increased viscosity by less than 20% compared to water (Fig. 5). At a concentration of 1000 mg/L, high MW β-glucans increased the viscosity values by 30–70% relative to water. Most of the β-glucan viscosities relative to water viscosity were lower than the specific viscosity of the β-glucan-free wort (i.e., the viscosity caused by non-β-glucan components relative to water viscosity). This suggests that purified β-glucan polymers affect the wort viscosity less than other wort components as a whole. It is interesting that the specific viscosity of wort containing no β-glucans increased at higher temperatures (the VRW value was 42 ± 1, 52 ± 0, 73 ± 3, and 115 ± 0% at 5, 20, 48, and 76°C, respectively) although the wort viscosity decreased (Fig. 3). The decrease in viscosity of the β-glucan-free wort at high temperatures was therefore caused by decreased solvent (water) viscosity. The major components of a 12°P wort are maltose and proteins. Thus the increased specific viscosity of the β-glucan-free wort may be due to heat enhanced protein-protein hydrophobic interactions. It is hypothesized that the interactions among β-glucan molecules were dominated by hydrogen bonding, which is weaker at higher temperatures.

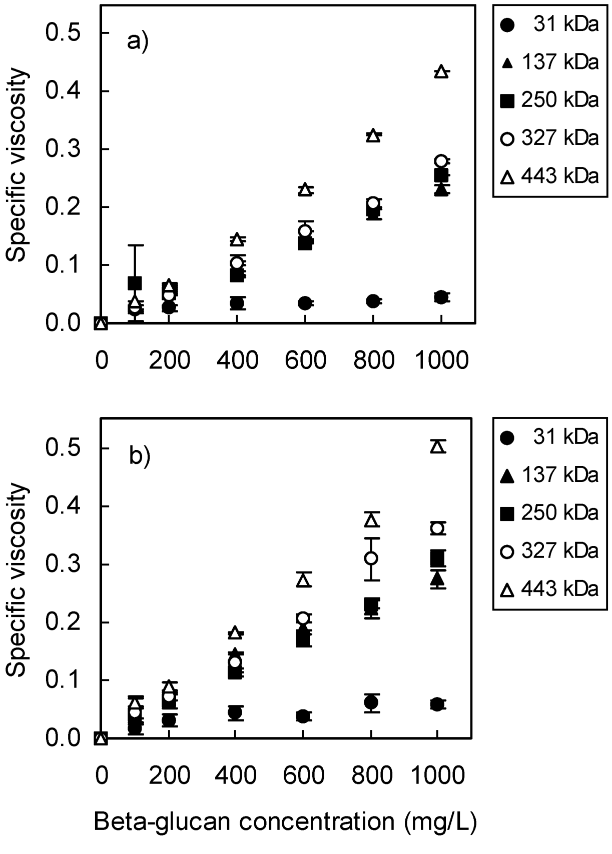

Fig. 4. Effects of molecular weight and concentration on the η_{sp} of β-glucans in 12°P wort at (a) 20°C and (b) 5°C.

Effect of shearing at 20°C on wort viscosity

Shearing has been reported to enhance "gel formation" (i.e., entanglement and precipitation) of β-glucans[29,38,43,44]. Shearing of 0.5% w/w β-glucan solutions also significantly ($p < 0.05$) retarded 0.45 μm membrane filtration[43]. To determine if and how shearing would affect the viscosity of wort containing β-glucans, samples were sheared at 20°C and the viscosities determined at 20°C. Related studies demonstrated that the viscosities of sheared wort increased compared to the unsheared wort at the same temperature[25] ($p < 0.001$). The viscosity of the β-glucan-free wort increased from 1.52 mPa · s to 2.16 mPa · s after shearing. Interestingly in the β-glucan-free wort, the viscosity was caused by wort components other than β-glucans. However, the β-glucan η_{sp} (i.e., the viscosity caused by β-glucans relative to the wort "solvent") decreased after being sheared ($p < 0.001$). This agrees with the results of shearing β-glucans in model worts[43]. As shearing can cause unfolding and denaturation of protein molecules, the unfolded proteins may have swept out a greater space in the aqueous solution resulting in a higher viscosity. More hydrophobic groups on the protein molecules could have been exposed after shearing and enhanced protein-protein interactions with increased solution viscosity. Conversely, the decrease in β-glucan-caused viscosity after shearing may be due to association of β-glucan molecules to form larger more compact particles[25] resulting in a lower volume concentration of the particles and thus a lower viscosity.

