Received: 23/01/2023 Revised: 11/04/2023 Accepted: 21/05/2023 Published: 05/10/2023

ORIGINAL ARTICLE

DOI 10.58430/jib.v129i3.27

INSTITUTE OF BREWING & DISTILLING

Filtration enzymes applied during mashing affect beer composition and viscosity

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Abstract

Why was the work done: Filtration enzymes targeting the degradation of arabinoxylan and β-glucan are widely used in the brewing industry to improve wort and beer filtration. Although these enzymes have proven their effectiveness in improving lautering efficiency and beer filterability, the effect of varying dosage and type of enzyme preparation on beer composition and quality has not been described.
How was the work done: The impact of dose of different filtration enzyme preparations (Laminex®750, Laminex®C2K, and Laminex®MaxFlow4G) was investigated on the free ferulic acid content in wort, chemical composition and viscosity of beer, together with the content and structure of arabinoxylan and β-glucan.
What are the main findings: The structural features of arabinoxylan and β-glucan in beer were strongly influenced by the dosage and type of filtration enzyme. In general, the high-molecular weight (HMW) arabinoxylan and HMW β-glucan and total β-glucan content in beers decreased with increasing enzyme dosage, while the total arabinoxylan levels increased. The HMW arabinoxylan content was strongly related to beer viscosity. The use of filtration enzymes decreased HMW arabinoxylan content and beer viscosity, which could affect the palate fullness. Overdosing filtration enzymes resulted in more ferulic acid, the precursor (in the presence of phenolic yeast) of the clove-like 4-vinyl guaiacol.
Why is the work important: This work provides brewers with insight as to how filtration enzymes affect beer composition and viscosity. Further it can help make an informed choice of the type of filtration enzyme and the dosage applied during mashing.

Keywords:

beer viscosity, filtration enzymes, mashing, arabinoxylan, β-glucan

Introduction

The role of arabinoxylan and β-glucan in adverse processing events during beer production are well known, although both non-starch carbohydrates can have a positive effect on beer quality. Arabinoxylan and β-glucan are both hemicellulose polymers that are derived from barley, barley malt, and other cereals during beer production. Structurally, arabinoxylan is composed of a linear backbone consisting of β-(1,4)-glycosidic linked β-D-xylopyranosyl monomers, to which L-arabinofuranose is substituted by α-(1,2) and/or α-(1,3) linkages. Furthermore, ferulic acid and acetic acid can be esterified to L-arabinofuranose (Henry 1988; Viëtor et al. 1992; Debyser et al. 1997; Han 2000). Barley β-glucan is structurally composed of building blocks consisting of three or four β-(1,4)-linked glucose monomers which are connected by β-(1,3) bonds (Zielke et al. 2019). In barley and barley malt, the endosperm cell walls are composed of approximately 70% β-glucan and 30% arabinoxylan, while the proportions are reversed in the aleurone cell walls (Bamforth and Kanauchi 2001; Langenaeken et al. 2020c).

During mashing, the aim is for the maximal conversion of starch into fermentable sugars, while other constituents are extracted and degraded by endogenous enzymes. With arabinoxylan and β-glucan, only the fraction that is water extractable or solubilised by the action of enzymes result in the wort, while the water unextractable and unsolubilised arabinoxylan and β-glucan fractions are in the spent grains (Debyser et al. 1998; Han and Schwarz 2018; Krahl et al. 2009; Langenaeken et al. 2020a; Li et al. 2005).

Both water extractable and unextractable non-starch carbohydrates negatively affect the separation of the wort from spent grains. The high viscosity forming potential of high-molecular weight (HMW) non-starch carbohydrates in wort and the high water holding capacity of water unextractable non-starch carbohydrates in the spent grains both have a detrimental effect on the wort filtration rate (Lu and Li 2006; Cui et al. 2013; Gastl et al. 2020). During beer filtration, HMW arabinoxylan and HMW β-glucan (> 80 kDa) are related to membrane clogging and poor beer filterability due to their potential contribution to beer colloid formation (Sadosky et al. 2002; Speers et al. 2003; Stewart et al. 1998; Egi et al. 2004; Jin et al. 2004; Lu et al. 2005; van der Sman et al. 2012; Kupetz et al. 2015; Martinez Amezaga et al. 2016).

In addition to the adverse effects on wort and beer filtration, arabinoxylan can also be associated indirectly with the production of off flavours during fermentation. Firstly, HMW arabinoxylan is involved in premature yeast flocculation, which is linked to elevated SO_2 levels in beer (Herrera et al. 1991; Herrera and Axcell 1991; Shang et al. 2020; Shang et al. 2015). Secondly, the release of ferulic acid from arabinoxylan during mashing can be decarboxylated by phenolic off-flavour positive yeast (Coghe et al. 2004; Szwajgier et al. 2005) resulting in 4-vinyl guaiacol – a clove-like off flavour- in beer.

Over the years, barley breeding programmes have led to the reduction in the β-glucan content of barley, while maltsters and brewers have optimised their processes to maximize β-glucan degradation (Yousif and Evans 2020). As a result, filtration problems are rarely attributed to β-glucan, but more often to arabinoxylan. Gastl et al (2020) showed that a higher arabinoxylan content and a higher molecular weight significantly reduce filter cake permeability. Therefore, commercial filtration enzymes, a mixture of microbial β-glucanases and endoxylanases, are frequently added during mashing (Bamforth 2009; Malfliet 2013). The use of filtration enzymes have proved to be effective, but their impact on the structural characteristics of the non-starch carbohydrates in beer has yet to be investigated (Evans et al. 2011). The content and molecular weight of non-starch carbohydrates in beer can be important for several beer quality parameters. These include the positive contribution of HMW arabinoxylan to the foam stability of beer (Evans et al. 1999; Li and Du 2019; Song et al. 2022). Moreover, the viscosity enhancing potential of arabinoxylan and β-glucan may contribute to the improved palate fullness of beer (Lyly et al. 2003; Langenaeken et al. 2020b). With alcohol-free and low alcohol beers, the absence of ethanol results in low viscosity and reduced palate fullness. Therefore, a higher beer viscosity is required in these beers to improve palate fullness (Sohrabvandi et al. 2010). Accordingly, the use of filtration enzymes during

mashing may be detrimental to the palate fullness of beers.

Here, the impact of filtration enzymes originating from different microbial sources on the final beer quality is investigated. The initial aim was to gain insight into the effect of dosage of filtration enzymes on the content and structural features of the non-starch carbohydrates and how this affects the beer viscosity. Secondly, the impact of feruloyl esterase activity in enzyme preparations on ferulic acid release during mashing was investigated. This research mainly focused on the secondary effects of filtration enzymes, as their efficacy in filtration is well established. The findings of this research are intended to provide brewers with information about how beer composition and quality can be influenced by filtration enzymes. This can help to improve the control of final beer composition together with recipe development.

