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Effective strategies to maximise dextrin formation in brewing

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Abstract

Why was the work done: Dextrin is the non-fermentable product of starch hydrolysis and plays a role in enhancing the perceived palate fullness of beer. Therefore, increasing dextrin formation is a promising strategy to improve palate fullness, particularly in non-alcoholic and low-alcohol beers.
How was the work done: This study investigated the impact of adjusting the mashing profile of a 100% barley malt mash on the dextrin content and molecular weight distribution in the wort. Mash thickness, heating rate, and mashing-in temperature with and without the addition of a thermostable α-amylase were adjusted during mashing to evaluate the impact on dextrin content and molecular weight distribution. To benchmark this work, the dextrin content and molecular weight distribution was determined in five pilsener beers and their non-alcoholic counterparts.
What are the main findings: With the exception of one non-alcoholic beer which contained 72 g/L, the concentration of dextrin ranged from 15 to 30 g/L in the five commercial pilsner-type beers and their non-alcoholic equivalents. The molecular weight distribution of dextrin among the beers was similar, with 85-98% of the dextrin population characterised by a degree of polymerisation below 35. Various strategies were applied during mashing to evaluate the impact on the content and the molecular weight distribution of dextrin. A strategy that promoted dextrin formation was mashing with a lower water-to-grist ratio. This resulted in delayed starch gelatinisation influenced by increased solid extract content in wort. Furthermore, at a low water-to-grist ratio, faster mash heating (up to 2°C/min) or isothermal mashing at temperatures below 72°C had no impact on dextrin formation. Isothermal mashing at 78°C supplemented with thermostable α-amylase increased the dextrin level in wort up to 60 g/L, while the molecular weight distribution of dextrin was similar to that found in commercial beers.
Why is the work important: This study demonstrates that increased dextrin formation is achievable in beer but requires significant changes to the mashing process. These insights will enable brewers to enhance the palate fullness of beers, especially those which are non-alcoholic or low in alcohol.

Keywords

dextrin, starch hydrolysis, mashing, pilsner-type beers, non-alcoholic beers

Introduction

In this study, dextrin is defined as a collection of structures compromising four or more glucose monomers, interconnected by α-(1,4) and α-(1,6) bonds. During the brewing process, dextrin is formed during mashing. This stage involves subjecting a mixture of milled barley malt and water to a temperature-time profile. When the mash temperature exceeds the starch gelatinisation temperature, starch granules lose their crystallinity, and amylose leaches out. The starch gelatinisation process renders starch granules more susceptible to hydrolysis by hydrolysing enzymes, which convert starch into fermentable sugars and dextrin (MacGregor et al. 2002; Delcour and Hoseney 2010). It is important to highlight that, except for diastatic strains of *Saccharomyces cerevisiae*, conventional yeast strains do not ferment dextrin (Laluce et al. 1988; Park et al. 2014). Consequently, dextrin remains in the final beer. Moreover, dextrin constitutes the most abundant solute in beer, often reaching levels of up to 70 g/L (Ragot et al. 1989; Bauwens et al. 2021).

Dextrin in beer are small molecules, with the degree of polymerisation ranging from 20 to 40 (Enevoldsen and Bathgate 1969; Vriesekoop et al. 2010; Langenaeken et al. 2020; Kato et al. 2021). At the concentrations found in beer, there is a positive correlation between dextrin content and perceived palate fullness (an important sensory characteristic of beer). Moreover, the larger dextrin structures in beer have been observed to be more effective in enhancing palate fullness (Ragot et al. 1989; Rübsam et al. 2013; Krebs et al. 2019; Kato et al. 2021). However, the positive effect of dextrin on palate fullness has not been consistently confirmed (Langstaff and Lewis 1993; Bauwens et al. 2021). Recently, there has been increased attention on dextrin formation, given its potential to enhance the palate fullness of non-alcoholic and low-alcoholic beers, which often suffer from being percieved as 'thin' due to the absence or very low concentration of ethanol (Sohrabvandi et al. 2010; Piornos et al. 2023). Despite brewers employing different strategies during mashing to influence the formation of dextrin (and fermentables), the scientific understanding of effective strategies for influencing starch hydrolysis during mashing remains limited.

The general factors that influence starch hydrolysis have been extensively covered in the brewing literature. The first consideration is the type of hydrolysing enzyme that acts on starch (Hu et al. 2014). In brewing, malt α-amylase and beta-amylase are the most important starch hydrolysing enzymes (De Schepper et al. 2021, 2022a; Viader et al. 2021). Additionally, Evans et al (2010, 2022) have highlighted the significance of limit dextrinase which is positively related to the content of fermentable sugar and negatively related to the dextrin content of the wort. Malt α-amylase randomly cleaves the α-(1,4) bonds of gelatinised and non-gelatinised starch, whereas malt beta-amylase liberates β-maltose from the non-reducing ends of gelatinised starch (Bijttebier et al. 2008; Evans et al. 2010; Vriesekoop et al. 2010; Laus et al. 2022). Malt limit dextrinase is a debranching enzyme hydrolysing the α-(1,6) branching sites of starch. Notably, malt beta-amylase, limit dextrinase, and α-amylase are optimally active at distinct temperature ranges of (respectively) 60-65, 60-63, and 65-75°C and are inactivated once these temperatures are exceeded (Muller 1991; Evans and Fox 2017; De Schepper et al. 2022b; Laus et al. 2022).