Effect of pH, maltose level and shearing temperature on wort viscosity

The presence of β-glucan (443 kDa at 600 mg/L) increased wort viscosity ($p < 0.001$; Fig. 6a; data at pH 4.0 and 6.8 are not shown). In addition to the maltose and pH levels, shearing temperature affected viscosity of wort ($p < 0.001$) containing 600 mg/L of 443 kDa β-glucan. Shearing of worts at 20°C resulted in lower viscosities than at 48°C and 76°C ($p < 0.001$). The viscosity of

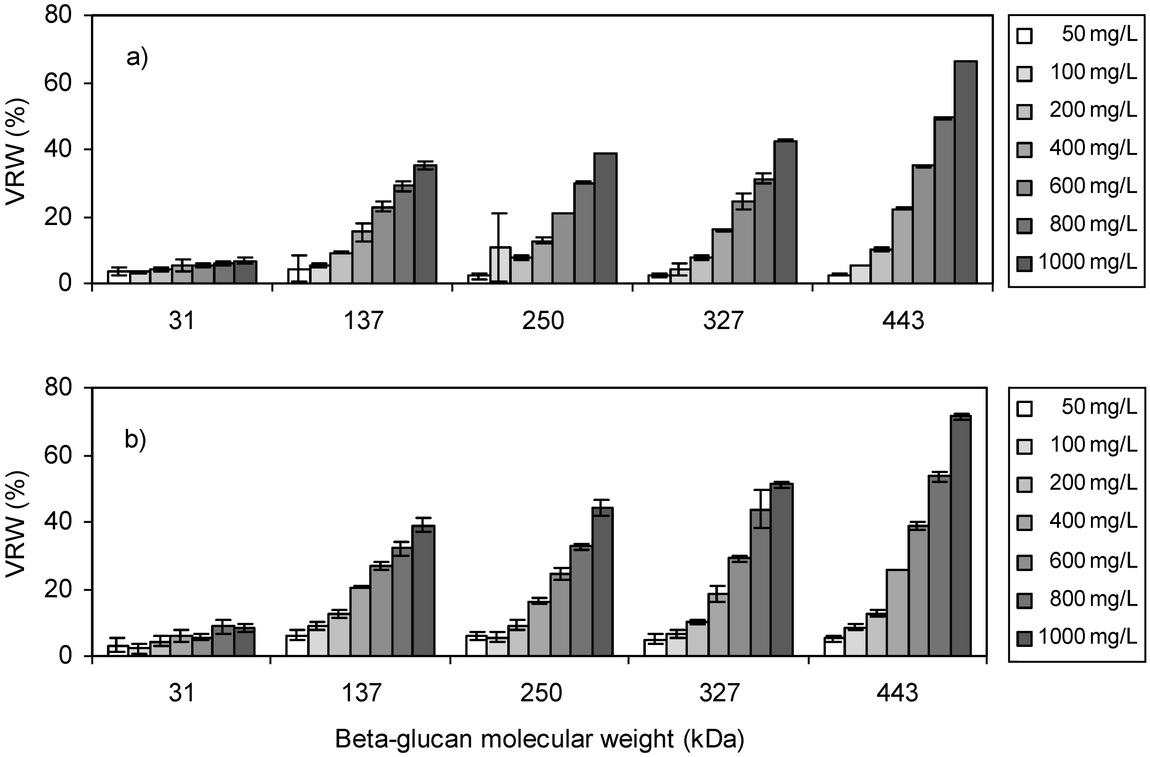

Fig. 5. Wort viscosity caused by β-glucans relative to water viscosity (VRW) at (a) 20°C and (b) 5°C.

