Arabinoxylan from non-malted cereals can act as mouthfeel contributor in beer

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Highlights:

- Arabinoxylan is responsible for an increase in beer viscosity
- Non-malted rye can be used to improve mouthfeel in beer
- Fine milling increases rye arabinoxylan solubilisation to a limited extent
- Addition of xylanases during mashing reduces rye beer viscosity only slightly
- A sensory panel can differentiate a rye beer as beer with higher fullness

Abstract

A satisfying mouthfeel is essential for the production of non-alcoholic, low-alcohol beers and light beers. This paper highlights the importance of non-starch carbohydrates as mouthfeel contributors in this context. Beers were brewed with a substitution of 20% barley malt grits by non-malted barley, rye or oats compared to a control. For the beer brewed with rye, both a 53% increase in arabinoxylan content and an increase in the average degree of polymerization from 29 to 50 were observed. Compared to the control beer (1.48 mm^2/s), viscosity was the highest for the rye beer (1.85 mm^2/s). Multivariate data analysis underlined the role of arabinoxylan content and degree of polymerization as determinants of beer viscosity. A sensory panel distinguished a low-alcohol rye beer as the one with increased fullness compared to a 100% malt beer. These experiments suggest that rye addition can be used as a strategy to increase the beer fullness.

Keywords: Arabinoxylan, β-glucan, dextrin, rye, oat, barley, beer viscosity, mouthfeel, NABLAB

1. Introduction

The movement towards a healthier lifestyle of modern-day society explains the rise of no- and low-alcohol beers (NABLAB's) and low-calorie beers. However, the removal of alcohol and/or dextrin in the production of these beers seems to go hand in hand with a decrease in mouthfeel. Consumers often perceive these beers as "low in body", "watery" or "empty" (Krebs, Müller, Becker, & Gastl, 2019; Malfliet, Goiris, Aerts, & De Cooman, 2009).

The evaluation of beer mouthfeel is complex. Langstaff & Lewis described mouthfeel as consisting of three elements, being carbonation, fullness and afterfeel (Langstaff & Lewis, 1993). Chronologically, carbonation is the first sensation that is noticed by a beer taster. Besides the foam head, this also relates to sting, bubble size and total carbon dioxide content of the beer. Subsequently, the taster will evaluate the fullness of a beer using terminology like viscosity and density. Finally, the remaining taste in the oral cavity is referred to as afterfeel. This includes astringency, stickiness and mouth coating properties of beer. The warming effect of ethanol is also described within the segment of afterfeel (Schmelzle, 2009).

Ethanol content is the main factor that influences the sensation of mouthfeel. This might be due to the viscosity of ethanol, but also to the strengthening effect of ethanol on the perceived sweetness, bitterness and sourness (Ramsey et al., 2018). The vast amount of data indicating the lack of mouthfeel of NABLAB's underlines the role of ethanol in mouthfeel perception (Ghasemi-Varnamkhasti et al., 2012; Krebs et al., 2019; Ramsey et al., 2018). During the production of low-calorie beers, dextrin is degraded. This results again in a decreased perception of mouthfeel (Malfliet et al., 2009; Rübsam, Gastl, & Becker, 2013). However, sensory trials with beers that were spiked with dextrin, led to the conclusion that an increase in viscosity was detectable by a sensory panel only starting at a concentration of 52 g/L (Ragot, Guinard, Shoemaker, & Lewis, 1989). Besides ethanol and dextrin, β-glucan, protein, polyphenols, chloride ions, glycerol are reported to impact mouthfeel as well (Goiris et al., 2014; Langstaff & Lewis, 1993).

The potential of cereal carbohydrate polymers and more in particular of non-starch carbohydrates such as β-glucan and arabinoxylan is not yet fully exploited. Mixed-linkage-β-glucan, or in short β-glucan, consists of β-1,3 bound cellotriosyl and cellotetraosyl units. This stepped structure results in a high degree of solubility for β-glucan despite its relatively high degree of polymerization. Commonly, the β-glucan content in beer is below 0.5 g/L (McCleary & Nurthen, 1986) and molecular weights between 10^4 and 10^7 are reported in barley malt (Rimsten, Stenberg, Andersson, Andersson, & Åman, 2003; Tomasi,

Marconi, Sileoni, & Perretti, 2017). The high molecular weight β-glucan is notorious for blocking wort filtration and has the tendency to form aggregates upon beer ageing (Speers, Jin, Paulson, & Stewart, 2003; Tügel, Runyon, Gómez Galindo, & Nilsson, 2015). These aggregates can be observed as beer haze (Bamforth, 1999). On the contrary, also mouthfeel related aspects are reported as spiking experiments with β-glucan extracts in beverage systems showed that β-glucan improved fullness, but yielded a rather slimy texture in the mouth (Lyly et al., 2003). Arabinoxylan is composed of a backbone of xylopyranosyl units, of which some are unsubstituted and others linked with one or two arabinofuranosyl units. This degree of substitution determines its tendency to aggregate on a molecular level and thus its impact on viscosity. Arabinoxylan levels range between 0.5 and 1.9 g/L in commercial beers and molecular weights between 150 and 10^4 are reported in beer (Courtin et al., 2009). This is equivalent to a degree of polymerization (DP) between 1 and 60. Arabinoxylan in wort can exceed a molecular weight of $2.5 \cdot 10^5$, but this high molecular weight fraction is often removed during the filter process (Sadosky, Schwarz, & Horsley, 2002). Despite the relatively smaller size of arabinoxylan compared with β-glucan, the higher arabinoxylan levels might play a considerable role in beer viscosity. The role of arabinoxylan on beer turbidity is more ambiguous. It is reported that protein and most possibly arabinoxylan are responsible for the formation of visible beer haze (Bamforth, 1999; Coote & Kirsop, 1976). However, the colloidal haze in white beers, that are brewed with high amounts of wheat and thus wheat arabinoxylan, was shown to consist of starch and protein, while β-glucan and arabinoxylan were only minor constituents (Delvaux, Delvaux, & Delcour, 2000). Most probably, the degree of substitution and degree of polymerisation play an important role in the aggregation behaviour of arabinoxylan (Cleemput et al., 1995).

