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Impact of flavouring substances on the aggregation behaviour of dissolved barley β-glucans in a model beer

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ABSTRACT

Structural polymers such as cereal β-glucan may cause various processing problems in beverage industry depending on concentration, molar size distribution and agglomeration behaviour. In this context, influences of the beer volatiles dodecanoic acid, octyl butanoate, ethyl decanoate and decyl acetate on molar mass and radii of barley β-glucan were investigated in ethanolic (4% w/w) model solution. After addition of 100 mg/l ethyl decanoate and decyl acetate to the β-glucan solution, a wider-ranging molar mass distribution could be observed by means of asymmetric field-flow-fractionation. Due to agglomeration, average molar mass of β-glucan standard ($M_W = 6.8 \times 10^6$ g/mol) increased by 2×10^6 g/mol ($P < 0.05$) in solution containing decyl acetate. Furthermore, a significant growth ($P < 0.05$) from 86 to 102 nm in gyration radius was measured. The obtained results elucidate the importance of fatty acid derived flavouring substance composition in beer regarding the aggregation behaviour of β-glucan.

1. Introduction

Polysaccharides are a key component of virtually all foods and beverages. Depending on the structure and composition, these polymers are important for the nutritional, health and taste value of foods such as pasta or bakery products as well as beverages like beer (Anttila, Sontag-Strohm, & Salovaara, 2004; Sahan, Yasar, & Hayaloglu, 2008). Besides texture and appearance polysaccharides could modify the rate and intensity of flavour molecule release due to specific and non-specific interactions (Boland, Buhr, Giannouli, & van Ruth, 2004; Shin, Lee, Chang, Lee, & Kim, 2014). The nutritional and health value is influenced by the amount of soluble fibres in the foods (Collins et al., 2010). Such fibres exist in beer in form of β-glucans, arabinoxylans and other polysaccharides resulting from barley malt as main source (Sadosky & Schwarz, 2002; Tiwari & Cummins, 2011). The β-glucans are (1→3)(1→4)-β-D-linked linear chains and occur in barley in a concentration between 3% and 10% (Nielsen, Karlsson, & Engelsen, 2008). Because this β-bond is not digestible by enzymes in human gastrointestinal tract, these polysaccharides have a moderating effect on postprandial blood glucose, insulin response and reduce elevated blood cholesterol levels (Burkus & Temelli, 2005; Sahan et al., 2008). Although, β-glucans have a positive effect on human metabolism, the processing of glucan-containing raw materials often results in various problems because of its viscoelastic properties. Especially in terms of beer filtration β-glucan is a well-known substance, whereas the presence of higher concentrations yields in a decline of filterability (Bamforth, 1982; Kreisz, Spieleder, & Back, 2003). Particularly high molar fractions (>10^5 g/mol) are known to have a negative impact on beer filtration (Jin, Speers, Paulson, & Stewart, 2004b; Stewart, Hawthorne, & Evans, 1998). The molar mass distribution of β-glucans in beer depends, e.g., on differences in raw materials, malt modification, action of native enzymes, milling process, mashing intensity as well as succeeding manufacturing processes (Grimm & Krüger, 1995; Manzanares, Navarro, Sendra, & Carbonell, 1991; Marconi, Tomasi, Dionisio, Perretti, & Fantozzi, 2014). Marconi et al. (2014) showed a decreasing molar mass during malting depending upon germination time of barley. During mashing, these malt β-glucans are released by enzymatic and thermal hydrolysis in a molar mass range between 10^3 and 10^7 g/mol (Anderson, 1990; Bamforth & Martin, 1983; Foldager & Jørgonson, 1984). Comparable molar size area distributions of β-glucans were also detected in beer (Grimm & Krüger, 1995; Manzanares et al., 1991). Cereal β-glucans exhibit the ability to form gels, an association or cross-linking of polysaccharide chains via hydrogen bonds to form a 3-dimensional network (Burkus & Temelli, 1999; Clasen & Kulicke,

Abbreviations: AsFiFFF, Asymmetric flow-field-flow-fractionation; HMM, High molar mass; log *P*, partition-coefficient; LS, Light scattering; MALLS, multiangle laser light scattering; MCFA, Medium chain fatty acid; RI, refractive index; x_M, degree of association; x_r, aggregation number; ρ, ρ-parameter.

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2003; Tosh, Wood, & Wang, 2003; Vaikousi, Biliaderis, & Izydorczyk, 2004). These agglomerates have a negative impact on filtration due to their augmented particle sizes (Fischer, 2005). Besides enhancing effects of shear forces and reaction time, an influence of low sugar and high ethanol concentrations as well as low temperatures could be described on gel formation (Grimm, Krüger, & Burchard, 1995; Tosh et al., 2003; Ulmius, Önning, & Nilsson, 2012; Vaikousi et al., 2004). Other effects regarding agglomeration could be observed in terms of volatility of several flavour substances (Voilley, Lamer, Dubois, & Feuillat, 1990). The addition of β-glucans resulted in a lower volatility of aroma compounds which could be shown to be declining with increasing lipophilicity (log *P* value) of the aroma compound in question (Christensen, Trindade Leitão, Petersen, Jesperson, & Engelsen, 2009; Shin et al., 2014). Similar results were observed during filtration of beer model solutions, where a decrease of flavouring substances with rising log *P* value could be measured (Kupetz, Zarnkow, et al., 2015). In addition to the loss of aroma substances filterability was impaired. These flavour compounds are in addition to acetate esters (e.g., ethyl acetate and isoamyl acetate) also medium-chain fatty acid (MCFA) ethyl esters, formed during alcoholic fermentation (Procopio, Qian, & Becker, 2011). In alcoholic beverages, these esters carry fatty acid residues between C_6 and C_{12}, whereas their concentrations differ with respect to yeast fermentation performance (Jiang & Zhang, 2010; Saerens, Verstrepen, Thevelein, & Delvaux, 2008; Suomalainen, 1981).