The second factor is the gelatinisation of starch, which is influenced by its molecular fine structure and the conditions in the mash (Yu et al. 2020; De Schepper and Courtin 2022b). Notably, small starch granules exhibit gelatinisation at temperatures about 3.1°C higher than the large starch granules, thereby raising questions about their impact on the outcomes of starch hydrolysis (Langenaeken et al. 2019; De Schepper and Courtin 2022b, 2023). Moreover, differences in the ratio of starch granule have been identified among different barley malt varieties (De Schepper et al. 2020).

A third consideration is the interplay between starch gelatinisation and starch hydrolysis. It is important to consider that starch gelatinisation occurs in a temperature domain with onset and conclusion temperatures as borders and reflects the heterogeneous gelatinisation behaviour of a group of starch granules. Moreover, enzyme activity represents the net result of increased activity at higher temperatures corrected for the degree of inactivation at that temperature (De Schepper et al. 2021). It is important to note that enzyme

inactivation is a dynamic process, dependent on variables including the applied temperature and solute concentration (Henson and Duke 2007). Moreover, kinetic parameters can characterise thermal inactivation, including the rate of thermal inactivation and decimal reduction time (De Schepper et al. 2022a). Conceptually, the balance established at each temperature-time point during mashing between starch gelatinisation and the enzymes acting on gelatinised starch may affect the content of dextrin and fermentable sugars together with the structure of dextrins in wort (MacGregor et al. 2002; De Rouck et al. 2013; Fox et al. 2019; Saarni et al. 2020).

A fourth aspect, which adds further complexity to starch hydrolysis during mashing, is the impact of the mashing conditions and the changing wort composition on starch gelatinisation and enzyme activity. For instance, a higher extract content in the wort, caused by fermentable sugars or by brewing at a lower water-to-grist ratio, increased the gelatinisation temperature (De Schepper and Courtin 2022b). Moreover, wort composition can affect starch hydrolysis as (i) lower water-to-grist ratios can stabilise starch hydrolysing enzymes and (ii) higher maltose concentrations can reduce beta-amylase activity through inhibitory mechanisms (De Schepper et al. 2022b). Beside the influence of different factors on starch hydrolysis during mashing, the dextrin content and structure in the final beer, the addition of dextrin is a further factor that can determine the occurrence and structure of dextrins in beer.

This study sought to identify effective strategies for enhancing dextrin formation during mashing. The dextrin content and structure in wort, by adjusting the mashing scheme, were compared to the dextrin content and structure in commercial pilsner-type beers and their non-alcoholic beer counterparts. Different strategies to influence the balance between starch gelatinisation and starch degradation were applied during mashing to investigate changes in dextrin content and molecular weight distribution.

The emphasis was placed on a high dextrin content as this would contribute positively to the palate fullness of beers. To achieve this, the impact of varying water-to-grist ratio (‘mash thickness’), heating rate between isothermal periods, and mashing-in temperature (<72°C) were investigated. Additionally, the use of mashing-in temperatures above 78°C with the supplementation of thermostable α-amylase was explored, as it is one of the strategies proposed for the production of non-alcoholic and low-alcohol beers (Vanderhaegen 2013). The content of dextrin and fermentable sugar were measured in the first worts without sparging as this process would contribute unnecessary variability. Accordingly, conclusions regarding wort composition can be drawn, rather than those about the mashing yield. By providing brewers with scientifically informed insights and practical methodologies, this study aims to provide knowledge and tools to enhance dextrin formation, leading to an enhanced palate fullness of beers.

Materials and methods

Materials

Five pilsner-type beers and their non-alcoholic equivalents were purchased at local stores. Barley malt (*Hordeum vulgare*, variety Planet, harvest year 2019) was kindly provided by Boortmalt (Herent, Belgium). The chemical composition of the malt and the endogenous starch hydrolysing activities are reported in the Supplementary Information Table S1. A commercial thermostable α-amylase solution, Termamyl BrewQ, was kindly provided by Novozymes (Bagsværd, Denmark). All chemicals, reagents, and solvents used were of analytical grade and purchased from Sigma-Aldrich (Bornem, Belgium).

Determination of starch gelatinisation properties

Starch gelatinisation was assessed using a Q2000 Differential Scanning Calorimetry (DSC) instrument (TA Instruments, New Castle, USA). Barley malt was finely milled using a Tecator Cyclotec 1093 (Foss, Hillerod, Denmark) equipped with a sieve of pore size 0.5 mm. Water was added in excess to ± 3.00 mg dry matter finely milled barley malt in aluminium DSC pans (Hitachi High-Technologies, Tokyo, Japan). The water-to-grist ratio in the DSC pans was 2.5, reflecting the high gravity mashing conditions used in this study. DSC pans were heated from 0 to 130°C at a heating rate of 1°C/min. After the integration of the endotherms, the 'onset', 'peak' and 'conclusion'

gelatinisation temperatures, termed 'To', 'Tp', and 'Tc', respectively, were determined. For further details see De Schepper and Courtin (2022b).

Laboratory scale mashing

Mashing was performed in a Lochner LB8 mashing device (Lochner Labor+Technik, Berching, Germany). Barley malt was disk milled (Buhler, Uzwil, Switzerland) using a disk spacing of 0.2 mm. Brewing liquor (deionised water with 2.55 mM calcium chloride and 0.75 mM sulphuric acid) was preheated to the mashing-in temperature. On reaching the mashing-in temperature, the milled barley malt was added. The mixture was subjected to a time-temperature profile, as specified for each mashing condition applied, with the mash stirred at 100 rpm. At the end of mashing, the mash was filtered through a pre-folded paper filter (Whatman 597 1/2 filter, Whatman, Maidstone, UK). Sparging of the filter cake was not performed as this work focused on the influence of varying mashing conditions on the starch hydrolysis products during mashing. This approach was taken to eliminate any variations that could affect sugar and dextrin concentrations. As a result, only the first worts were collected.