Adjuncts are often referred to as starch-rich cereals other than barley and wheat malt that are used for several reasons (Glatthar, Heinisch, & Senn, 2002). Cost-effectiveness is an important one for breweries, but the use of adjuncts has also a specific impact on the taste of the end product. Adjuncts can be part of a specific beer style as determined by traditions or because the crops are locally grown. While the drinkability is improved when using rice or corn as an adjunct, specific taste profiles or better foam retention are achieved with cereals like barley, wheat, sorghum, rye or oats (Bamforth, 2006). The latter has gained interest for the production of beers for people suffering from Coeliac disease (Kordialik-Bogacka, Bogdan, & Diowksz, 2014). Corn or rice will increase extract yield due to their high starch content, while other adjuncts lead to a reduced extract yield, higher turbidity and higher wort viscosity resulting in filtration issues (Kordialik-Bogacka et al., 2014).

The use of non-malted cereals in the context of NABLAB's or low-calorie beers is especially interesting as higher molecular weight arabinoxylan, β-glucan and protein are released during the brewing process (Li, Lu, Gu, Shi, & Mao, 2005; Schnitzenbaumer, Kerpes, Titze, Jacob, & Arendt, 2012). This might provide a clean label solution by making use of the advantages of specific raw materials.

We hypothesise that carbohydrates play an important role in beer viscosity and that the addition of adjuncts is a promising way to improve the mouthfeel of NABLAB's or low-calorie beers. Moreover, the relative contribution of arabinoxylan, β-glucan or dextrins to beer viscosity and mouthfeel has not been elucidated yet. In this study, the impact of non-malted barley, rye and oats as adjuncts on the carbohydrate content in beer and their impact on beer viscosity is compared. Non-malted barley will deliver beers with increased β-glucan content, while rye will result in an increased arabinoxylan content. Beers produced with oats were also included as a beer with 10% oats was previously characterised with an intense, creamy mouthfeel (Schnitzenbaumer et al., 2012). As most soluble non-starch carbohydrates are present in the endosperm cell walls (Kanauchi & Bamforth, 2002), the impact of fine-milling and xylanase addition is also investigated. This research is concluded with a sensory analysis of rye beers.

2. Materials & Methods

2.1. Materials

Barley malt (Malzfabrik Mich. Weyermann GmbH & Co. KG, Bamberg, Germany), oat flakes (Brewferm, Beverlo, Belgium) and rye (var. Dukato, harvest 2018) were purchased at local stores. Non-malted barley (var. Sebastian, harvest 2015) was provided by Cargill France SAS (Herent, Belgium). To avoid confusion, barley malt will be referred to as malt in this paper, while the non-malted adjunct is referred to as barley. Hops and yeast were purchased at Brouwland bvba (Beverlo, Belgium). The xylanase preparation Grindamyl Powerbake 900 was from Dupont (Mechelen, Belgium) and the dextrin hydrolysing preparation Attenuzyme Pro was from Novozymes (Bagsværd, Denmark). All other chemicals, reagents and solvents used were of analytical grade and purchased from Sigma-Aldrich (Bornem, Belgium).

2.2. Milling

Malt was milled with a laboratory disk mill (Buhler, Uzwil, Switzerland) with a disk spacing of 0.2 mm. All adjuncts were milled to fine particle sizes in order to improve non-starch carbohydrate solubilisation. Barley and rye were milled with a Tecator Cyclotec 1093 laboratory impact mill (Foss A/S, Hillerød, Denmark) with a mesh size of 0.5 mm, while oats were milled with a mesh size of 1.0 mm. In subsequent

experiments, the impact of particle size on rye arabinoxylan solubilisation was investigated. Pre-milled rye was milled using a PM100 ball mill (Retsch, Aartselaar, Belgium) for 2 times 15 minutes at 500 rpm. The resulting product is referred to as finely milled rye. Coarsely milled rye was obtained using the laboratory disk mill as described for malt with a disk spacing of 0.2 mm.

2.3. Particle size distribution

Particle size distribution was measured by a Beckman Coulter LS 13 320 particle size analyser (Fullerton, California, USA). The dry powder system module was used for all samples except for the ball-milled rye, for which the universal liquid module was used. Median particle sizes are expressed on a calculated volume basis of duplicate measurements.

2.4. Microscopic analysis of arabinoxylan in rye endosperm cell walls

Cereal samples were embedded in HistoResin, sliced with a microtome and stained with a catalytically inactivated xylanase coupled with Alexa Fluor 488 (Dornez et al., 2011). Slides were analysed using a Nikon Eclipse 80i Epifluorescence microscope (Melville, NY, USA) equipped with a FITC filter cube that allowed excitation between 465-495 nm. A dichroic mirror with a long pass 505 nm filter and emission filter for wavelengths between 515 and 555 nm allowed visualization of the Alexa Fluor 488. Digital images were shot with a Nikon Digital Sight DS-U2 camera.