sheared worts containing 600 mg/L of 443 kDa β-glucan could be expressed by the following relationship (R^2 – adj = 0.978; $n = 108$; $p < 0.001$):

$$\eta_{20°C} = 0.9960 + 0.001\ T_s + 0.061\ Mal + 0.018\ pH \quad (15)$$

where $\eta_{20°C}$ is wort viscosity at 20°C (mPa · s); T_s is the shearing temperature (°C) and Mal is the maltose concentration (% w/w) of wort. However, the level of wort β-glucans should be considered as well. The data of all the sheared worts, with and without β-glucans, could be modeled by the following relationship (R^2 – adj = 0.976; $n = 108$; $p < 0.001$):

$$\eta_{20°C} = 0.878 + 3.751 \times 10^{-4}\ C + 7.564 \times 10^{-4}\ T_s + 0.059\ Mal \quad (16)$$

where C is the concentration of 443 kDa β-glucan. Standard coefficients of the multiple regression indicated that maltose concentration was the most important factor, followed by β-glucan concentration and shearing temperature. It is not surprising that wort viscosity was primarily controlled by the maltose level as maltose is a major component in brewers' wort and was varied over a large range (6.1–16.1% w/w) in this study. It was found that maltose dissolved in water (20°C) at 6.1%, 10.1% and 16.1% w/w had viscosities of 1.252 ± 0.002, 1.420 ± 0.002 and 1.756 ± 0.015 mPa · s ($n = 2$), respectively. Since purified β-glucans are neutral molecules, their viscosities were not affected by the change in pH. For instance, it was reported in previous work that viscosity of an oat β-glucan solution was not affected by pH (in the range of 2–10)[10]. It is noteworthy that high molecular weight β-glucans caused higher wort viscosity (equations 9 to 13) although only the 443 kDa β-glucan was tested in equation 16.

To examine if maltose affected the viscosity caused by β-glucans in wort, the specific viscosities of the samples were further compared. Results are shown in Fig. 6b. Shearing lowered the specific viscosities of wort samples ($p < 0.001$) in the range of pH 4.0–6.8 and maltose at 6.1–16.1% w/w. An analysis of variance (ANOVA) of the η_{sp} for sheared worts indicated that η_{sp} values at 20°C (i.e., wort viscosity due to 443 kDa β-glucan at 600 mg/L) increased with pH and maltose levels, as well as shearing temperature ($p < 0.001$; Fig. 6b). Shearing of wort at 20°C reduced the β-glucan η_{sp} more than shearing at 48°C and 76°C ($p < 0.001$). The decreased β-glucan η_{sp} is hypothesized to be a result of the smaller β-glucan particle size distribution in sheared wort when sheared at 20°C[25].

Effect of β-glucan MW and concentration on beer viscosity

The effect of β-glucans at various MWs and concentrations on beer viscosity at 5°C was also examined (Fig. 7a). As could be expected (similar to wort), beer viscosity increased linearly with increased MW and concentration of β-glucans ($p < 0.001$). The following relationship governed the beer viscosity (5°C) in the presence of β-glucans (R^2 – adj = 0.989; $n = 80$; $p < 0.001$):

$$\eta = 2.106 + 8.041 \times 10^{-7}\ MW^2 + 2.518\ C^2 + 4.714 \times 10^{-6}\ MW \times C - 6.1 \times 10^{-4}\ MW - 1.581 \times 10^{-4}\ C. \quad (17)$$

Also, the viscosity due to β-glucan (at identical MW and concentration) was higher in beer than in wort ($p < 0.001$; Figs. 4b and 7b). When the specific viscosities were examined using the β-glucan-free beer as a solvent, the changes could be explained by MW and concentration of β-glucans studied (R^2 – adj = 0.991; $n = 80$; $p < 0.001$):

$$\eta_{sp} = 2.215 \text{ MW} \times \text{C} + 9.729 \times 10^{-8} \text{ C}^2. \qquad (18)$$

As with wort, specific viscosity is useful in evaluating the contribution of β-glucans to beer viscosity. When the concentration of β-glucans was low (i.e., ≤200 mg/L), the viscosity caused by β-glucans accounted for less than 20% of that of β-glucan-free beer (Fig. 7b). Even at a concentration as high as 800 mg/L, β-glucans only increased the beer viscosity by less than 80%. Beer viscosity caused by all other components was higher than that by β-glucans alone.

The specific viscosity of β-glucans in beer was approximately twice as high as that in wort (Figs. 4b and 7b). The results suggest that beer (containing 3.3% w/w of real extract and 5.0% v/v of ethanol, pH 4.2) is a better solvent for barley β-glucans than wort (12% w/w of extract of which 9.2% w/w was maltose, pH 5.4). In good solvents, solvent-polymer interactions are preferred over intrachain and interchain interactions, whereas in poor solvents the latter are favoured[18]. Polymer molecules occupy a large volume in good solvents[18] resulting in a higher viscosity. However, the comparison of η_{sp} values of β-glucans in wort and beer were based on different solvent systems (i.e., β-glucan free wort and beer). A common reference system such as water is more useful to compare the viscosity behaviour of β-glucans as well as their solvents.