Mashing with varying mash thickness

A water-to-grist ratio of 6:1 ('low gravity mashing') and 2.5:1 ('high gravity mashing') were used (Puligundla et al. 2020; De Schutter et al. 2022). 50 g ('low gravity mashing') or 120 g ('high gravity mashing') milled barley malt (as dry matter) was added to brewing water (300 g). The temperature-time profile consisted of isothermal periods at 45 (15 min), 62 (30 min), 72 (30 min), and 78°C (10 min) with a heating rate of 1°C/min. This was the control mashing scheme, performed in duplicate for each condition.

Mashing with varying heating rate

Mashing was performed under high gravity mashing conditions (water-to-grist ratio of 2.5:1) with a grist of barley malt. The mash was preheated at 45°C and held for 15 min. The mash was heated to 78°C using two different heating rates: 0.5°C/min and 2°C/min. On reaching 78°C, the mash was maintained at this temperature for 10 minutes and then filtered. Mashing was performed in duplicate for both heating rates.

Mashing-in at elevated temperature

Two different mashing profiles were applied using high gravity mashing conditions (water-to-grist ratio of 2.5:1) of milled barley malt. The first mashing profile consisted of isothermal steps at 62°C (30 min), 72°C (30 min), and 78°C (10 min), or the '62°C mashing-in' profile. The second mashing profile consisted of isothermal steps at 72°C (30 min) and 78°C (10 min), or the '72°C mashing-in' profile. A heating rate of 1°C/min was applied in between the steps of both profiles. For each profile, mashing was performed in duplicate.

Isothermal mashing at elevated temperatures with added thermostable α-amylase

Isothermal mashing at 78°C (45 min) and 85°C (45 min) was applied using high gravity mashing conditions (water-to-grist ratio of 2.5:1). After the addition of the grist to the water at mashing in temperature, thermostable α-amylase (400 µL, Termamyl BrewQ) was added. Both procedures were performed in duplicate.

Measurement of dextrin and fermentable sugar content in wort and beer

The content of fermentable sugar and dextrin in wort and beer were determined using a Dionex ICS-5000 high-performance anion-exchange chromatography system with integrated pulsed amperometric detection (Thermo Fisher, Waltham, USA). A CarboPac PA-100 pre-column (4 x 50 mm) and a CarboPac PA-100 analytical column (4 x 250 mm) were used. The column was equilibrated for 5 min with a 100 mM NaOH mobile phase and sample (12.5 µL) injected. The elution program consisted of an isocratic flow of 100 mM NaOH, and between 5 and 30 min of analysis a sodium acetate gradient at a rate of 3.6 mM/min was applied. The flow rate was kept constant at 1 mL/min (Langenaeken et al. 2019).

To determine the content of fermentable sugar in the wort, an internal standard solution (500 µL of 20 mg rhamnose monohydrate/mL ultrapure water) was added to 500 µL of wort sample and the mixture filtered through a Millex-GP 0.22 µm PES syringe filter (Millipore Sigma, Burlington, MA, USA). With the commercial beers, the sample preparation was similar, except for the lower concentration of the internal standard solution (1 mg rhamnose monohydrate/mL ultrapure water). A sugar solution (rhamnose, glucose, fructose, sucrose, and maltose at 10 µg/mL and maltotriose at 5 µg/mL in ultrapure water) was analysed for peak identification and sugar quantification.

The glucose potential of the fermentable sugars (GP_{FS}) comprises all glucose present in the fermentable sugars and is calculated according to formula (1):

$$(1)\ GP_{FS}\left(\frac{g}{L}\right) = c_{glucose} + c_{sucrose} * CF_{sucrose} + c_{maltose} * CF_{maltose} + c_{maltotriose} * CF_{maltotriose}$$

where GP_{FS}: the total amount of glucose as fermentable sugars expressed in g/L wort/beer,
cx: concentration of sugar x expressed in g/L wort/beer, and correction factors (CF) $CF_{sucrose}$ (=180/342), $CF_{maltose}$ (=360/342), and $CF_{maltotriose}$ (=540/504), for glucose released after acid hydrolysis of the corresponding sugars, allowing for the incorporation of water during hydrolysis.

The dextrin content in wort and beer was determined by correcting the total glucose content after an acid hydrolysis treatment, to provide the 'total glucose potential' (GP_{TOT}), with the GP_{FS}. The GP_{TOT} was determined by mixing 250 µL of diluted wort (1:10 v/v) or beer sample (1:4 v/v) in ultrapure water, 250 µL internal standard solution (20 mg rhamnose monohydrate/mL ultrapure water) and 500 µL 4N trifluoroacetic acid. This mixture was heated at 110°C for 1 hour. After cooling, an aliquot was diluted with ultrapure water until a rhamnose concentration of 10 µg/mL was obtained and then filtered through a Millex-GP 0.22 µm PES syringe filter (Millipore Sigma, Burlington, MA, USA). A standard solution was prepared by heating a mixture of 500 µL calibration solution (10 mg rhamnose and 10 mg glucose per mL ultrapure water) and 500 µL 4N trifluoroacetic acid at 110°C for 1 hour. The sample was cooled, diluted to a final rhamnose concentration of 10 µL/mL, and filtered through a Millex-GP 0.22 µm PES syringe filter (Millipore Sigma, Burlington, MA, USA). The total amount of glucose measured after acid hydrolysis was referred to as 'GP_{TOT}' and comprises of glucose from the fermentable sugars and dextrin. Therefore, the difference between GP_{TOT} and GP_{FS}, is the amount of glucose in the dextrin population, as used in formula (2) to calculate the dextrin content (g/L). The factor 0.9 corrects for the exclusion of a water molecule in the polymerisation reaction of glucose into dextrin.

$$(2)\ \text{Dextrin content}\left(\frac{g}{L}\right) = 0.9 * (GP_{TOT} - GP_{FS})$$

Beer samples were analysed in triplicate for fermentable sugar content and GP_{TOT}. Wort samples were analysed in duplicate. As wort samples were produced in duplicate, this represents a quadruplicate measurement for each mashing scheme.