2.5. Lab-scale brewing

Lab-scale mashing was performed in duplicate in a Lochner mashing bath LB 8 (Berching, Germany) as described by Langenaeken et al. (2019). Malt (50 g on dry matter) was weighed and 300 mL brewing water was added, which was adapted with 2.55 mM $CaCl_2$ and 0.75 mM H_2SO_4 (Langenaeken, De Schepper, De Schutter, & Courtin, 2019). With adjunct brews, 20% of malt was substituted with adjuncts on equal extract content. Wort filtration was carried out with a folded paper filter MN 615 ¼ (Macherey-Nagel, Düren, Germany), after which the resulting wort was diluted to 10 °P with demineralised water. Subsequently, Saaz hops (0.2 g) were added and boiling was mimicked with 200 mL of the hopped worts in Schott bottles (Schott AG, Mainz, Germany) by autoclaving for 15 minutes at 121 °C, including cool down to 80 °C. Evaporation loss was between 8.3 and 8.5 %. To the cooled wort, 0.2 g yeast BE-134 (Lesaffre, Marcq-en-Barœul, France) was added. This yeast ferments all maltotriose. Primary fermentation was carried out at an ambient temperature of 24 °C followed by secondary fermentation

at 4 °C. The samples were centrifuged (1000*g*, 10 min, 4°C) to remove solids. Samples were not pressurised to limit carbonation to a minimum.

2.6. Pilot-scale brewing

A promising beer recipe using 30% rye adjunct substitution was validated sensorially and compared with a 100% malt beer control. Therefore, two food-grade low-calorie beers (3.5% ABV) were brewed on pilot-scale (30L). The mashing scheme consisted of 30 minutes at 62 °C and 72 °C each with 10 minutes mashing-off at 78 °C. Intermediate heating took place at 1 °C/min. At the mashing stage, 190 µL/L Attenuzyme Pro was added to hydrolyse dextrin. This commercial enzyme mixture was found to exhibit also β-glucanase side activity (results not shown). Beers were dry-hopped to mask aroma differences as a result of the rye substitution.

2.7. Chemical and viscosity analysis of beer samples

Density, ethanol content and all values derived from these, including the caloric value of the beers, were determined by an Alcolyzer Beer Analysing System (Anton Paar, Graz, Austria). Arabinoxylan and dextrin content were estimated by hydrolysing the polysaccharides and reducing and derivatising the formed monosaccharides to alditol acetates to be measured by gas chromatography (Courtin et al., 2009). To this end, first 2 mL of degassed beer was hydrolysed with 2 mL trifluoroacetic acid 4M. Secondly, the monomers are reduced using sodium borohydride. Finally, the reduced monomers are acetylated with acetic anhydride with n-methylimidazole as a catalyst. The alditol acetates were analysed with an Agilent 6890 series gas chromatograph (Santa Clara, California, USA) equipped with a Supelco SP 2380- column (30 m x 0.32 mm x 0.2 µm film thickness). An injection volume of 1 µL was used, with a split ratio of 1:20. The separation was carried out at 225 °C, while injection and detection occurred at 270 °C. Detection was performed with a flame ionization detector with hydrogen gas (flow rate: 30 mL/min) as fuel and dry air (flow rate: 400 mL/min) as oxidant gas. Helium (flow rate: 26 mL/min) and nitrogen gas (flow rate: 25 mL/min) were used as the carrier and the make-up gas, respectively. Reducing sugars were analysed using the same general procedure, but with reduction prior to hydrolysis, while for free sugars the hydrolysis step was omitted. This allowed calculating an average degree of polymerization (avDP) for arabinoxylan and dextrin. β-glucan was measured calorimetrically as glucose after enzymatic degradation according to EBC method 8.13.1. Lyophilised beer was accurately weighed in tin cups and analysed for nitrogen using a Carlo Erba EA1108 elemental analyser (Milano, Italy). A conversion factor of 6.25 was used to estimate the protein content (Celus, Brijs, & Delcour, 2006). Kinematic viscosity of

beer was measured at 20.00 °C with an Ubbelohde viscometer (iVisc, Lauda DR. R. Wobser GmbH & Co. KG, Königshofen, Germany) to evaluate an instrumental parameter for fullness. Carbon dioxide content of the pilot-scale beers was estimated according to EBC method 9.28.2.

2.8. Sensory evaluation

A one-sided pairwise comparison was carried out with the rye and control beers. Samples were anonymised and presented in a randomised order to a non-trained test panel of 28 people. They were asked to select the sample with the most predominant sensation of fullness (Meilgaard, Civille, & Carr, 2016). Informed consent of all participants was obtained before the sensory evaluation.

2.9. Statistical analysis

All pair Tukey's honestly significant difference (HSD) test was used after a positive omnibus test ($p < 0.05$). Principal component analysis was carried out on the data of the different adjunct beers. The statistical software JMP (version 12 Pro, SAS Institute Inc., Cary, North Carolina, USA) was used to perform these analyses.

3. Results & Discussion

Lab-scale beers were brewed in duplicate and analysed. The results are summarised in Table 1. Negligible differences between beers were detected for original extract, which can be explained by the fact that firstly, the amount of adjunct was added on an equal extract content and secondly, all beers were diluted to 10 w/w% with demineralised water before boiling. Interestingly, the apparent extract and thus beer density was the highest for the beer with rye as an adjunct, followed by barley and oat beer. This already gives an indication that more non-fermentable substances are present in these beers, compared to the control. This inevitably led to a slightly decreased alcohol content and thus real degree of fermentation (RDF) for the adjunct beers. The pH value did not change for the adjunct beers, while the colour value was reduced to lighter coloured beers. The low standard deviations indicate that the lab-scale brewing process was reproducible.