Materials and Methods

Materials

Barley malt (variety Planet, harvest 2019) was supplied by Boortmalt N.V. (Herent, Belgium). Filtration enzymes (Laminex® MaxFlow 4G, Laminex® 750 and Laminex® C2K) were kindly provided by International Flavors and Fragrances Inc. (New York, USA). Barley malt was finely milled with a laboratory disk mill with a gap of 0.2 mm between two disks rotating in opposite directions (Buhler, Uzwil, Switzerland). SafeAle™ S-04 (Lesaffre, Marcq-en-Baroeul, France) and Cascade hop (harvest 2019) pellets (Brewferm, Belgium) were purchased at Brouwland bvba (Beverlo, Belgium).

Determination of xylanase and β-glucanase activity in enzyme preparations

The XylX6 method and MBG4 method (Megazyme, Bray, Ireland) were used to determine the activity of endo-(1,4)-β-xylanases (hereafter 'xylanases') and endo-(1,3:1,4)-β-glucanases (hereafter 'β-glucanases'), in the commercial filtration enzyme preparations. For the measurement of the xylanase and β-glucanase activity, the procedures are as described by Mangan et al (2018) and Cornaggia et al (2019), with some minor adaptations.

Acetate buffer (0.1 M, pH 5.5, 0.5 mg/mL BSA) was used as a dilution buffer for both enzymes. The filtration enzymes were diluted until a suitable concentration was reached for the conditions (30°C, pH 5.5). Each measurement was performed in triplicate. Xylanase and β-glucanase activity are expressed as the amount of XylX6 and MBG4 as Units per mL of enzyme.

Laboratory scale brewing

Mashing was performed in a laboratory scale mashing unit LB 8 (Lochner Labor und Technik GmbH, Berching, Germany). Deionised water acidified with 0.75 mM sulphuric acid and modified with 2.55 mM calcium chloride was used as brewing liquor to achieve a mash pH of 5.6. Finely milled barley malt (50 g dry matter) was added to pre-heated liquor (200 mL) at 45°C to achieve a liquor-to-grist ratio of four. The mash was continuously stirred (100 rpm) during a temperature time mashing scheme consisting of four isothermal periods at 45°C (15 min), 62°C (30 min), 72°C (30 min), and 78°C (10 min) with an intermediate heating rate of 1°C/min. At the start of the mashing programme, filtration enzymes (Laminex® MaxFlow 4G, Laminex® 750, and Laminex® C2K) were added to the mash. A wort sample without addition of filtration enzyme was prepared in duplicate for each mashing trial (control wort). At the end of mashing, an additional 100 mL of brewing water at 78°C was added to the mash before wort filtration. Wort filtration was performed using a pre-folded filter (Whatman 597 ½ 320 mm) for 30 min. Pelletised hops were added to 200 mL wort, and wort boiling was mimicked in an autoclave at 121°C for 15 min and allowed to cool down to 80°C. Although an unconventional temperature time profile for wort boiling, wort sterilisation, α-acid isomerisation and protein-polyphenol complexation occurred. However, this treatment may affect colour formation and aroma/flavour of the wort and beer. The boiled wort was filtered with a pre-folded filter (Whatman 597 ½ 320 mm). Mass losses during wort boiling due to evaporation varied between 7.1 and 8.1%. Yeast (0.2 g, SafeAle S-04) was added to the wort (200 mL) in a 250 mL Schott bottle, and static fermentation was performed at a constant temperature of 23°C. After 14 days of fermentation, the yeast was removed by filtration using a pre-folded filter

(Whatman 597 ½ 320 mm). The mass losses during fermentation varied between 3.9-4.1% (Langenaeken et al, 2020a).

Analysis of free phenolic acids in wort

Free phenolic acids in wort were analysed using RP-HPLC and a UV detector. Wort samples (2.5 mL) were acidified with 1.0 M HCl to pH 2. Caffeic acid was added as the internal standard (50 µL of a solution of 10 mg caffeic acid in 20 mL methanol). Phenolic acids were extracted three times with 2.5 mL diethyl ether. Between the extraction steps, the samples were vortexed and centrifuged (1000 x *g*, 5 min, 20°C). The diethyl ether phases were collected and evaporated under nitrogen. The dried extract was dissolved in methanol (0.5mL) and filtered (0.45 µm) before analysis. A calibration sample containing caffeic acid, 4-hydroxybenzaldehyde, trans-p-coumaric acid, vanillin, and trans-ferulic acid in methanol was analysed for peak identification and quantification of the phenolic acids in wort (Antoine et al. 2003; Snelders et al. 2013). Separation of phenolic acids was performed using a Luna phenyl hexyl column (250 mm x 4.6 mm, 5 µm particle size). Samples (20 µL) were injected and separated at 45°C. A ternary gradient consisting of aqueous trifluoroacetic acid (1 mM), acetonitrile, and methanol at a flow rate of 1.0 mL/min was used. Signals were monitored at 280 nm using a UV detector. Each wort sample was prepared in duplicate for each condition and analysed in duplicate resulting in a quadruplicate measurement.

Beer composition and viscosity

The ethanol content (% v/v), density (g/mL), real extract (% w/w), original extract (% w/w), and the real degree of fermentation were analysed using an Alcolyzer Beer Analysing System (Anton Paar, Graz, Austria). Fermentable sugar and dextrin content were analysed according to Langenaeken et al (2019). For the analysis of protein, beer (60 µL) was transferred to a tin capsule and dried overnight at 50°C. The total nitrogen content of the residue was measured using an elemental analyser (EA 1108 CHNS–O elemental analyser, CE Instruments/ Thermo Scientific, Waltham, MA, USA) and the Dumas method. The protein content was calculated by multiplying the total nitrogen content by 6.25 (Celus et al. 2006). The total arabinoxylan content, average degree of polymerisation and average degree of substitution of arabinoxylan were analysed according to Courtin et al (2009). A distinction was made between HMW arabinoxylan, precipitating with ethanol at 65% (v/v), and low-molecular weight (LMW) arabinoxylan which remains in solution under those conditions. The HMW β-glucan content was analysed according to EBC Method 8.13.2 ('high molecular weight ß-glucan content of wort: fluorimetric method'). Here, the measured fluorescence intensity ($\lambda_{excitation}$ = 360 nm, $\lambda_{emission}$ = 450-460 nm), is a result of the specific binding between the fluorochrome calcolfuor and HMW β-glucan (> 10 kDa). The total β-glucan content in beer was measured according to EBC Method 8.13.1 ('high molecular weight β-glucan content of wort enzymatic method') with modifications. These led to increased precipitation of β-glucan, resulting in the better measurement of total β-glucan content in beer. To degassed beer (5 mL), ethanol (97% v/v) was added to a final concentration of 80% v/v, with precipitation for 24 hours at 7°C. The precipitate was isolated by centrifugation (30 min, 4000 x *g*) and the pellet washed with ethanol (10 mL, 97% v/v). The supernatants were discarded and the pellets were lyophilised. Next, β-glucan was degraded into glucose by lichenase and β-glucosidase, and the amount of glucose quantified as described in EBC Method 8.13.1 (McCleary and Nurthen 1986). The viscosity of beer was measured using an Ostwald viscometer (SI Analytics GmbH, Germany) in a water bath at 25°C. All measurements were performed in duplicate on each beer and as each beer sample was produced in duplicate, each value is the result of quadruplicate measurement.