Molecular weight distribution of dextrins in wort and beer

The molecular weight distribution of dextrin in beer and wort was analysed by separation, based on hydrodynamic volume using high-performance size exclusion chromatography with refractive index detection. Beer samples were diluted five-fold with ultrapure water. Wort samples were diluted ten-fold ('low gravity mashing') or twenty-fold ('high gravity mashing') after centrifugation (15 min, 7°C, 12,000g). Samples were filtered through a Millex-GP 0.45 µm PES syringe filter (Millipore Sigma, Burlington, MA, USA) before injection. A sample volume of 50 µL was injected (SIL-HTc Auto 153 sampler, Shimadzu, Kyoto, Japan) and separated on a Shodex SB-803 HQ column (Showa Denko KK, Tokyo, Japan) with a Polysep GFC-P guard column (Phenomenex, Torrance, CA, USA), both kept at 30°C in a column oven (CTO-20A, Shimadzu, Kyoto, Japan). Elution was performed with a 0.3% (w/v) NaCl solution at a flow rate of 0.5 mL/min for 45 min using a modular Shimadzu SIL-HTC unit equipped with an LC-20AT pump and a DGU-20A5 degasser. Detection was performed using a RID-10A detector operating at 40°C (Shimadzu, Kyoto, Japan). Maltotetraose (1 mg/mL) and pullulan standards

with a molecular mass of 1080, 6100, 9600, 22000, and 47100 Da were individually analysed as calibration samples.

Wort extract and density

Wort extract was measured singly for each wort produced, using an EasyDens density meter (Anton Paar, Graz, Austria). Extract was expressed using the °P scale, where 1°P has the same density as a solution of 1 g of sucrose in 100 g water (Thesseling et al. 2019). This generally corresponds to 1 g of solid mass in 100 g of wort.

Statistical analysis

Statistical analyses were performed in JMP 16 Pro (SAS Institute, Cray, NC, USA). Significant differences were compared using a Tukey-Kramer HSD test after a positive omnibus test ($p < 0.05$).

Results and discussion

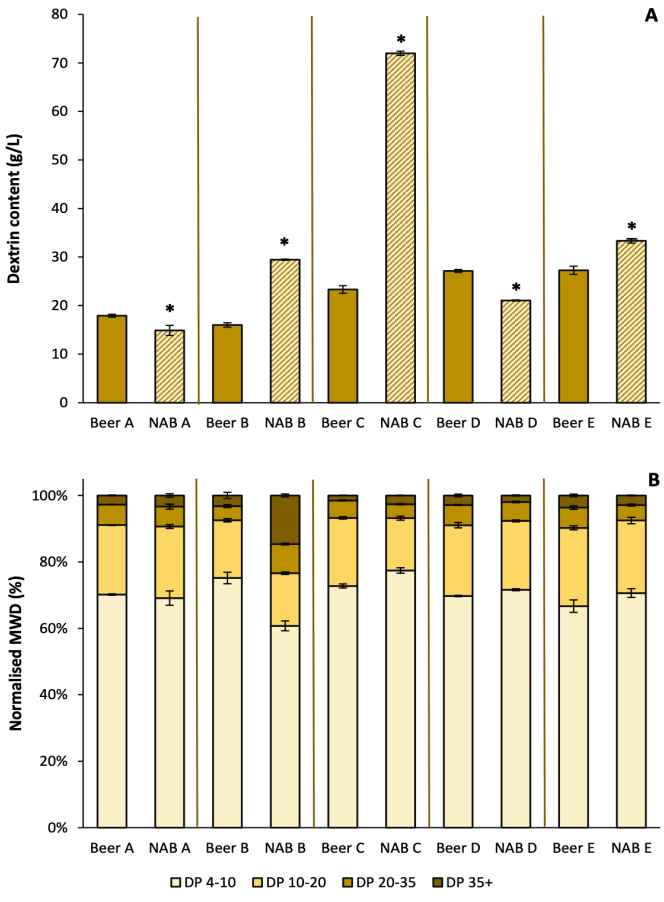
Dextrin content and molecular weight distribution in commercial beers

In Figure 1, the dextrin content and molecular weight distribution of five alcoholic beers and their respective non-alcoholic counterparts are shown. The dextrin content of the alcoholic beers was within the range of 16-27 g/L, while for the non-alcoholic beers (NABs), the range was broader (15-72 g/L). This broader range reflects the dextrin content of three NABs (B, C, and E), which were significantly higher than for their alcoholic counterparts. In contrast, the dextrin content of NAB_D and NAB_A were 6 g/L and 3 g/L lower, respectively, compared to their alcoholic counterpart. Notably, NAB_C had a higher dextrin content of 72 g/L, which exceeded that of all the other beers. This suggests that the production process of NAB_C used a different approach than the other NABs.