Table 1: Brew-technical data for the 100% malt control beer and the beers made with 20% adjunct substitution (non-malted barley, rye or oat). Beers were brewed in duplicate and average values are given with standard deviations.

	Beer made with 100% malt (control)	Beer made with 20% barley substitution	Beer made with 20% rye substitution	Beer made with 20% oats substitution
Original extract [w/w%]	11.24 ± 0.01	11.13 ± 0.00	11.11 ± 0.08	11.25 ± 0.02
Apparent extract [w/w%]	0.76 ± 0.03	0.93 ± 0.01	1.05 ± 0.06	0.88 ± 0.01
Alcohol [v/v%]	5.52 ± 0.01	5.37 ± 0.01	5.29 ± 0.01	5.46 ± 0.01
RDF [%]	76.4 ± 0.2	75.2 ± 0.1	74.3 ± 0.3	75.6 ± 0.2
Density [g/mL]	1.0012 ± 0.0001	1.0018 ± 0.0001	1.0023 ± 0.0002	1.0016 ± 0.0001
pH Value [-]	3.96 ± 0.00	4.02 ± 0.00	4.00 ± 0.00	3.94 ± 0.00
Colour Value [EBC]	15.1 ± 0.0	13.2 ± 0.0	13.6 ± 0.1	12.7 ± 0.0

These beers were characterised on a carbohydrate level (Figure 1). The maltodextrin content, which is defined here as all non-fermentable soluble starch and derivatives, is increased for all adjunct beers. This might be due to the reduction in starch degrading enzymes because of the 20% malt substitution. No significant differences were detected for the maltodextrin average degree of polymerization. The control beer contained 0.96 g/L arabinoxylan. Slightly lower values were found for the barley and oat beers. The arabinoxylan avDP did not differ between those three beers. The rye beer, on the contrary,

has a 53% increased arabinoxylan content compared to the control. Moreover, the arabinoxylan avDP increased from 29 to 50, indicating that the arabinoxylan derived from the rye is much higher in molecular weight compared that of barley. Based on the initial arabinoxylan levels in the raw material, we calculated that 7.6% of total barley malt arabinoxylan was solubilised in beer, while 20.1% of the total rye arabinoxylan was released (data not shown). Assuming that the impact of the reduced xylanase activity or the possible impact of xylanase inhibitors in rye is minor, one can calculate that the arabinoxylan derived from rye in this beer had an avDP of 134. This corresponds to an average molecular weight of about $2.2 \cdot 10^5$. Therefore, we can say that rye is an excellent source of high molecular weight arabinoxylan. The average degree of substitution (avDS) of arabinoxylan is a measure for the degree of arabinose substitution on the xylose backbone (Courtin et al., 2009). This value decreased slightly for the adjunct beers to 0.58 ± 0.00 for rye and 0.56 ± 0.01 for barley and oats, compared to the control beer (0.61 ± 0.00). Although the differences are small, it reflects that the adjunct arabinoxylan was less substituted compared to that of malt. Due to the high quantity of β-glucan in non-malted barley, the β-glucan content in this beer reached the highest concentration. Compared to the control beer, this is more than a fourfold increase. The adjunct beer with oats had the second-highest concentration of β-glucan followed by the beer with rye.

Given that these beers had a similar alcohol content, one can compare on a carbohydrate level which type of carbohydrate affects beer viscosity the most. The control beer sample had an average kinematic viscosity of 1.48 mm^2/s, which slightly increased for a beer brewed with barley (+3.4%) or oats (+1.7%) as an adjunct. The most substantial viscosity increase was detected for the rye beer, with an increase to 1.85 mm^2/s.