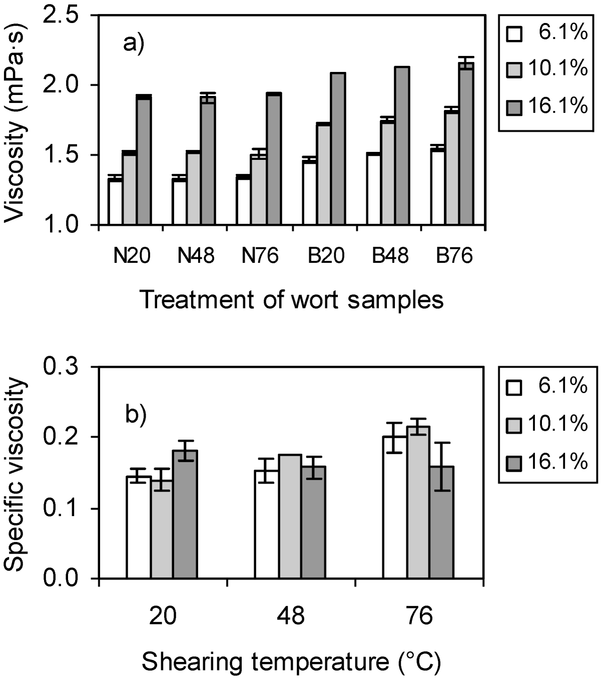

Fig. 6. Effects of β-glucan (443 kDa at 600 mg/L), maltose content (6.1%, 10.1% and 16.1% w/w), and shearing temperature on (a) viscosity and (b) specific viscosity of wort at pH 5.4. N20, N48, and N76 indicate samples containing no β-glucan were sheared at 20°C, 48°C, and 76°C, respectively; B20, B48, and B76 represent samples (containing 443 kDa β-glucan at 600 mg/L) sheared at 20°C, 48°C, and 76°C, respectively.

Beta-glucan-caused viscosity relative to water was therefore used to compare the contributions of both β-glucans and their "solvent" to the viscosities of the wort and beer (Figs. 5 and 8). When water was used as a reference, non-β-glucan components of beer were more viscous (130% relative to water viscosity) than that of wort components which was 42% of water viscosity ($p < 0.001$). Thus, β-glucans played a less important role in beer than in wort at 5°C although β-glucans exhibited higher viscosities in beer than in wort.

Effect of β-glucan, shearing, shearing temperature, pH and ethanol content on beer viscosity

Effect of shearing at various β-glucan concentrations on beer viscosity. When beer samples containing 0–1000 mg/L β-glucans (31–443 kDa) were sheared at 5°C, viscosity declined significantly compared to the unsheared beers ($p < 0.001$). This was opposite to the effect of shearing wort at 20°C on the wort $\eta_{20°C}$ which increased after shearing. For both sheared and unsheared beer samples, a significant relationship (R^2 – adj = 0.703; $n = 160$; $p < 0.001$) was found:

$$\begin{aligned}\eta = {} & 2.028 + 1.5 \times 10^{-4} \text{ C} + 4.443 \times 10^{-6} \text{ S} \times \text{MW} \times \text{C} \\ & - 1.3 \times 10^{-4} \text{ MW} - 1.2 \times 10^{-4} \text{ S} \times \text{MW} \\ & - 1.1 \times 10^{-4} \text{ S} \times \text{C} \qquad (19)\end{aligned}$$