Statistical analysis

The software JMP Pro 16 (SAS Institute Inc., Cary, North Carolina, USA) was used for statistical data analysis. A one-way ANOVA test with a comparison of mean values using a Tukey-Kramer posthoc test (p-value <0.05) was performed to verify significant differences between means from quadruplicate measurements. To create predictive models for beer viscosity, multi-linear forward regression was applied.

Results and discussion

Differences in the specification of filtration enzymes

Table 1 details the specifications of the filtration enzyme preparations. The xylanase and the β-glucanase activities differ to a large extent. Laminex® MaxFlow 4G contained the highest xylanase activity (1248 XylX6 U/mL), which was 13- and 6-fold higher than the xylanase activity as measured in Laminex® 750 and Laminex® C2K, respectively. Similarly, the β-glucanase activity in MaxFlow 4G (18.19 MBG4 U/mL) was higher compared to Laminex® 750 (0.08 MBG4 U/mL) and Laminex® C2K (1.80 MBG4 U/mL). However, the comparison between xylanase and β-glucanase activity is not appropriate due to the likely difference in the kinetics between the enzyme and substrate. The optimal working conditions for Laminex®750 (80°C, pH 3.5-5.5), Laminex®C2K (60°C, pH 4.5), and Laminex® MaxFlow 4G (70-75°C, pH 6.0) were different, as were the microbial origins of the enzymes. As a consequence, the substrate specificity of the xylanase and the β-glucanase to barley arabinoxylan and barley β-glucan may differ. Thus, the total enzyme activity, their optimal working conditions, and their substrate specificity influence the performance of each filtration enzyme preparation. The recommended dosage of Laminex® 750 (0.05-0.30 kg/1000 kg of grist), Laminex® C2K (0.05-0.30 kg/1000 kg of grist) and Laminex® MaxFlow 4G (0.05-0.40 kg/1000 kg of grist) was slightly different.

To investigate the impact of dosage of the enzymes on beer composition, the lower and upper limit of the recommended dosage were added at the start of mashing and accordingly are referred to as 'low' and 'high' dosage. Therefore, the results reported here are relevant to brewing practice. Furthermore, a very high dosage (ten times the recommended maximum dosage), or 'very high' was added to demonstrate the maximum potential of the enzymes.

Filtration enzymes and the occurrence and structural properties of non-starch carbohydrates

Figure 1 shows the arabinoxylan content of the beers produced by the addition of filtration enzymes at the start of mashing. Compared to the control beer, a low dosage addition of Laminex® 750, Laminex® C2K, and Laminex® MaxFlow 4G, increased the arabinoxylan content by 7%, 12%, and 5%, respectively. At high dosage, a 14, 21, and 24% increase in arabinoxylan content was observed for Laminex® 750, Laminex® C2K, and Laminex® MaxFlow 4G, compared to the arabinoxylan in the control sample. This demonstrates that high dosages, still within the recommended dosage,

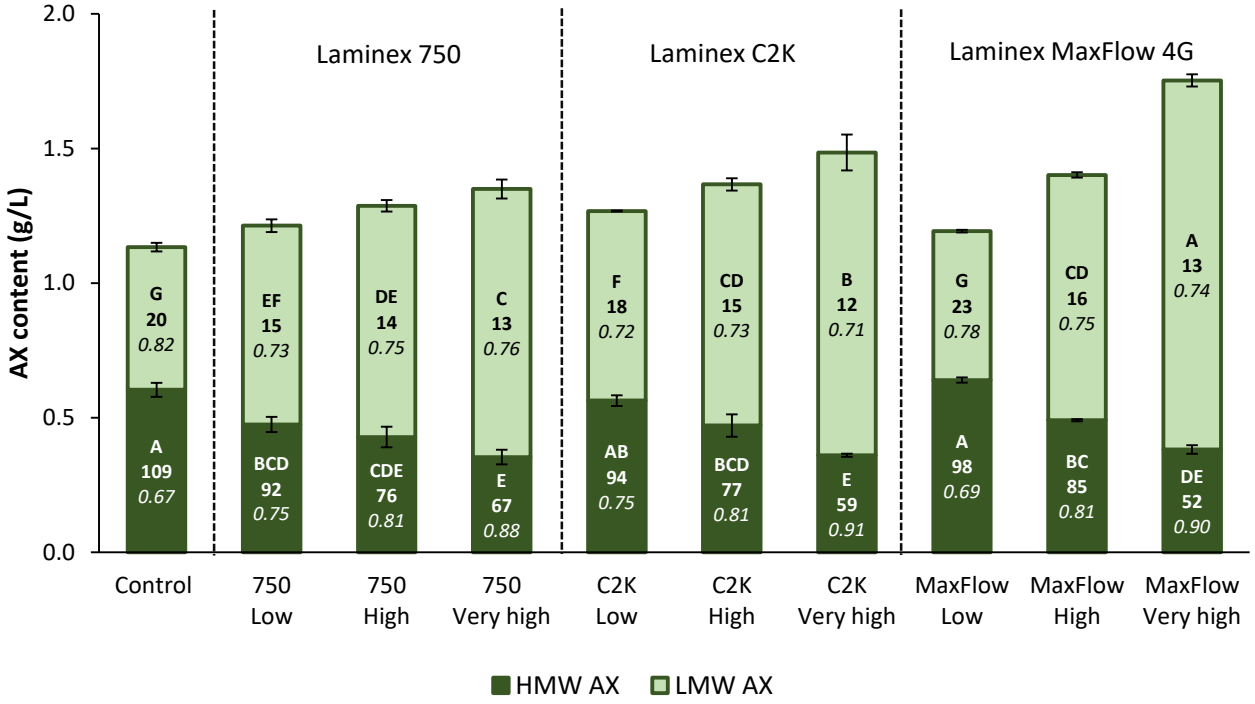
Table 1. Specification of the filtration enzymes.

Optimal working conditions (pH and temperature), microbial origin, and recommended usage of the purified enzymes were provided by the supplier.

	Laminex® 750	Laminex® C2K	Laminex® MaxFlow 4G
Xylanase activity (XylX6 U/mL)	98 ± 3	207 ± 3	1248 ± 32
β-glucanase activity (MBG4 U/mL)	0.08 ± 0.00	1.80 ± 0.05	18.19 ± 0.30
Optimal working temperature	80°C	60°C	70-75°C
Optimal pH	3.5-5.5	4.5	6.0
Microbial origin	*Geosmithia emersonii*	*Penicillum funiculosum*	*Trichoderma reesei* and *Bacillus subtilis*
Recommended usage amount (kg/1000 kg of grist)	0.05-0.30	0.05-0.30	0.05-0.40

Figure 1. The AX content in beers produced with the addition of commercial filtration enzymes at the start of mashing, divided into low-molecular weight (LMW) and high-molecular weight arabinoxylan (HMW AX) content (g/L).