The molecular weight distribution of dextrin differed to a limited extent between the different beers (Figure 1B). The dextrin populations of the beers were divided into four subclasses based on their degree of polymerisation (DP) (4-10, 10-20, 20-35, 35+). The smallest fraction (DP 4-10) accounted for 61-77% of the dextrin population, DP 10-20 for 16-24%, DP 20-35 for 4-9%, and DP 35+ for 2-15%. Accordingly, 77 to 93% of the dextrin population had a DP equal to or lower than 20. Furthermore, only 2 to 15% of the dextrin population had a DP of 35 or more. The molecular weight distribution of dextrins in NAB_B and the other beers differed, with NAB_B containing larger dextrins. The presence of these larger dextrins may be explained by in/post-process addition of larger dextrins. Overall, this shows the dextrin population typically consists of small molecules, which are likely to reflect extensive starch hydrolysis during the mashing process.

Figure 1.

Dextrin content and normalised molecular weight distribution in commercial beers (n=10). (A) Dextrin content is reported as g/L beer. Standard error bars are calculated based on triplicate measurements. (B) The molecular weight distribution of dextrin is normalised and fractionated into four classes based on the degree of polymerisation (DP): DP 4-10, DP 10-20, DP 20-35, and DP 35+. Standard error is based on duplicate measurements. Significant differences (α = 0.05) in dextrin content between a beer and its non-alcoholic counterpart are indicated by an asterisk (*).



Dextrin content and molecular weight distribution in commercial beers

Different mashing strategies were used to influence the formation and molecular weight distribution of dextrin in 100% barley malt mashes. The parameters determined above in commercial beers provide a benchmark for the results obtained below.

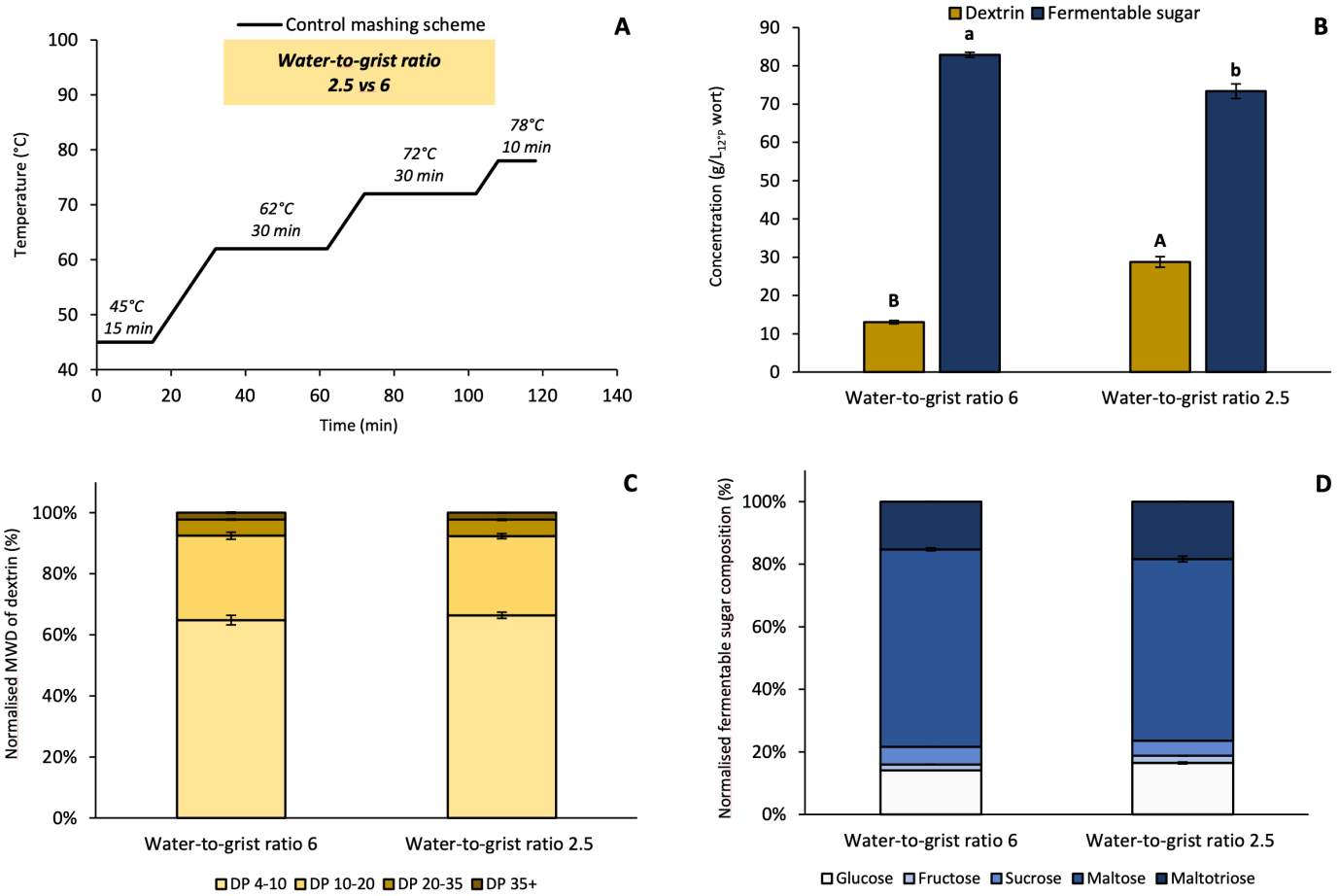
Impact of mashing thickness

The impact of low - and high gravity mashing on the dextrin and fermentable sugar composition in wort was examined. Low and high gravity mashing corresponded to a water-to-grist ratio of 6 and 2.5 with the control mashing profile (Figure 2A) applied for both thicknesses. Regardless of the mashing conditions, the content of fermentable sugar and dextrin in wort are expressed for a 12°P wort, allowing a standardised comparison for samples of varying extract. The dextrin content was also expressed on a 12°P basis as this is a common target for pilsner beers with an ethanol content of 5% (v/v). This facilitates comparing the dextrin content in wort with that of the commercial beers.