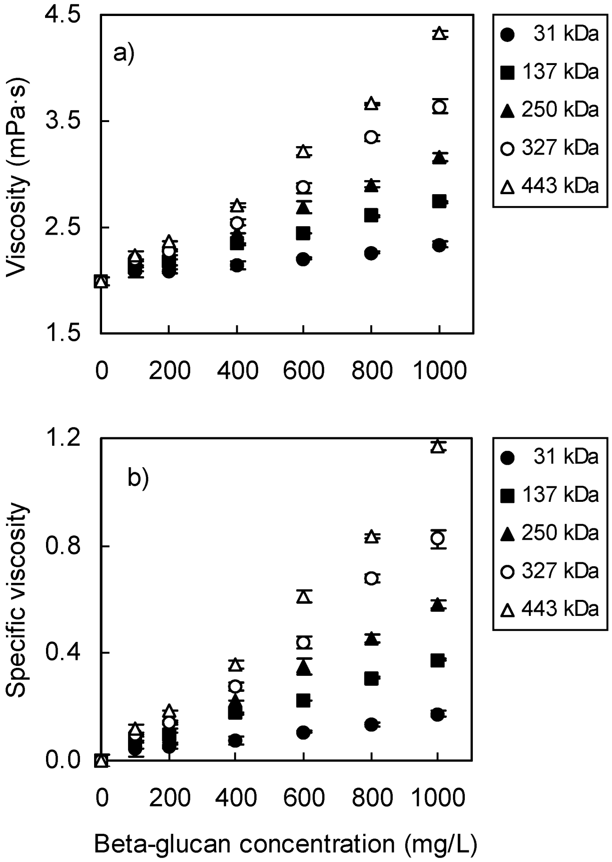

Fig. 7. Effects of MW and concentration of β-glucans on (a) beer viscosity and (b) specific viscosity of β-glucans in beer (5°C).

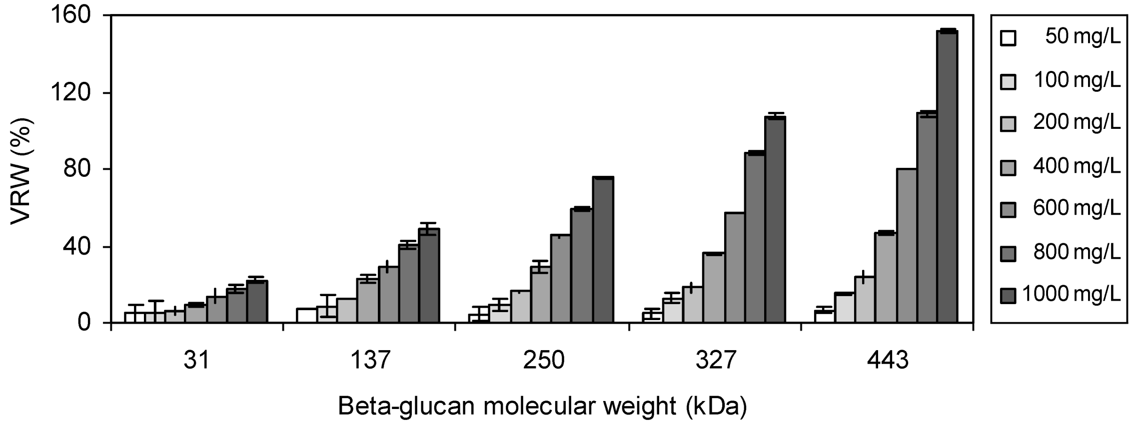

Fig. 8. Comparison of beer viscosity caused by β-glucans relative to water viscosity (VRW) at 5°C. The non-β-glucan components had a value of 130% water viscosity.

where S = 1 for sheared beers and S = 0 for unsheared beer samples. Further examination of specific viscosities of β-glucans (β-glucan-free beer as a solvent; Fig. 7b) found that the specific viscosity at 5°C was affected by MW and concentration of β-glucans ($p < 0.001$), but not affected by shearing ($p > 0.05$). It is noteworthy that shearing of wort at 20°C resulted in lower η_{sp} of wort at 20°C ($p < 0.001$). The different effects of shearing on β-glucan viscosities in wort and beer were due to the difference in "solvent" conditions (i.e., sugar and extract content, ethanol and pH levels).

Effect of shearing temperature, ethanol and pH levels on beer viscosity. The viscosity of the unsheared beer samples was examined at various pHs, temperatures, and ethanol concentrations for beer with or without β-glucan (Fig. 9a). The presence of 443 kDa β-glucan at 600 mg/L increased beer viscosity with all samples ($p < 0.001$). Beer pH in the range of 3.8–4.6 had no significant influence on viscosity ($p > 0.05$; data at pH 3.8 and 4.6 are not shown). It is noteworthy that preparations of barley β-glucans contained various amounts (0.72–9.35%) of protein[25]. In the literature[50], purified oat aleurone β-glucans containing 4.0–8.0% protein showed an average of five charged groups in each β-glucan molecule with pK_a values of 4–4.5, and four groups with pK_a values of 5–7.5. If the proteins are attached to the β-glucan molecules, a broad change in pH should affect the viscosity of β-glucan solutions. However, pH between 3.8 and 4.6 did not affect the viscosity of β-glucans and other components such as beer proteins. Since the 443 kDa β-glucan used in this study contained only a small amount (3.34 ± 0.28%) of protein[25], it is not surprising that beer viscosity was not affected by pH in a narrow range of 3.8–4.6. This pH range was selected for study to reflect the normal variation of beer pH[20]. It has been reported that the viscosities of model beer samples containing 0.5% w/w β-glucan (327 kDa) were different at pH values of 3.6 and 5.2 ($p = 0.05$)[43] probably due to the effect by protein when such a high concentration of β-glucan was added. In theory, however, the β-glucan molecules are neutral in charge and their viscosities are independent to pH.