Filtration enzymes were added at the start of mashing at the lower (low) and upper (high) limit of the recommended dosage, as well as a very high dose (10 times the upper limit). The control beer was produced without adding filtration enzymes. The first letter label on the histogram bars assigns a significant difference between the LMW AX or HMW AX content between the samples as determined by a Tukey-Kramer HSD test (α<0.05). The average degree of polymerisation AX (bold) and the average degree of substitution (italics) of both the LMW and HMW AX fractions are shown as the second and third labels, respectively.



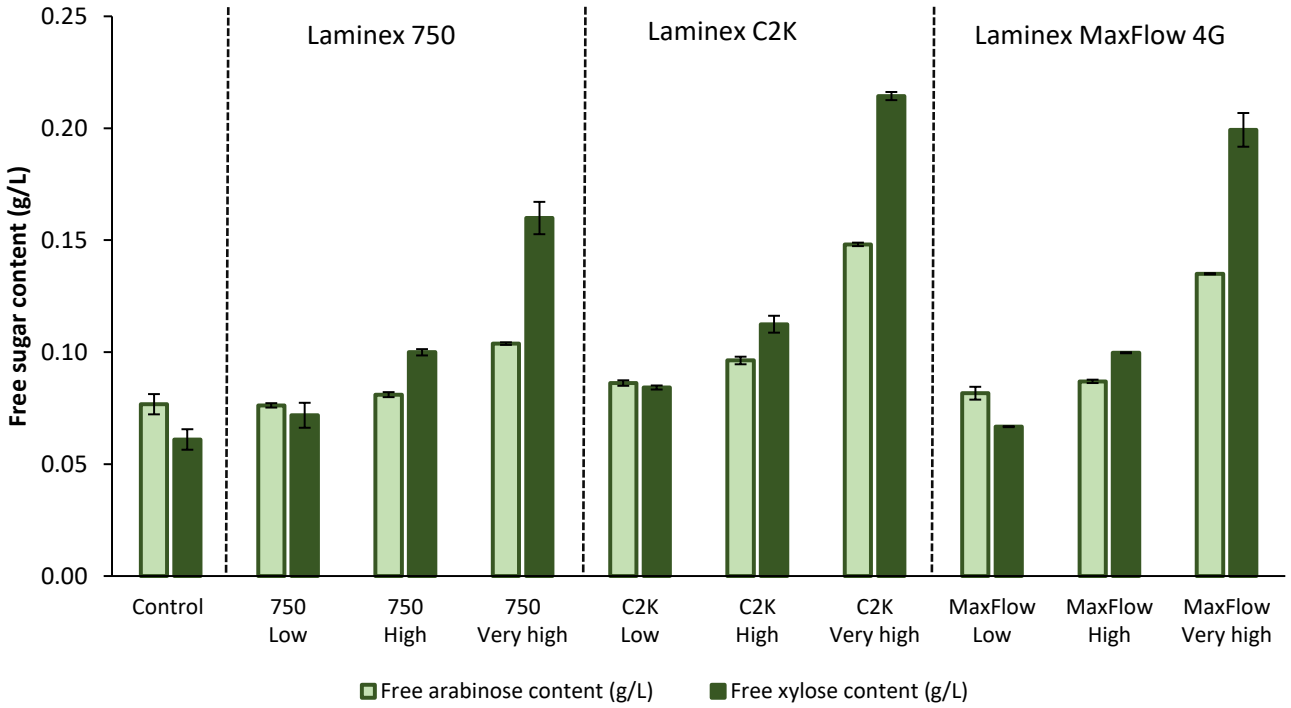
would lead to 14 to 24% more arabinoxylan in the final beer. A maximum value of 1.75 g/L was observed at a very high dosage of Laminex® MaxFlow 4G, which was 55% higher than the arabinoxylan content of the control beer without the addition of exogenous enzymes. It can be concluded that the amount and the type of filtration enzyme preparation used influences the arabinoxylan content in beer.

The higher arabinoxylan content of beers produced by the addition of filtration enzyme can be explained by xylanases acting on water unextractable arabinoxylan, leading to increased extraction during mashing. The addition of a low amount of Laminex® MaxFlow 4G increased the total arabinoxylan content by only 5%, while it exhibited the highest xylanase activity, compared to an increase of 7 and 12% at a low dosage for Laminex® 750 and Laminex® C2K, respectively. On the contrary, 19, 31, and 55% more arabinoxylan was in the final beer after adding at a very high dosage, Laminex® 750, Laminex® C2K, and Laminex® MaxFlow 4G, which was in line with the xylanase activity. Based on these observations, it can be concluded that arabinoxylan solubilisation was a function of total xylanase activity but also depends on the specificity of enzymes towards water unextractable arabinoxylan together with their optimal working conditions.

The HMW arabinoxylan content of beer decreased on enzyme addition (Figure 1). While the average degree of polymerisation of arabinoxylan of the HMW fraction after enzyme addition was lower compared to the control sample, the average degree of substitution of arabinoxylan of the HMW fraction increased. This can be explained by the preference of xylanases to hydrolyse low-substituted arabinoxylan structures, while their action on high-substituted arabinoxylan molecules was limited. Indeed, at a very high dosage, HMW arabinoxylan structures with an average degree of substitution of 0.88-0.91 were found. At this very high dosage, it can be assumed that the degradation of the HMW arabinoxylan molecules was limited due to the

Figure 2. The free arabinose and xylose content (g/L) of beers produced with and without the addition of filtration enzymes at the start of mashing.

Filtration enzymes were added at the start of mashing at the lower (low) and upper (high) limit of the recommended dosage, as well as a very high dose (10 times the upper limit). The control beer was produced without adding filtration enzymes.