Figure 2B reports the dextrin and fermentable sugar content of the first worts obtained from control mashing at low and high gravity mashing. Low gravity mashing yielded a dextrin and total fermentable sugar content of 13 and 83 g/L respectively. High gravity mashing conditions yielded a significantly higher dextrin content (29 g/L) and a significantly lower total fermentable sugar content (73 g/L) in comparison to low gravity mashing ($p < 0.05$).

Figure 2.

Dextrin and fermentable sugar profile of wort from a control mashing scheme (A) applied at low (water-to-grist ratio of 6) and high-gravity mashing conditions (water-to-grist ratio of 2.5). Dextrin content (g/L) and total fermentable sugar content (g/L) of wort are reported on a 12°P basis (B). Standard error bars are calculated based on triplicate measurements. The normalised molecular weight distribution (MWD) of dextrin (C) and the fermentable sugar composition in the wort (D) are presented. Standard error bars are calculated based on in duplicate and triplicate measurements for the molecular weight distribution of dextrin and fermentable sugar profile. A different capital and lowercase letter indicates a significant difference (α=0.05) in the content of dextrin and fermentable sugar.



To gain insight into the factors contributing to the differences in the dextrin content between low and high gravity mashing, the evolution of the dextrin content during mashing was determined (Figure 3). For both conditions, the dextrin content in wort increased during the heating-up phase to 62°C. In the case of high gravity mashing, the dextrin content increased significantly ($p < 0.05$) during the heating-up phase to 72°C, whereas the dextrin content remained constant in the low gravity mashing. As in previous work, the starch gelatinisation temperature rises with an increased extract content in wort (De Schepper and Courtin 2022b). The initial concentration of extractable components was greater at high gravity than with low gravity mashing conditions. This results in the leverage of the gelatinisation temperature of not-yet-gelatinised starch granules. Consequently, part of the starch does not gelatinise until beta-amylase and limit dextrinase inactivation occurs (Stenholm and Home 1999; De Schepper and Courtin 2022b), resulting in wort with lower fermentable sugar content and higher dextrin content.

Despite the well documented positive influence of the solute/maltose concentration on the thermal stability of β-amylase (Duke and Henson 2008; Henson et al. 2020), recent studies have revealed the inhibitory effect of maltose on β-amylase activity under high gravity mashing conditions (De Schepper et al. 2022b). Consequently, it can be inferred that the combined factors of delayed starch gelatinisation and product inhibition of β-amylase surpass the protective effect of more concentrated mashes and thermal degradation of enzymes. Figure 2D presents the fermentable sugar composition of the first worts obtained from control mashing at low and high gravity. The wort obtained from high gravity mashing contained relatively more maltotriose and less maltose compared to low gravity mashing. This observation strengthens the argument that starch is proportionally more hydrolysed by α-amylase at high gravity mashing conditions. Consequently, a wort with higher dextrin content was obtained at high gravity mashing conditions at the expense of fermentable sugar content.

Although the dextrin content in the wort for both conditions was different, the molecular weight distribution of dextrin was similar (Figure 2C). At both low and high gravity mashing conditions, sufficient α-amylase activity remained in the temperature window at which starch was gelatinised. Moreover, the molecular fine structure of starch was consistent across both conditions. The difference between low- and high gravity mashing lies in the fact that a portion of the starch population only gelatinises in a temperature domain at which beta-amylase and limit dextrinase inactivation occurred. Consequently, the portion of starch gelatinised at higher temperatures under high gravity mashing conditions would have been proportionally more hydrolysed by α-amylase. Therefore, irrespective of differences in gelatinisation behaviour, α-amylase generates dextrins with an equivalent molecular weight distribution. Given that more dextrin was

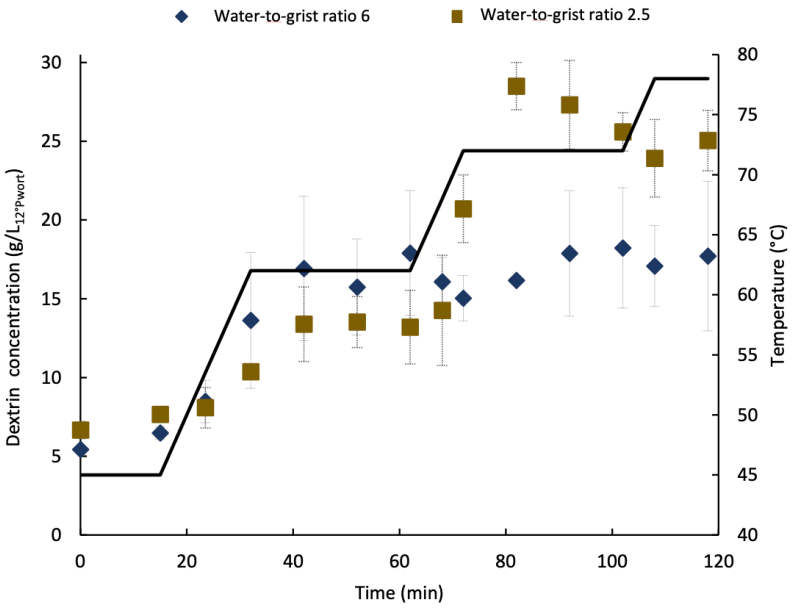


Figure 3.