There was no significant difference ($p > 0.05$) in the viscosities among samples sheared at 0, 5 and 10°C (Fig. 9a; data at pH 3.8 and 4.6 are not shown) since the temperature range was narrow. The increase in beer viscosity caused by ethanol was significant with all samples ($p < 0.001$). The ethanol content (0–10% v/v) reflected the levels from non-alcohol beer to strong beer or high gravity beer before dilution. Ethanol in beer is able to lower dielectric constant of the medium[46]. The decreased dielectric constant causes a lower solubility of proteins. Addition of miscible solvents such as ethanol may also reduce the interactions between β-glucan and water molecules

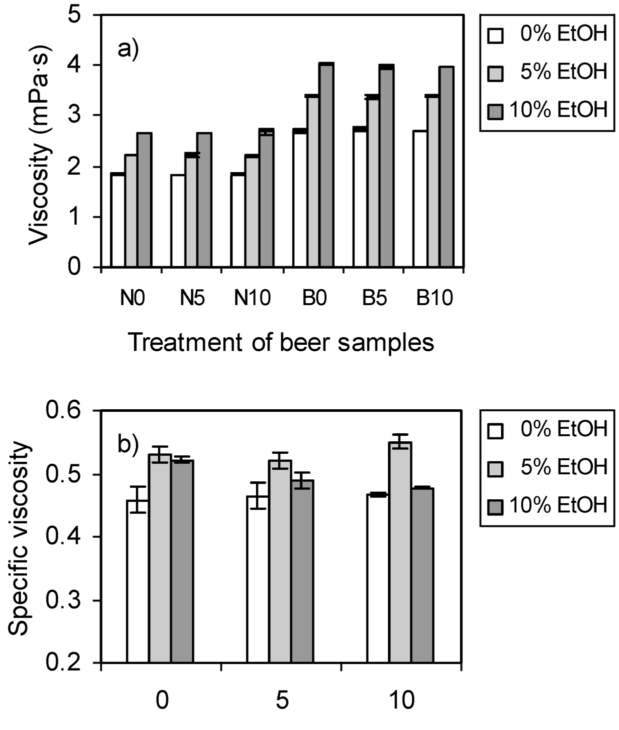

Fig. 9. Effects of β-glucan (443 kDa at 600 mg/L), ethanol content, and shearing temperature on (a) viscosity and (b) specific viscosity of beer at 5°C and pH 4.2. N0, N5, and N10 represent samples (containing no β-glucan) sheared at 0°C, 5°C, and 10°C, respectively; B0, B5, and B10 indicate beer samples (containing 443 kDa β-glucan at 600 mg/L) which were sheared at 0°C, 5°C, and 10°C, respectively.

and increase the interactions among β-glucan molecules leading to higher solution viscosities. It was noted that ethanol (5°C) has a viscosity of 2.003 ± 0.033 mPa · s which is higher than water viscosity (1.516 mPa · s). Ethanol at 5% and 10% v/v in water had viscosities of 1.841 ± 0.040 mPa · s and 2.176 ± 0.018 mPa · s, respectively. The interaction between ethanol (10% v/v) and water molecules resulted in a higher viscosity than that of either water or ethanol. A multiple linear regression suggested that only β-glucan and ethanol content determined the level of beer viscosity at 5°C while pH and shearing temperature in the ranges studied did not (R^2 – adj = 0.957; n = 108; $p < 0.001$):