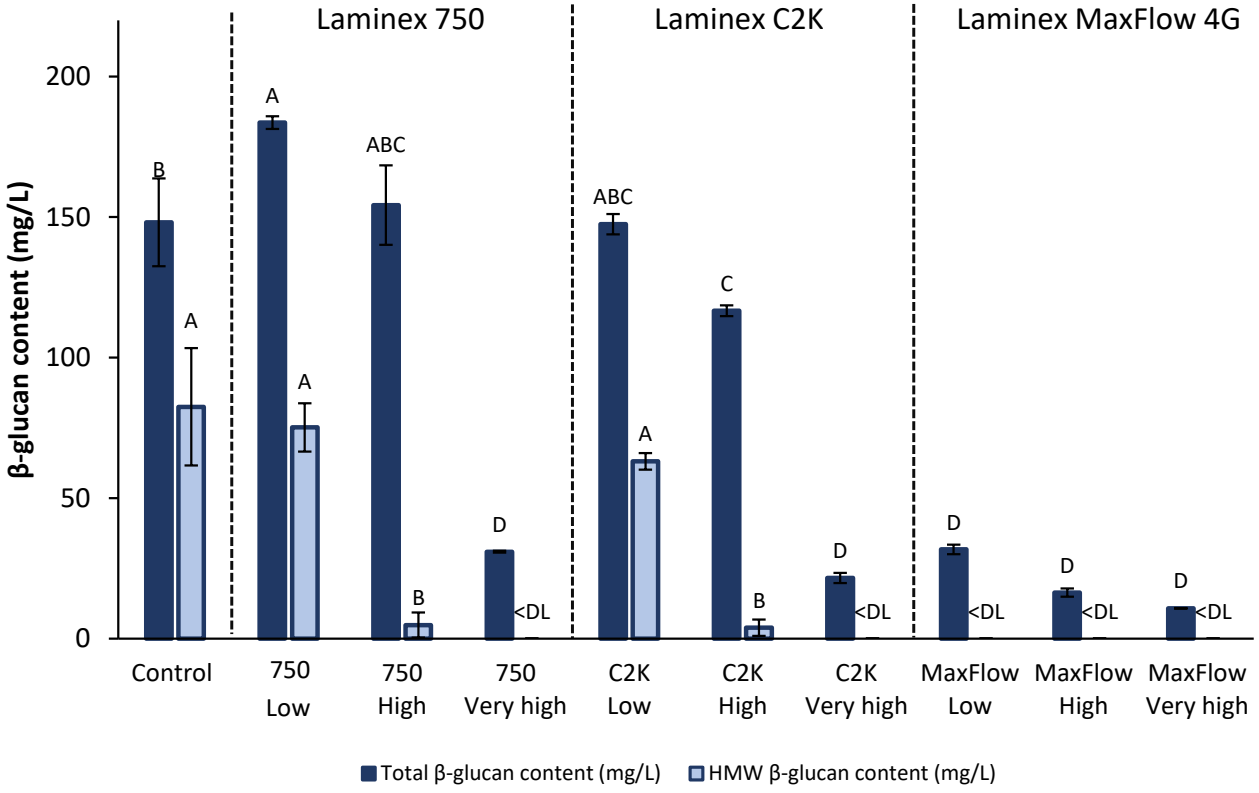


remaining highly substituted arabinoxylan substrate. As a consequence, even at an excess of xylanase added, arabinoxylan structures with an average degree of polymerisation between 52 and 67 were still present. However, it is unclear to what extent those highly degraded arabinoxylan molecules might negatively affect lautering and beer filtration performance.

The LMW arabinoxylan content in beer increased by dosing more filtration enzymes. This can be explained by the increased solubilisation of water unextractable arabinoxylan by the exogenous enzymes and the simultaneous degradation of HMW to LMW arabinoxylan. Further, the average degree of polymerisation of the LMW arabinoxylan fractions was lower when more filtration enzymes were added during mashing, indicating the more extensive degradation of arabinoxylan with increasing dosage. The greater release of free arabinose and xylose when adding more filtration enzymes (Figure 2), reflects the activity of arabinofuranosidase and xylosidase in the enzyme preparations. As a result, it can be argued that the combined action of xylanase, arabinofuranosidase, and xylosidase leads to a more intensive degradation of arabinoxylan structures. However, recent research by Evans et al (2022) found no significant correlation between endogenous malt arabinofuranosidase activity and the lautering efficiency of 96 commercial barley malts and the corresponding mash. As endogenous malt arabinofuranosidase activity rapidly decreases above 50°C and mashing-in temperatures of 65°C were used, this could be expected (Debyser et al. 1998). Nevertheless, when using microbial arabinofuranosidase in filtration enzyme preparations which are more stable at higher temperatures, the effect on arabinoxylan degradation could be more important. Moreover, Debyser et al (1998) reported that a high endogenous xylanase activity positively impacted lautering efficiency, which suggests that more intensive arabinoxylan degradation improves lautering efficiency. The average degree of substitution of the LMW arabinoxylan fraction was lower in beers with the addition of filtration enzymes, regardless of the dosage, compared to the control. This can be explained by the action of arabinofuranosidase, releasing more arabinose from the arabinoxylan molecules which is supported by the higher arabinose content in beers with increasing enzyme dosage (Figure 2). A further explanation might be

Figure 3. Total and high-molecular weight (HMW) β-glucan content (mg/L) of beers produced with the addition of filtration enzymes at the start of mashing.

Filtration enzymes were added at the start of mashing in the lower (low) and upper (high) limit of the recommended dosage, as well as a very high dose (10 times the upper limit). The control beer was produced without adding filtration enzymes. A different letter code is used to assign a significant difference in high-molecular weight or total β-glucan content as determined by a Tukey-Kramer HSD test (α<0.05).



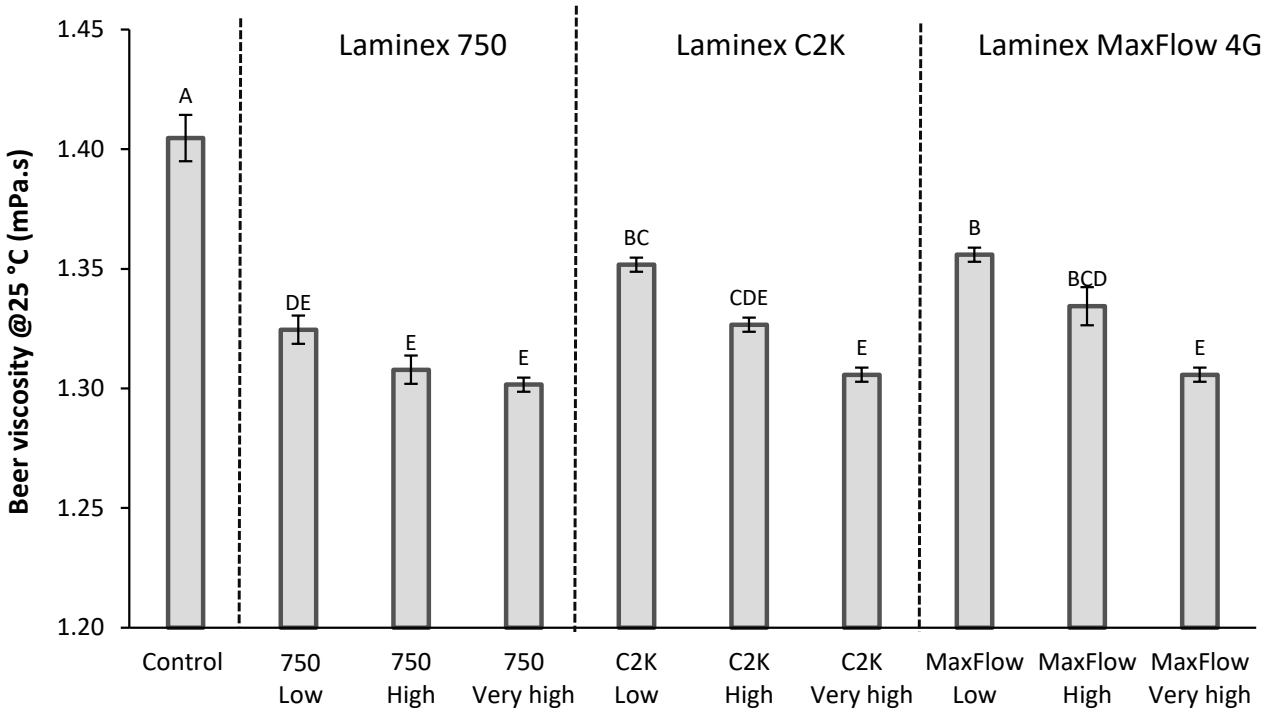
the increased solubilisation of low-substituted arabinoxylan molecules when enzyme dosage is increased. Finally, the faster degradation of low-substituted HMW to LMW arabinoxylan molecules can lead to the enrichment of lower substituted molecules in the LMW arabinoxylan fraction.