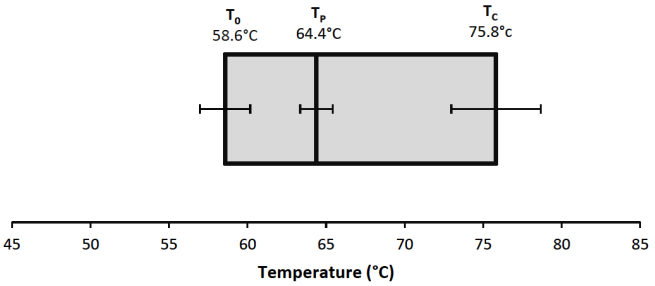
Changes in dextrin content (g/L in a 12°P extract), **during mashing at low and high gravity conditions** (water-to-grist ratio of 6 and 2.5, respectively). The mashing profile (black line) includes isothermal steps at 45°C (15 min), 62°C (30 min), 72°C (30 min), and 78°C (10 min), with intermediate heating at a rate of 1°C/min. Standard errors are based on duplicate measurements.

formed under high gravity mashing conditions, this might be attributed to the absence or limited activity of limit dextrinase activity on the portion of starch gelatinised at higher temperatures. Based on this observation, it can be concluded that different gelatinisation behaviour during mashing leads to different amount of dextrin but not to a difference in distribution of molecular weight.

These results are in agreement with the reports of De Rouck et al (2013) and De Schepper and Courtin (2022b), who observed a decreased fermentable sugar content in the wort with increasing mashing thickness. De Rouck et al (2013) noted an increased extract yield, decreased apparent attenuation limit (AAL), and increased fermentable sugar content in wort with increasing mashing thickness. This indirectly suggests that more dextrin was formed under high gravity conditions. Importantly, the temperature-time profiles where starch gelatinisation occurs were comparable between these studies and the work reported here, involving an isothermal period at 62-64°C, followed by an isothermal rest at 72°C, and a final mashing temperature at 78°C. Muller (1991) observed increasing wort fermentability with increasing mashing thickness using isothermal mashing at 70, 75, 80, and 85°C. In these mashing systems and irrespective of the mashing thickness applied, a major proportion of malt starch instantly gelatinises and becomes accessible for enzymic degradation. Furthermore, increased solute concentration in thicker mashes reduces enzyme sensitivity to thermal inactivation (Evans et al. 2005; Duke and Henson 2016). This suggests with high gravity mashing, enzymes are thermally stabilised by higher osmolyte concentrations leading to a longer activity window, whilst undergoing product inhibition leading to a reduced production of fermentable sugars. Depending on the raw materials and mashing protocol used, one or both phenomena might drive wort composition. This is exemplified by the results of Evans et al (2011) who noted an increased AAL with increasing mashing thickness, although the magnitude of the impact varied among different malt varieties. Here, the mashing profile consisted of a mashing-in temperature of 65°C (50 min), followed by mashing-out at 74°C (10 min). As demonstrated in Figure 4, it is important to note that starch gelatinisation occurs over a broad range of temperature between 58.6 to 75.8°C. This suggests that as long as the terminal gelatinisation temperature is not reached, a portion of starch remains inaccessible for degradation, and ends up in spent grain (Langenaeken et al. 2019). Thus, reaching a final mashing temperature of 78°C, instead of 74°C, can increase the dextrin content rather than the fermentable sugar content, resulting in a decrease in the AAL. Therefore, it can be anticipated that the mashing profile applied may differently affect starch gelatinisation and enzyme activity, resulting in contradictory observations.

Figure 4.

The starch gelatinisation temperature domain of malt (Planet, 2019) **during high gravity mashing**. Onset (T_o), peak (T_p) and conclusion (T_c) temperatures are provided in a boxplot and are the mean results from triplicate measurements.



Nevertheless, an increased mash thickness with the mashing profile used here, enhances the formation of dextrin at the expense of fermentable sugars. Moreover, the results do not suggest that high mashing conditions are impractical or economically unsustainable during industrial mashing practices. In this study, the first worts were analysed, suggesting that sparging of the filter cake after high gravity mashing would enhance the extraction of sugars/dextrin, resulting in increased yield. This would offset the reduced formation of fermentable sugars under high gravity mashing conditions, ultimately leading to a greater ethanol content in the final beer.

The further work in this study used only high gravity mashing conditions, given their common use in the industry (Puligundla et al. 2020). It was assumed that during control mashing both α-amylase and beta-amylase had sufficient time at their optimal temperature range to efficiently hydrolyse starch (Figure 2A). Therefore, high gravity mashing using the control mashing scheme is considered as the reference method.