$$\eta_{5°C} = 1.709 + 0.002\,C + 0.105\,E \qquad (20)$$

where E is the ethanol concentration (% v/v). Ethanol at 5% and 10% also increased the viscosity of beer containing no β-glucan (Fig. 9a). It is therefore necessary to compare the specific viscosities of β-glucan (443 kDa) using the β-glucan-free beer as a solvent (Fig. 9b; data at pH 3.8 and 4.6 are not shown). Ethanol not only increased the viscosity of the solvent (i.e., beer without β-glucan), but also enhanced the viscosity exhibited by β-glucan molecules ($p < 0.001$). Interestingly, 5% v/v of ethanol caused a higher β-glucan viscosity than 10% v/v of ethanol. This finding may partly explain the slower beer membrane filtration at 5% v/v of ethanol observed in a related study[25]. Shearing of beer samples lowered the viscosity due to β-glucan ($p < 0.001$) but shearing temperature had no significant effect ($p > 0.05$). Beer pH in the range of 3.8–4.6 showed no influence on the viscosities caused by 443 kDa β-glucan at 600 mg/L. This may be because the 443 kDa β-glucan contained a limited amount of charged proteins and/or because the pH range examined was narrow.

Intrinsic viscosity of β-glucans in wort and beer

The intrinsic viscosities of β-glucans in wort (12°P, containing 9.2% maltose, pH 5.4) and beer (3.3% w/w of real extract, 5.0% v/v of ethanol, pH 4.2) at 5°C were determined by using both Kraemer's and Huggins' equations. Mean values are presented in Fig. 10a. The intrinsic viscosity is a characteristic of a polymer in a given solvent system. Literature reports on β-glucan intrinsic viscosities varied from 0.7 to 31.0 dL/g[25]. Unfortunately, few reports have studied β-glucans in solvent systems which mimic the wort or beer compositions. It is more valuable to determine [η] in real wort and beer to examine the behaviour of β-glucans in brewing. Results in Fig. 10a can be described by the Mark-Houwink relationship (Eq. 6). With the 31–443 kDa β-glucans in wort, the intrinsic viscosity was proportional to its weight average molecular weight (M_w) used ($R^2 = 0.927$; $n = 10$; $p < 0.001$):

$$[\eta] = 0.467\,M_w^{0.371} \qquad (21)$$

For β-glucans in beer, the relationship between intrinsic viscosity and MW was ($R^2 = 0.982$; $n = 10$; $p < 0.001$):

$$[\eta] = 0.465\,M_w^{0.466} \qquad (22)$$

The values of constant K and exponent α are functions of both polymer characteristics and solvent systems, and only valid for the particular polymer in a given solvent system. These two parameters are related to the local stiffness of the polymer chains and polymer-solvent interactions. The exponent α for coil-like polymers is around 0.8 in a good solvent and 0.5 in poor solvents[12]. The α values for β-glucans observed in this study are 0.37 and 0.47 in wort and beer, respectively (equations 21 and 22). These values are lower than that of barley β-glucans studied in buffer systems (0.53 and 0.73)[17,40]. These findings suggest that β-glucans (31–443 kDa) exhibit coil-like conformations. Also, wort and beer are poor solvents for β-glucans when judged by the value of Mark-Houwink exponent. In poor solvents, polymers tend to reduce their contact with the solvent and adopt more compact conformations[18].

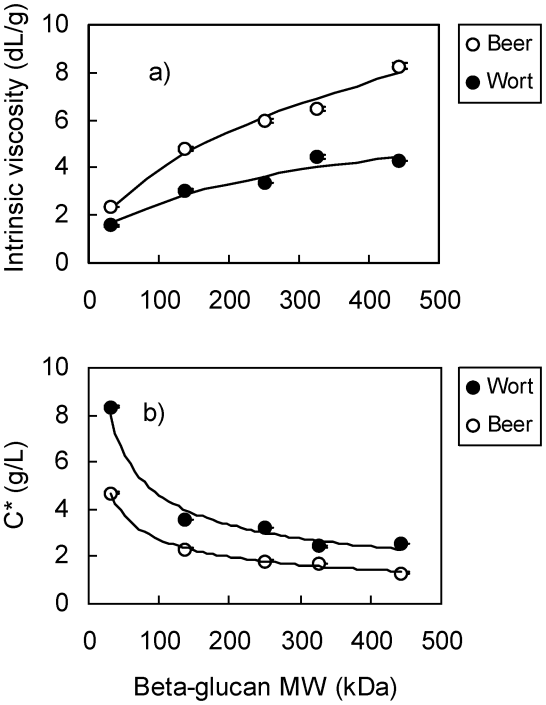

Fig. 10. Effects of β-glucan molecular weight on (a) intrinsic viscosity and (b) critical overlap concentration (C*) values in wort and beer at 5°C. Lines indicate the best fit of the models to the data.