The 'very high' dosage of filtration enzymes led to more extensive degradation and increased solubilisation of arabinoxylan compared to 'high' dosage. This suggests that at a high dosage (defined as the upper limit of the dosage recommended by the supplier), the full potential of xylanases to degrade arabinoxylan or solubilise arabinoxylan was not reached. Accordingly, overdosing enzymes will not improve filtration performance.

In addition to xylanase activity, the enzyme preparations contain β-glucanase activity, which impacts on the β-glucan content of the beers. High and very high dosages resulted in an undetectable or very low HMW β-glucan content in beer (Figure 3). However, a significant decrease in the HMW β-glucan content was not achieved with a low dosage of Laminex® 750 and C2K, whereas low dosage of Laminex® MaxFlow 4G resulted in undetectable HMW β-glucan. High dosage of Laminex® 750 and Laminex® C2K resulted in respectively 117 and 154 mg/L total β-glucan in the beer. This implies that high dosing leads to the degradation of HMW β-glucan but does not result in complete degradation of the β-glucan population, even with overdosing. Nevertheless, the total β-glucan content in the beer was low (11-31 mg/L) and it is postulated that a fraction of β-glucan was not susceptible to enzymatic hydrolysis, possibly because of the interaction of β-glucan with other molecules, such as proteins (Zielke et al. 2017). Interestingly, the higher total β-glucan content in beer with low dosage Laminex® 750 beer compared to the control. This suggests that part of water unextractable β-glucan was liberated from the solid fraction by the action of exogenous β-glucanases or enzymes acting as β-glucan solubilases during

Figure 4. Viscosity of beers at 25°C produced with and without the addition of filtration enzymes at the start of mashing.

Filtration enzymes were added at the start of mashing in the lower (low) and upper (high) limit of the recommended dosage, as well as a very high dose (10 times the upper limit). The control beer was produced without the addition of filtration enzymes. A different letter code is assigned to a significant difference in beer viscosity as determined by a Tukey-Kramer HSD test (α<0.05).



mashing. In the literature, it is suggested that β-glucan solubilisation is hindered by interaction with arabinoxylan in the endosperm cell walls (Bamforth, 2009; Bamforth and Kanauchi 2001; Langenaeken et al. 2020c). Therefore, the arabinoxylan degrading enzymes in the enzyme preparations (xylanase, arabinofuranosidase, β-xylosidase, and feruloyl esterase) may positively affect the solubilisation of β-glucan. However, further work is required to understand the dynamics behind β-glucan solubilisation and degradation during brewing.

In summary, filtration enzymes differently influence the content and structural characteristics of arabinoxylan and β-glucan content in beer. With arabinoxylan, parts appear resistant to enzymatic degradation due to their structural properties. In addition to the degradation of the water extractable compounds, the enzymes promote the release of water unextractable non-starch carbohydrates from the solid fraction during mashing. Thus, the use of filtration enzymes can explain the differences in structural characteristics of arabinoxylan in commercial beers (Michiels et al. 2023).

Moreover, the addition of filtration enzymes has a influence on the β-glucan content and their structural properties in beer. Given that filtration enzymes are effective in degrading HMW arabinoxylan and HMW β-glucan contributes to trouble free filtration.

Impact of filtration enzymes on viscosity and composition of beer

Figure 4 demonstrates the impact of filtration enzymes on beer viscosity. At low dosage, a decrease in beer viscosity between 0.04 mPa.s and 0.08 mPa.s was found compared to the control beer. Taking the viscosity of water as a minimum, this corresponds to a decrease in relative viscosity of between 9 and 16%. At very high dosage of enzymes, a maximum decrease in beer viscosity of 0.10 mPa.s was reached, which is equivalent to a reduction of 20% relative to the viscosity of water. Since wort and beer viscosity are both positively related to the time of lautering and beer filtration, this was not entirely unexpected. However, a lower beer viscosity can be detrimental to the palate fullness of beers (Krebs et al. 2021).

For non-alcoholic pilsner type beers, a low beer viscosity explains the lack of palate fullness. Indeed, the reduction in beer viscosity at 25°C caused by the removal of 5% ABV ethanol is 0.17 mPa.s When filtration enzymes are used, the decrease in beer viscosity at 25°C falls in the range of 0.04-0.10 mPa.s, which is equivalent to 23-58% of the decrease in beer viscosity caused by the removal of ethanol (5% v/v). This indicates that the use of filtration enzymes may affect the palate fullness of beers, which is noteworthy when producing non-alcoholic lagers.