Although, structure and agglomeration behaviour of β-glucans are abundantly studied to date, none of the authors referred to influences of hydrophobic substances in beer in this context. However, the described influences of β-glucan on volatile release and negative effects on filterability suggest on structural impact on the polysaccharide. The aim of this research is the investigation of changes in molecular shape, agglomeration behaviour and viscosity of dissolved barley β-glucan affected by hydrophobic volatiles. To exclude further beer ingredients that may affect the examination, model beer containing barley β-glucan and different volatiles (free fatty acid and fatty acid derived flavouring substances) were chosen. Furthermore, the log *P* value should be validated as criterion for interactions with β-glucans, which would provide important information for several processing steps, particularly regarding the performance of beer filtration.

2. Material and methods

2.1. Materials

(1,3;1,4)-β-D-Glucan from barley (high viscosity barley β-glucan, Megazyme International Ireland, Dublin, M_W: 4.95×10^6 g/mol, purity: >94% (dry weight basis)) from one batch was used to investigate the influence of polysaccharides in beer because of its broad molar size distribution. A molar mass of 4.95×10^6 g/mol was reported by manufacturer, measured using size-exclusion chromatography combined with multiangle laser light scattering (MALLS) in a solution containing 1 mg/ml polysaccharide, 0.1 M sodium nitrate and 5 mM sodium azide (Megazyme, 2013). To study the effects of hydrophobic substances on the structure of β-glucans, dodecanoic acid (Fluka GC reference 61609, Fluka Analytical, Switzerland), octyl butanoate (Sigma, MKBG 5186V, Sigma-Aldrich Co. LLC., Germany), ethyl decanoate (Fluka 13404930K, GC reference, Fluka Analytical, Switzerland) and decyl acetate (Schuchardt, Merck Schuchardt OHG, Germany) were tested. The physicochemical data of the aroma compounds are shown in Table 1. These substances were chosen because of a similar hydrophobic constant (log *P* value) between 4.96 and 5.03 and a flash point above 100 °C. In addition, absolute ethanol from Merck (Germany) was used to simulate the beer composition.

For preparation of the beer model solutions, 1 g/l of the powdered β-glucan was weighted and diluted in 0.5 kg double distilled water. The suspension was heated on a hot plate until boiling. The temperature was maintained for 30 min. The complete solubility of β-glucan was checked visually. After cooling to 20 °C, the polysaccharide stock solution was weighed to 1 kg with double distilled water. All analysis solutions contained 4% (w/w) ethanol. Ethanol concentration was chosen due to usual occurring contents in bottom-fermented beer types (Krüger & Anger, 1990). Polysaccharide content was deliberately chosen in upper region of occurring concentrations in beer to allow an analysis with field-flow-fractionation (Jin, Speers, Paulson, & Stewart, 2004a). The flavouring substances were added in a concentration of 50 and 100 mg/l to β-glucan stock solution. These concentrations were chosen because of the total lipid amount in unfiltered and filtered beer (Bravi, Perretti, Buzzini, Della Sera, & Fantozzi, 2009). The final samples had a slight opalescence. The pH values of the samples varied between 3.6 and 4.2 and thus were close to the beer-pH.

2.2. Viscosity measurement

Since β-glucan is known to influence beer viscosity due to concentration, molar mass and gel content, viscosity measurement was performed using a rotational viscometer (Stabinger viscometer SVM 3000, Anton Paar, Graz) at 20 °C according to Analytica-EBC analysing methods (Jin, Speers, Paulson, & Stewart, 2004c; Welten, 2013). 12 ml of beer model solutions were filled in a test tube, measurement cell got pre-wetted with sample and dynamic viscosity (η) as well as density was determined in triplicate. Method is based on torque and speed measurement of a rotating magnet in SVM 3000. Three cycles air injection for 200 s were used for measurement cell cleaning after each sample. Density was determined by means of an integrated density measurement cell based on an oscillating U-tube system. Sample was automatically drawn into the U-shaped test tube and caused to oscillate. Measured oscillating period corresponded to sample density. Influence of temperature was compensated by precise temperature measurement.

2.3. Determination of β-glucan and β-glucan-gel content

Among detection of fluorescence intensity due to interactions between β-glucans and Calcofluor, influences of hydrophobic substances on β-glucan concentration measurement should be investigated applying Analytica EBC method 9.31.2 (Welten, 2013). This method is based on interactions between the dye Calcofluor and β-glucan due to hydrogen bonds, ionic interactions and vander-Waals forces (Wu, Deng, Tian, & Xie, 2008). The microtitre assay was accomplished using calibration standard of SBL (Scandinavian Brewery Laboratory Ltd., Copenhagen) with a concentration of 500 mg/l β-glucan. Initially, 15 μl of the standard was transferred into a 96-well plate by means of pipetting robot BioTek Precision XS (BioTek Instruments, Inc., Winooski United States) to create a 7-point calibration. 300 μl dye solution containing 5 ml Calcofluor (Sigma) and 495 ml degassed Tris-HCl buffer (0.1 mol/l pH 8.0) were pipetted into each cavity of the 96-well plate. The fluorescence intensity was recorded at an excitation wavelength of 360 nm and a measurement at 445 nm using BioTek synergy H4 (BioTek Instruments, Inc., Winooski United States). For calculation of β-glucan content of the model beer samples, a second order non-linear regression curve converting fluorescence intensity in dependence to β-glucan concentration of the 7-point calibration curve was created. Because of the initial weight, all samples were diluted 1:3 before measurement with double distilled water. Samples were