Intrinsic viscosities can also be useful in deriving the critical overlap or entanglement concentration (C*) of a polymer in a given solvent system. The value of C* for β-glucans (31–443 kDa) derived from Eq. 7 was found to vary from 2.5 to 8.3 g/L in wort and from 1.3 to 4.7 g/L in beer, respectively (Fig. 10b). The relationship between M_w and C* followed the power law, and can be described by empirical models for β-glucans in wort (Eq. 23) and beer (Eq. 24) as follows:

$$C^* = 39.173\,M_w^{-0.4646} \qquad (R^2 = 0.960;\ n = 10;\ p < 0.001) \qquad (23)$$

$$C^* = 22.846\,M_w^{-0.4613} \qquad (R^2 = 0.993;\ n = 10;\ p < 0.001) \qquad (24)$$

Barley β-glucans (31–327 kDa) have been found to have lower C* values (0.53–3.11 g/L) in 0.1 *M* acetate buffer (pH 4.1) containing 5% v/v of ethanol and 0.5%

w/v maltose[40,42] using the approach of Linemann and Krüger[31]. A β-glucan sample isolated from beer (175 kDa) was reported to have a C* value of 0.42 g/L in water at 1.5°C[31]. The difference in reported C* values here versus literature reports is believed to be a result of different solvent systems used. Apparently, β-glucan molecules overlap or entangle one another more easily at lower concentrations in water or acetate buffers than under real beer and wort conditions.

The derivations of C* by using "1/log η_{rel}" vs. C[31] and "log β_{hsp}" vs. C[36] were also attempted. However, the plots only showed curves rather than broken lines because the β-glucan concentration studied was low. The non-linearity of the plots was also hypothesized to be due to the polydispersity of the β-glucans.

CONCLUSIONS

While various components in wort and beer contributed to their viscosities, β-glucans (31–443 kDa) increased solution viscosities. However, β-glucans did not "dominate" (compared to the viscosities caused by all other solutes) as long as the β-glucan concentration was lower than 800 mg/L. In the range of β-glucan concentrations studied (0–1000 mg/L), viscosity increased linearly with MW and concentration of β-glucans. The Arrhenius relationship governed the effect of temperature on wort viscosity. Shearing at a fixed rate (≈13,000 s^{-1}) for a given period of time (35 s) increased the wort viscosity but decreased beer viscosity ($p < 0.001$). However, the β-glucan viscosity was lowered after shearing in wort ($p < 0.001$) but not in beer ($p > 0.05$). Shearing wort at 20°C influenced β-glucan viscosity more than shearing at 48°C and 76°C ($p < 0.001$). Finally, shearing temperature (0, 5 and 10°C) showed no significant effect on beer viscosity ($p > 0.05$).

Wort viscosity was higher at high pHs in the range of pH 4.0, 5.4 and 6.8 ($p < 0.001$). The variation of ionic strength due to pH adjustment was 4.2–9.4 m*M* and was assumed not to affect viscosity. The viscosity of wort due to β-glucans was higher at pH 6.8 as well ($p < 0.001$). However, beer pH in the range of 3.8–4.6 did not affect its viscosity ($p > 0.05$). Increasing the concentration of maltose in wort and ethanol in beer enhanced the viscosity caused by β-glucan polymers ($p < 0.001$). At the same temperature (5°C), β-glucans had higher intrinsic viscosities in beer than in wort ($p < 0.001$). The Mark-Houwink equation was found to govern the influence of MW on the intrinsic viscosity of β-glucans. The critical overlap concentrations (C*) of β-glucans were lower in beer than in wort ($p < 0.001$), suggesting that interchain interactions are more readily to occur in beer.

ACKNOWLEDGEMENTS

A strategic grant awarded by the Natural Sciences and Engineering Research Council of Canada to RAS and ATP is acknowledged. Graduate Fellowships awarded by the American Association of Cereal Chemists (1999–2001) to YLJ are also appreciated. Gist-Brocades Frances S.A. and Canada Malting Company Ltd. are acknowledged for providing commercial β-glucanase and malt, respectively.

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(Manuscript accepted for publication February 2004)