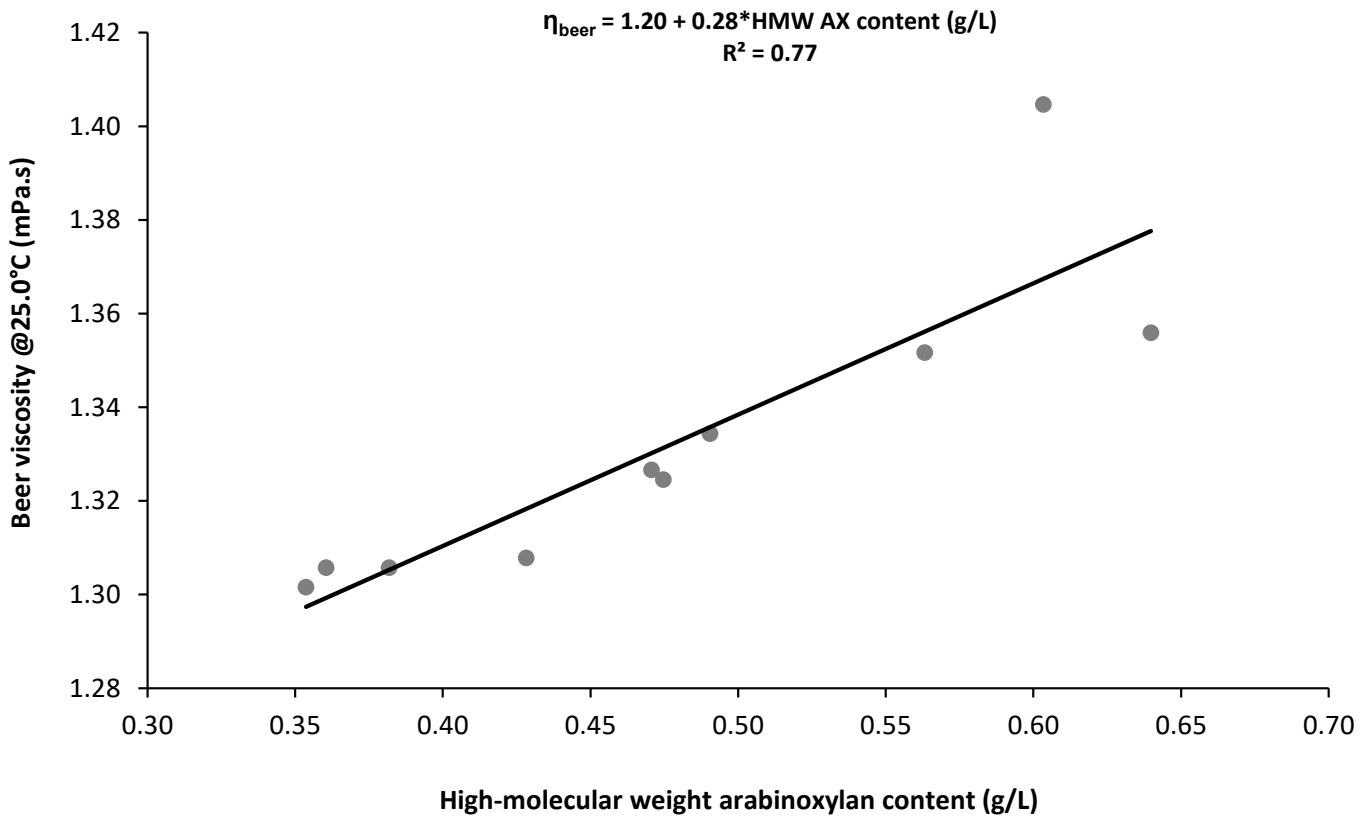
The ethanol, protein, sugar, and dextrin content of the beers produced with and without the addition of filtration enzymes are shown in Table 2. The ethanol (% v/v) and the protein content in the beers varied from 5.6-5.8% v/v and 4.6-4.8 g/L, respectively. For the dextrin and fermentable sugar content, a wider range was found, 24.9-27.5 g/L and 2.9-4.8 g/L. No relationship was found between the filtration enzymes at different dosages and the ethanol, protein, free sugar, and dextrin content in the respective beers. A relationship to predict the final beer viscosity with the measured beer components (ethanol, HMW arabinoxylan, HMW β-glucan, LMW arabinoxylan, LMW β-glucan, dextrin, protein, fermentable sugar content) as variables was assessed by forward predictive modelling. The HMW arabinoxylan content was the only significant ($\alpha<0.05$) component predicting the beer viscosity. In Figure 5, the relationship between the beer viscosity and the HMW arabinoxylan content is shown (η_{beer} (mPa.s) = 1.20 + 0.28*HMW arabinoxylan content (g/L)). A coefficient of variance (R^2) of 0.77 shows the relationship between HMW arabinoxylan and the viscosity of the beers produced with the addition of filtration enzymes and underlines the role of HMW arabinoxylan in beer viscosity. Based on this relationship, an increase of ca. 700-800 mg HMW arabinoxylan/L would compensate for the reduction in beer viscosity of 0.17 mPa.s through the removal of ethanol (5% v/v). Suggesting that HMW arabinoxylan could be of interest to increase the palate fullness of non-alcoholic beers. This supports the observations by Langenaeken et al (2020b), who proposed that arabinoxylan from non-malted cereals could act as a contributor to mouthfeel.

Table 2. Composition of beer produced with and without the addition of filtration enzymes at the start of mashing.

Filtration enzymes were added at the start of mashing in the lower (low) and upper (high) limit of the recommended dosage. The 'very high' dosage is 10 x the recommended upper limit.

Sample	Ethanol (% v/v)	Free sugar (g/L)	Dextrin (g/L)	Protein (g/L)
Control	5.7 ± 0.1	3.5 ± 0.7	26.0 ± 1.3	4.7 ± 0.1
Laminex 750 Low	5.6 ± 0.0	4.8 ± 0.0	24.9 ± 0.2	4.8 ± 0.0
Laminex 750 High	5.7 ± 0.0	4.2 ± 0.0	25.0 ± 0.5	4.8 ± 0.1
Laminex 750 Very high	5.6 ± 0.0	4.2 ± 0.4	26.6 ± 0.2	4.7 ± 0.1
Laminex C2K Low	5.7 ± 0.0	3.5 ± 0.2	25.7 ± 1.1	4.9 ± 0.1
Laminex C2K High	5.8 ± 0.0	3.4 ± 0.3	25.8 ± 0.5	4.8 ± 0.0
Laminex C2K Very high	5.8 ± 0.0	3.4 ± 0.0	26.7 ± 0.7	4.8 ± 0.0
MaxFlow 4G Low	5.7 ± 0.0	3.1 ± 0.2	27.5 ± 1.3	4.6 ± 0.1
MaxFlow 4G High	5.7 ± 0.0	3.1 ± 0.3	27.1 ± 0.3	4.6 ± 0.1
MaxFlow 4G Very high	5.7 ± 0.0	2.9 ± 0.1	26.1 ± 0.8	4.6 ± 0.3

Figure 5. The relationship between the absolute beer viscosity and the high-molecular weight (HMW) arabinoxylan content in beer (g/L). The R^2 of the model is 0.77 ($\alpha<0.05$).