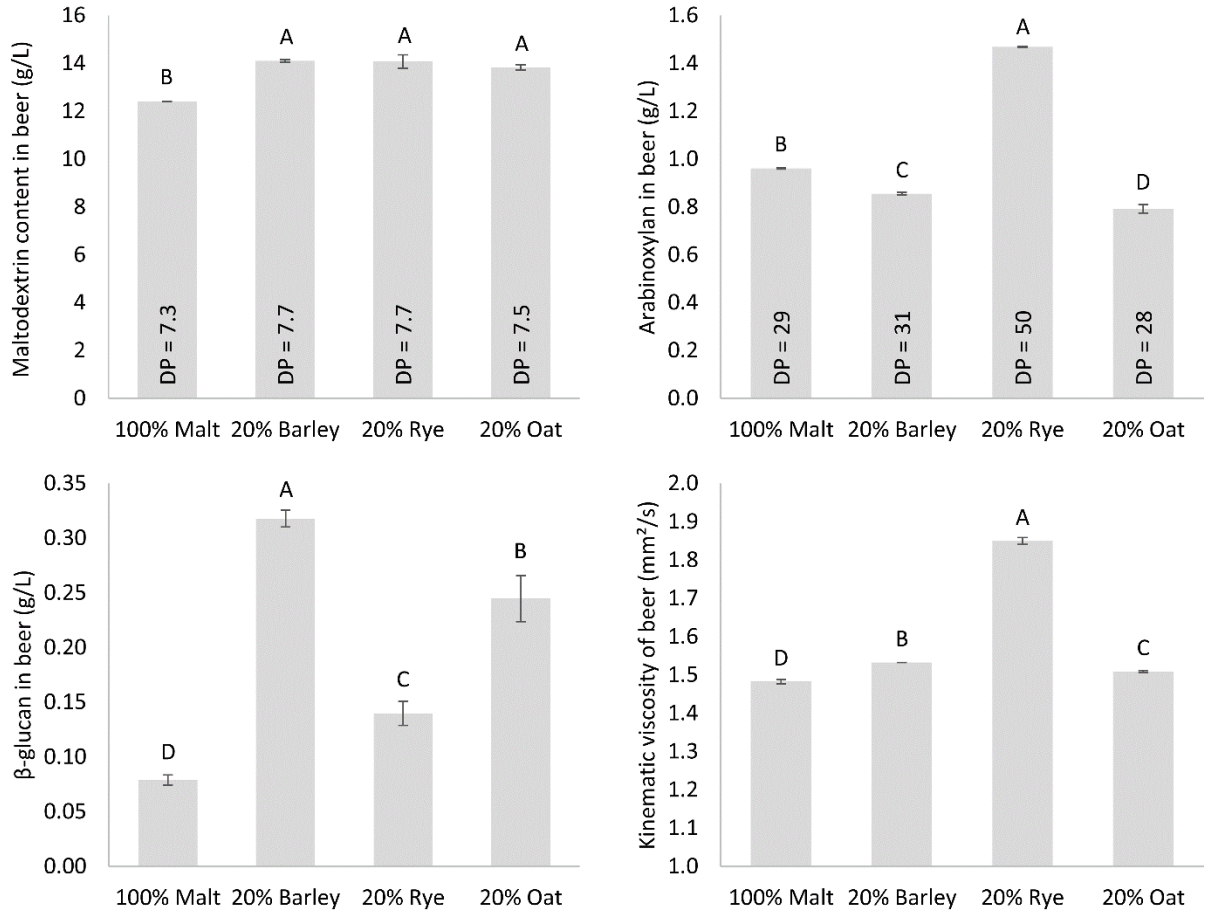

Figure 1: Carbohydrate content and kinematic viscosity of the 100% malt control beer and the beers made with 20% adjunct substitution (barley, rye or oat). Beers were brewed in duplicate. Averages are presented with standard deviations. Tukey's HSD assigns different letters to samples that are significantly different ($p < 0.05$).

Principal component analysis was used to present an overview of the correlations in this dataset. All duplicate beer samples cluster in the different quadrants of the bi-plot. Again, here, the arabinoxylan content, its avDP and kinematic viscosity seem to be strongly correlated. Placed perpendicularly on this are the loading vectors of avDS, protein content, maltodextrin content, avDP of maltodextrins and the β-glucan content. This demonstrates the subordinal role of protein, β-glucan and dextrin in this dataset in relation with beer viscosity. Self-evident, the RDF and alcohol content were closely correlated and inversely correlated with the beer density. Based on these results, brewing with non-malted rye seems to be a promising way to boost the beer viscosity in beers with reduced mouthfeel properties. Within this set-up, this was explained by the increased amount of high molecular weight arabinoxylan.

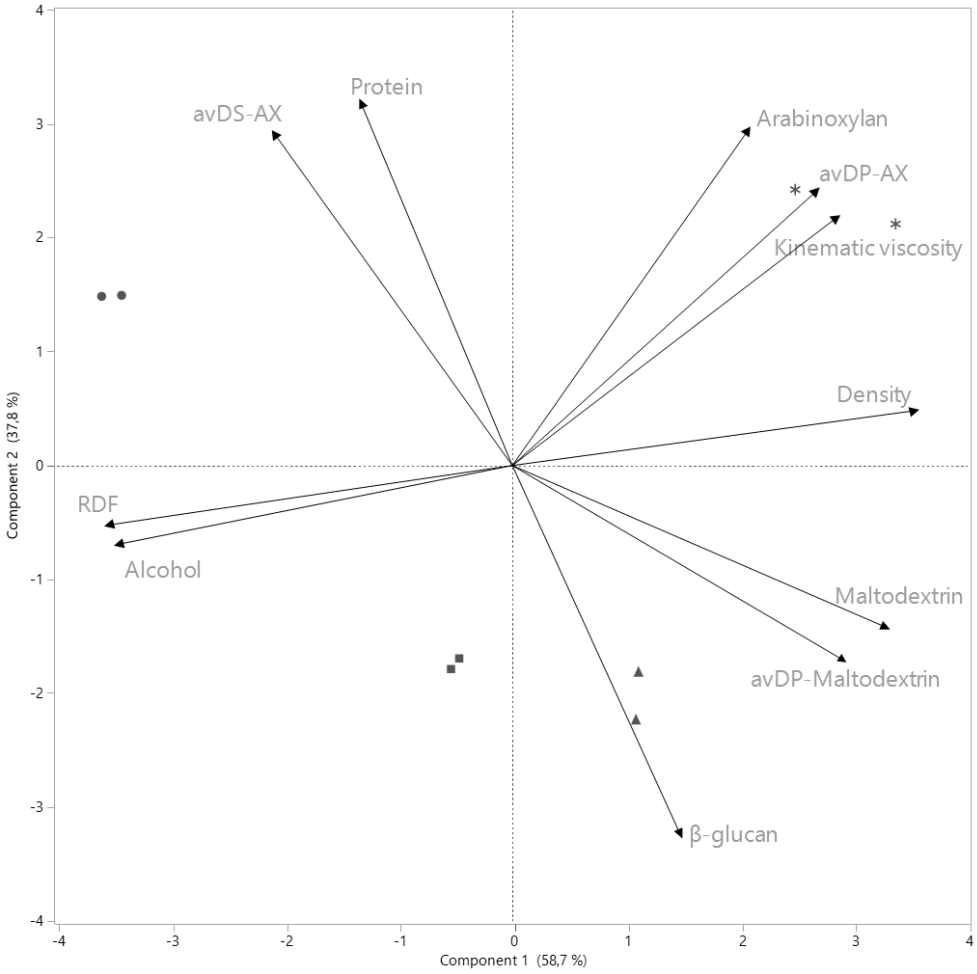

Figure 2: The bi-pot resulting from principal component analysis of the chemical composition, viscosity and density of 100% malt control beer and the beers made with 20% adjunct substitution (non-malted barley, rye or oat). The score plot shows the position of the lab-scale 100% malt control beer (round) and beers made with 20% adjunct substitution (barley: triangle; rye: asterisk; oats: square). (RDF: real degree of fermentation; avDS-AX: average degree of substitution of arabinoxylan; avDP: average degree of polymerization of arabinoxylan)

The rye that was used in previous experiments was milled with an impact mill, which resulted in a median particle size of 99.8 ± 1.1 µm. In order to improve the arabinoxylan solubilisation in rye beers even more, different milling techniques were used to disrupt the cell walls in which arabinoxylan is present (Vinkx & Delcour, 1996). Using pre-milled rye as a starting material, rye was ball-milled for 30 minutes in total. This resulted in a median particle size of 29.7 ± 0.0 µm. Coarsely milled rye was

obtained by laboratory disk-milling, with the same settings as used for malt. The median particle size was 741.3 ± 83.6 µm. The rationale behind this milling approach can be explained based on Figure 3. As endosperm cells in rye have sizes between 75 and 200 µm, these milling sizes cover different extents of milling. The finely milled sample was broken down to sub-endosperm cell level (Figure 3C), whereas the coarsely milled sample contained intact endosperm cells (Figure 3A). This difference in the degree of milling might influence the arabinoxylan solubilisation due to the improved accessibility towards endogenous enzymes. Besides the impact of milling, the impact of exogenous xylanase addition was studied. The impact-milled sample was used for this analysis.

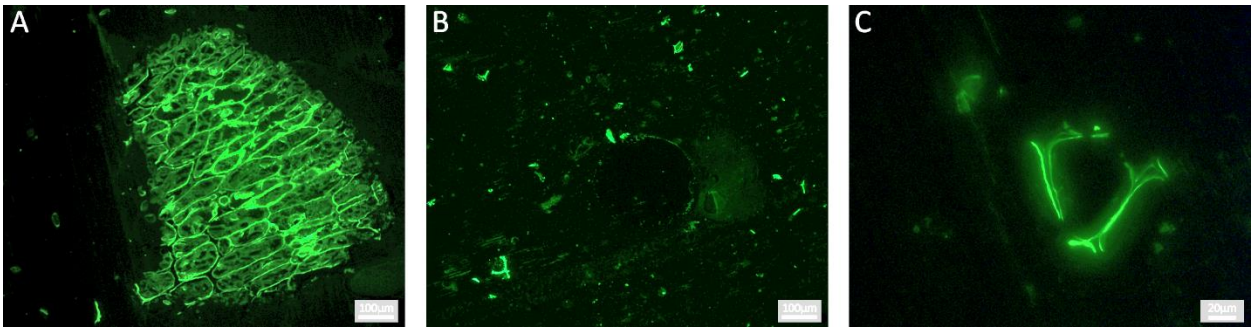

Figure 3: Fluorescence microscopy images of rye that was disk-milled (A) and ball-milled (B and C). Arabinoxylan, stained in green, is predominantly present in the cell walls. Image A shows an aggregate of intact endosperm cell walls after disk-milling. This structure is gone after ball-milling (B). The enlarged image C shows that the cell wall material is more fragmented after ball-milling than after disk-milling.

Duplicate lab-scale beers were brewed analogously to the previous experiment (Table 2). The values for the original and apparent extract were similar for all beers brewed. This resulted in an alcohol content of about 5.2 v/v% in all rye beers. Beer density was slightly higher for the beer with xylanase, although this difference is negligible. Beer colour and pH were nearly identical.

Table 2: Brew-technical data for the beers produced with 20% non-malted rye adjuncts with different milling sizes or with the addition of xylanase. Average values with standard deviations are given.

	Beer made with 20% finely milled rye substitution	Beer made with 20% coarsely milled rye substitution	Beer made with 20% rye substitution and xylanase
Original extract [w/w%]	10.80 ± 0.05	10.81 ± 0.05	10.92 ± 0.01
Apparent extract [w/w%]	0.97 ± 0.04	0.94 ± 0.01	1.04 ± 0.00
Alcohol [v/v%]	5.17 ± 0.05	5.19 ± 0.03	5.20 ± 0.01
RDF [%]	74.7 ± 0.3	74.9 ± 0.0	74.2 ± 0.0
Density [g/mL]	1.0019 ± 0.0001	1.0018 ± 0.0000	1.0022 ± 0.0000
pH Value [-]	4.13 ± 0.01	4.12 ± 0.00	4.12 ± 0.00
Colour Value [EBC]	13.8 ± 0.2	13.5 ± 0.0	13.8 ± 0.1

Carbohydrate content and kinematic viscosity were analysed for these different rye beers (Figure 4). The maltodextrin content and average degree of polymerization were comparable for all set-ups. In terms of milling degree, the arabinoxylan content was the highest for the finely milled rye sample, although it increased by only 8% compared to the coarsely milled sample. It seems that further solubilisation of arabinoxylan is possible but very limited. This is in line with the observation that arabinoxylan is tightly embedded in the cell walls and that severe conditions are necessary to release it (Vinkx & Delcour, 1996; Wood, 2010). The avDP of arabinoxylan is slightly increased for finely milled rye, which might indicate that some higher molecular weight arabinoxylan is solubilised or that the increased arabinoxylan content is solely responsible for that increase in avDP. The error on the β-glucan measurements is too large to draw conclusions, as no significant difference is detected. An analogous increase in β-glucan content upon finely milling, however, could be expected. The impact of these carbohydrate fractions on the kinematic beer viscosity is also rather limited, with a 1.7% increase for the finely milled sample.