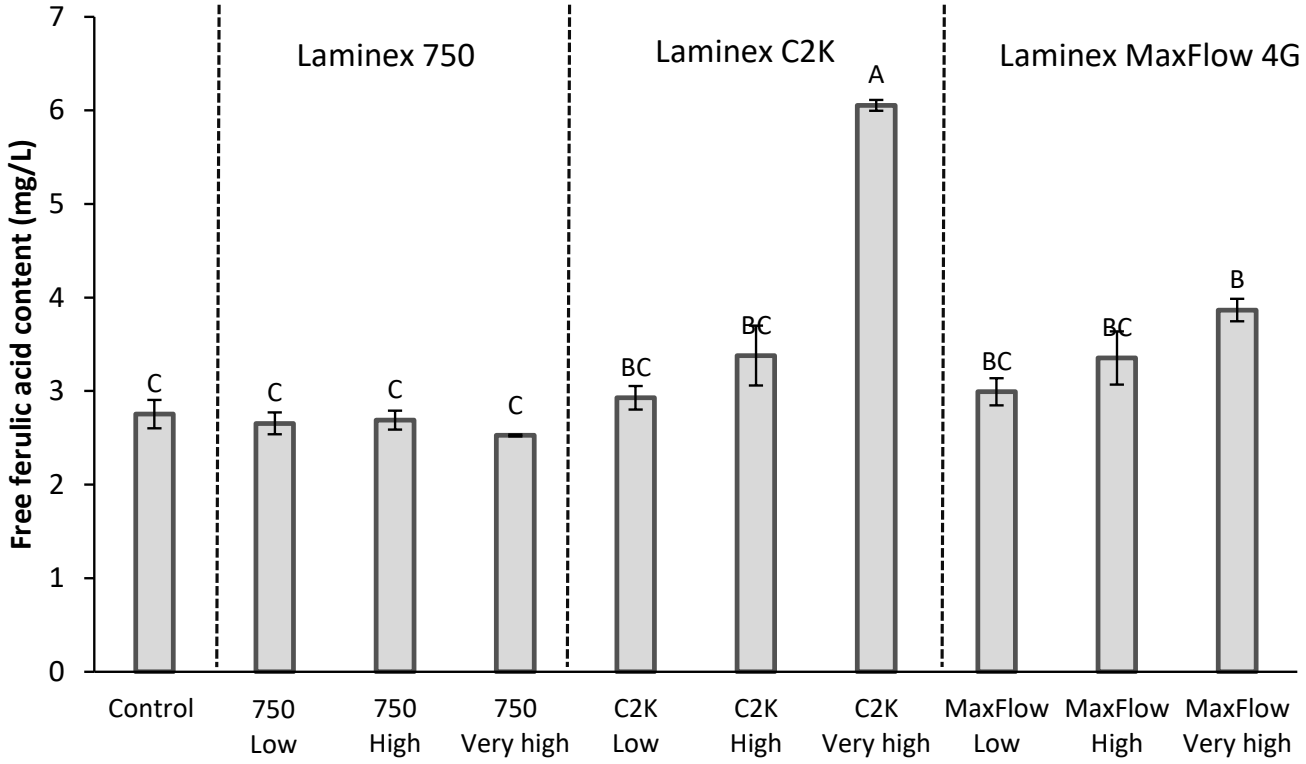


Impact of feruloyl esterase side activity on the release of ferulic acid in wort

The ferulic acid content of the wort samples varied from 2.53-6.05 mg/L (Figure 6). In the control wort, the ferulic acid content was 2.75 mg/L. The addition of Laminex® 750, at all dosage, did not increase ferulic acid release during mashing. On the contrary, the addition of Laminex® C2K and Laminex® MaxFlow4G increased the release of ferulic acid in the wort to 7 and 9%, (low dosing) with 32 and 22% at high dosing. At very high dosing of Laminex® C2K and Laminex® MaxFlow4G, 120 and 41% more free ferulic acid was present in the wort than in the control. Contrary to these results, Szwajgier et al (2005) did not observe an effect of adding filtration enzymes on free ferulic acid release during mashing. However, the type of filtration enzyme and dosage can impact the release of free ferulic acid during mashing. This is important for brewers who wish to control ferulic acid levels in the wort and, more importantly, the transformation by POF^+ yeasts to 4-vinyl guaiacol (Coghe et al. 2004). This is important in the production of wheat beers, where wheat or wheat malt, rich in ferulic acid, are used and where the control of 4-vinyl guaiacol formation is important to obtain a well-balanced flavour profile. In addition, ferulic acid release or increased phenolic acids, either free or bound, can result in the beer having an increased antioxidant capacity (Szwajgier et al. 2005; Yang and Gao 2021). Besides the release of free ferulic acid during mashing, the increased solubilisation of arabinoxylan to which ferulic acid is bound can result in increased levels of ferulic acid content in beer produced with filtration enzymes. Future studies focusing on the antioxidant capacity of beers in food and beverage systems may benefit from these findings (Broekaert et al. 2011; Izydorczyk and Dexter 2008; Szwajgier 2009; Walton et al. 2012; Yang and Gao, 2021).

Figure 6. Free ferulic acid content of wort produced with and without the addition of filtration enzymes at the start of mashing.

Filtration enzymes were added at the start of mashing in the lower (low) and upper (high) limit of the recommended dosage, as well as a very high dose (10 times the upper limit). The control beer was produced without adding filtration enzymes. A different letter code from a Tukey-Kramer HSD test indicates a significant difference (α<0.05).



Conclusions

Filtration enzymes were added during mashing and the impact of varying dosage and type determined on the viscosity and chemical composition of beer. Non-starch carbohydrates were affected by filtration enzymes, resulting in an increased arabinoxylan content, but decreased HMW arabinoxylan, total β-glucan, and HMW β-glucan content in beer. Multivariate data analysis showed that the decrease in the HMW arabinoxylan content was related to the decrease in beer viscosity, which may lead to a decreased palate fullness. The decrease in beer viscosity varied between 0.04-0.10 mPa.s, which corresponds to a decrease of 23-58% comparable to the drop in viscosity of non-alcoholic beer through the removal of ethanol (5% ABV). Besides this possible negative impact on palate fullness, the use of filtration enzymes may result in increased antioxidant capacity. The addition of filtration enzymes led to the production of beers with more arabinoxylan, rich in bound ferulic acid, and wort with more ferulic acid.

However, at very high dosage, increased free ferulic acid levels in wort can result (in the presence of POF^+ yeast) to the conversion of the clove-like 4-vinyl guaiacol. Accordingly, brewers are advised to consider the effects of filtration enzymes on the final beer characteristics.

Author contributions

P. Michiels: conceptualisation, methodology, formal analysis, investigation, data curation, writing (original draft), visualisation, funding acquisition.
W. Debyser: methodology, supervision, writing (review and editing).
C.M. Courtin: conceptualisation, methodology, resources, supervision, funding acquisition, writing (review and editing).
N. A. Langenaeken: methodology, conceptualisation, supervision, writing (review and editing), funding acquisition.

Acknowledgements

The authors thank Jannik Munksgaard (International Flavors & Fragnances Inc.) for providing the enzyme preparations. Professor Kevin Verstrepen, Sofie Mannaerts, and Lore Fondu are thanked for support with beer and protein analyses. VLAIO (Flanders Innovation and Entrepreneurship) and Ab InBev are acknowledged for their financial support in the form of a Baekeland mandate (Grant number: HBC.2020.2283). Niels A. Langenaeken acknowledges Internal Funds KU Leuven for PDM funding (PDMT1/21/021) and FWO (12A8723N) for financial support.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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