The addition of an exogenous xylanase resulted in an extensive increase in arabinoxylan content, up to 2.00 g/L. Interestingly or coincidentally, the avDP of arabinoxylan was again decreased to 29, which is the same avDP as found in the control beer. Despite the substantial xylanase concentration, the avDP was not further reduced. The remaining malt or rye arabinoxylan might have had a composition or substitution pattern that is unfavourable for enzymatic breakdown. The avDS of arabinoxylan was decreased to 0.52 ± 0.00, which might indicate that more less substituted arabinoxylan was set free because of the xylanase. Again, no effect on the β-glucan content was noticed. The addition of xylanase resulted in a decrease of 4.2% in kinematic beer viscosity compared to the rye adjunct sample of the

previous experiment. Given the high impact of enzymes on the avDP of arabinoxylan, this decrease seems rather moderate. Presumably, the higher concentration of lower molecular weight arabinoxylan compensates for the decrease in avDP.

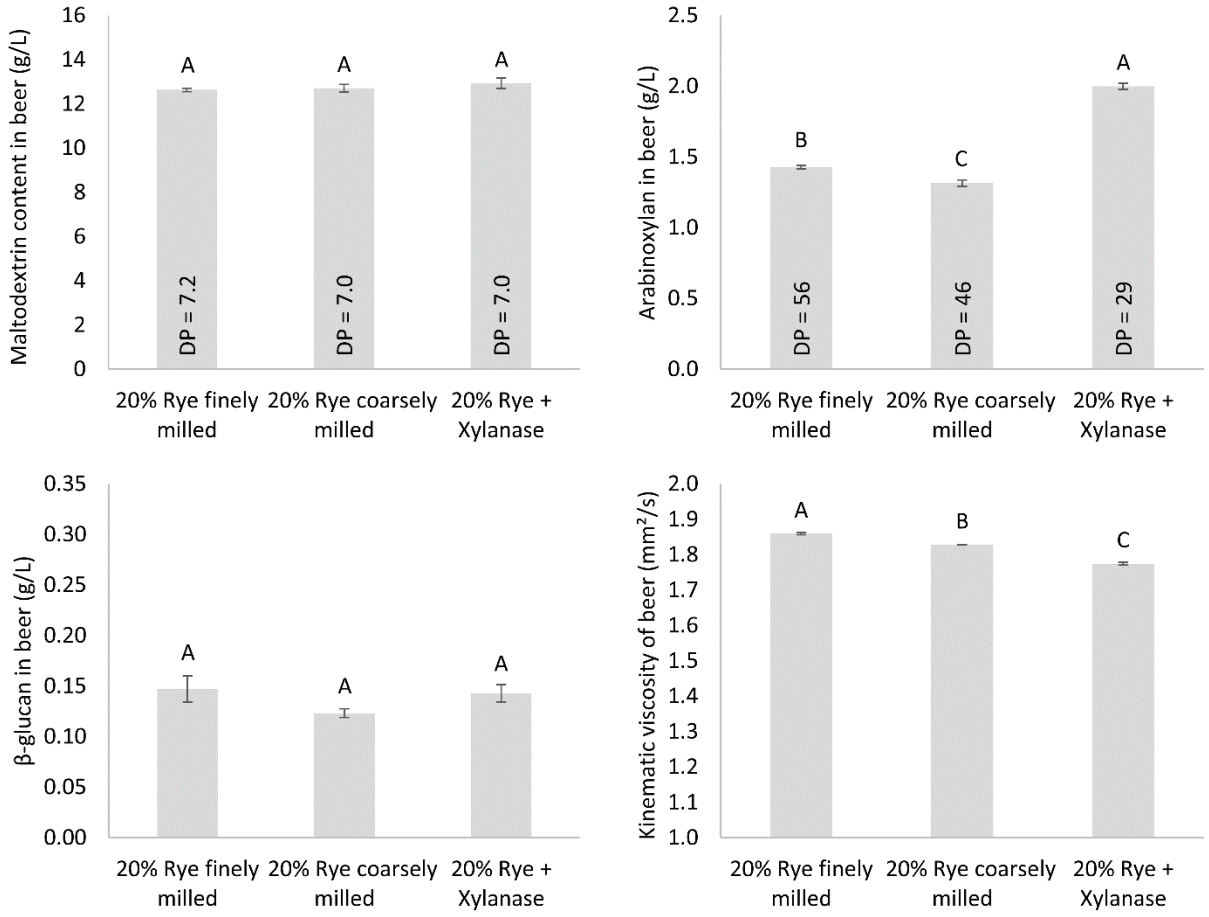

Figure 4: Maltodextrin, arabinoxylan and β-glucan content and kinematic viscosity of the beers produced with 20% rye adjuncts with different milling sizes or with the addition of xylanase. Beers were brewed in duplicate. Averages with standard deviations are presented. Tukey's HSD assigns different letters to samples that are significantly different ($p < 0.05$).

Integrating these results, rye is a valuable source of high molecular weight arabinoxylan that increases beer viscosity. The arabinoxylan solubilisation could be improved only moderately by fine milling, and the impact on beer viscosity was minor. It seemed that based on beer viscosity, xylanases can be used in the production of these rye beers and that the effect on beer viscosity is rather limited. This opens up perspectives for the production of these beers on a commercial scale, without the potential problems that might arise during filtration. To verify whether rye can improve mouthfeel characteristics, a sensory

317 analysis was executed. Two low-alcohol and low-calorie beers were brewed. The control beer consisted
318 of 100% malt, while for the experimental beer 30% of the malt was replaced by non-malted rye that was
319 coarsely milled to anticipate potential wort filtration issues. The results are summarised in Table 3.

320

321 The extract, alcohol, pH and colour values indicate that the beers were comparable to one another, with
322 a similar alcohol percentage and apparent extract. The caloric value was slightly increased for the 30%
323 rye beer, with 106.3 kJ/100 mL compared to 102.6 for the control. Although efforts were made to
324 ensure equal carbonation, the experimental rye beer ended up with slightly more carbon dioxide. The
325 commercial Attenuzyme PRO mixture was able to reduce the maltodextrin content to values around 2
326 g/L. The difference detected between both beers is minute compared to the much higher dextrin
327 content of regular beers. Both beers were brewed in a more dilute system to end up with a beer of 3.5%
328 alcohol. This immediately resulted in the dilution of all cereal carbohydrates, which is reflected in the
329 arabinoxylan content. To compare these beers with previous results, the arabinoxylan content is divided
330 by the original extract. This gives a measure for the amount of arabinoxylan that was released in the
331 system for each degree Plato, which on its turn is a measure for the amount of malt that was used. The
332 sensory control beer had a value of 74 mg arabinoxylan/L.°P, comparing with 85 mg arabinoxylan/L.°P
333 for the former control beer in Figure 1. Despite the fact that the rye substitution was increased to 30%,
334 the sensory beer ended up with 123 mg arabinoxylan/L.°P, which is similar to the coarsely milled rye
335 beer that had a value of 122 mg arabinoxylan/L.°P. This means that during the production of the sensory
336 beers, relatively less arabinoxylan ended up in the end product. This could be due to the difference in
337 the technical set-up of the wort filtration i.e. laboratory paper filter vs. lautering. The lower values of
338 arabinoxylan avDP might suggest that indeed higher molecular weight arabinoxylan is retained in the
339 spent grain fraction during the pilot-scale brewing process. No β-glucan was detected in both beers,
340 which can be explained by the β-glucanase side-activity of the Attenuzyme PRO mixture (data not
341 shown). This resulted in a beer viscosity of 1.21 mm^2/s of the control, compared to 1.35 mm^2/s for the
342 experimental beer. The rye arabinoxylan did not compensate completely in terms of beer viscosity
343 compared to the control of 6 v/v% alcohol and higher dextrin levels, although the improvement was
344 substantial.

345 An untrained test panel, consisting of 28 panellists, compared both beers and had to indicate which beer
346 was the fullest. Based on this one-sided directional difference test, 19 panellists indicated that the
347 experimental beer was fuller compared to the control, which is significant with $\alpha = 0.05$ (Meilgaard et

al., 2016). There was no indication for a slimy texture as was the case for beverages with added β-glucan (Lyly et al., 2003). No clear differences in aroma were observed as a result of the addition of rye and the overall flavour of the beers was rated as good by the panelists. The increase from 0.49 to 0.80 g/L of mainly high molecular weight arabinoxylan seemed to affect beer viscosity to the extent that it could be tasted by a sensory panel. Much higher contents of dextrin are needed to obtain equal results (Ragot et al., 1989), which also affects sweetness perception and caloric content. This emphasises the potential of arabinoxylan to improve beer mouthfeel in the context of NABLAB's and low-calorie beers.

Table 3: Brew-technical data, carbohydrate content and structural parameters and viscosity of food-grade 100% malt beer and beer produced with 30% non-malted rye adjunct substitution. The beer of which 30% of the malt was substituted with non-malted rye was identified as being higher in fullness ($p<0.05$).

	Beer made with 100% malt	Beer made with 30% rye substitution
Original extract [w/w%]	6.62	6.51
Apparent extract [w/w%]	-0.29	-0.24
Alcohol [v/v%]	3.54	3.45
pH Value [-]	4.54	4.54
Colour Value [EBC]	5.1	6.2
Caloric value [kJ/100mL]	102.6	106.3
CO_2 [g/L]	7.0	7.8
Maltodextrin (g/L)	1.97	2.14
avDP maltodextrin [-]	5	6
Arabinoxylan [g/L]	0.49	0.80
avDS arabinoxylan [-]	0.55	0.54
avDP arabinoxylan [-]	16	20
β-glucan [mg/L]	< LOD*	< LOD*
Viscosity [mm^2/s]	1.21	1.35
Participants that indicate this beer as the fullest in pairwise comparison	9	19

* LOD = 5 mg/L (McCleary & Nurthen, 1986)

4. Conclusion

Beers with different non-malted adjuncts were brewed to investigate their ability to improve mouthfeel in NABLAB's and low-calorie beers. Non-malted rye increased beer viscosity the most, which was attributed to the release of higher molecular weight arabinoxylan in the final end product. Fine milling or the addition of xylanases had an impact on arabinoxylan solubilisation and thus beer viscosity, although the impact was rather limited. During sensory analysis of low-alcohol and low-calorie beers, an experimental beer with 30% rye was assigned as the fullest beer. This underlines the role of arabinoxylan and its degree of polymerization in beer viscosity and hence fullness, compared to β-glucan and dextrin in adjunct brewing. Although brewing with rye might be challenging in an industrial context because of its notorious effect on filtration properties, this approach can be proposed as part of a clean label solution to improve mouthfeel characteristics of NABLAB's or low-calorie beers.

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6. Conflicts of interest

None.

7. References

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