Impact of heating rate

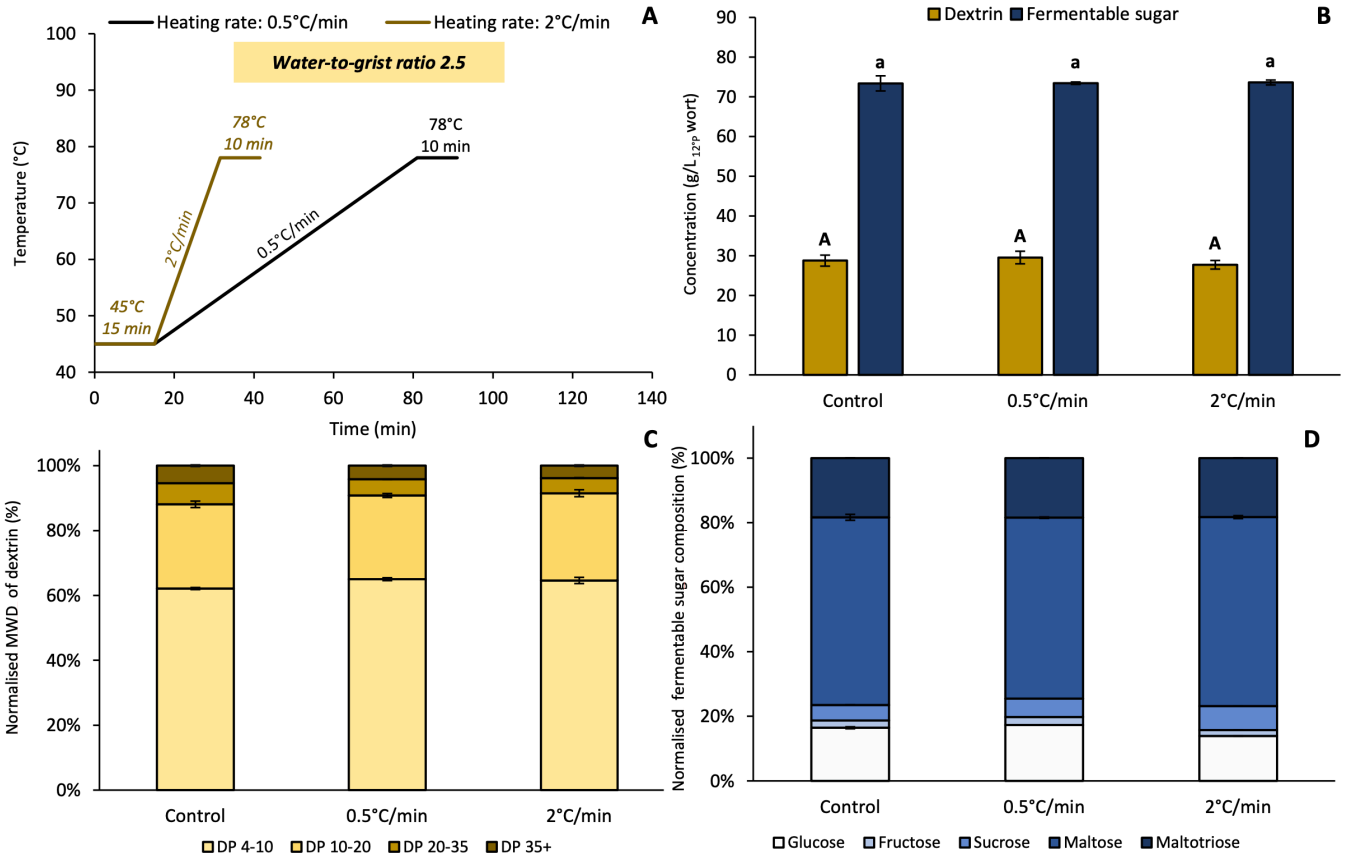
The impact of heating rate during mashing on dextrin formation was evaluated where the mash was heated from 45 to 78°C using two different heating rates: 0.5°C/min and 2°C/min or 'low' and 'high' heating rates (Figure 5A). Low and high heating rates were used to create conditions where both starch gelatinisation and enzyme (in)activation occurred over a short and long period.

Figure 5B shows the content of dextrin and fermentable sugar in wort after control mashing, and the low heating rate and fast-heating rate regimes. Additionally, Figures 5C and 5D present the normalised molecular weight distribution of dextrin and fermentable sugar composition in the wort under these various conditions.

Two main observations can be drawn. Firstly, the different heating rates did not influence the content of dextrin and total fermentable sugars in the wort (Figure 5B). Secondly, the heating rate had no impact on the molecular weight distribution of dextrin (Figure 5C). Nevertheless, a relatively higher content of sucrose and lower content of fructose and glucose were found in wort when subjected to the high heating rate compared to the low heating rate or the control mashing program (Figure 5D). These differences may be linked to the activity of invertase, which hydrolyses sucrose into glucose and fructose. Invertase is optimally active at 50°C and is rapidly inactivated at temperatures above 55°C (Laus et al. 2022). As a result, invertase was inactivated faster during the mashing scheme with a high heating rate, resulting in reduced sucrose hydrolysis (Langenaeken et al. 2020).

Figure 5.

Dextrin and fermentable sugar profiles of wort from a control mashing, with a low (0.5°C/min) and high heating profile (2°C/min) at high gravity (water-to-grist ratio 2.5) (A). Dextrin content (g/L) and total fermentable sugar content (g/L) of wort on a 12°P basis (B). Standard error bars are based on triplicate measurements. The molecular weight distribution (MWD) of dextrin (C) and the fermentable sugar composition in wort (D) are shown. Standard error bars are based on duplicate and triplicate measurements for the molecular weight distribution of dextrin and fermentable sugar profile, respectively. A different capital and lowercase letter indicates a significant difference (α=0.05) in the content of dextrin and fermentable sugar.



The total mashing time for the control, low heating rate, and high heating rate were (respectively) 118, 91, and 41.5 minutes. Nonetheless, no significant differences in dextrin, fermentable sugar content, and molecular weight distribution of dextrin were observed. This indicates that the enzyme load in a 100% barley malt mashing system was sufficient to completely hydrolyse the gelatinised starch, even when α-amylase and beta-amylase were inactivated more rapidly, and the total mashing time was reduced. It is important to note that the results provide insight into wort composition and do not reflect the sugar or dextrin yield. These depend on various factors, including the extent of starch hydrolysis, the coarseness of the grist, milling method, wort filtration system and its efficiency, together with the volume of sparge water.

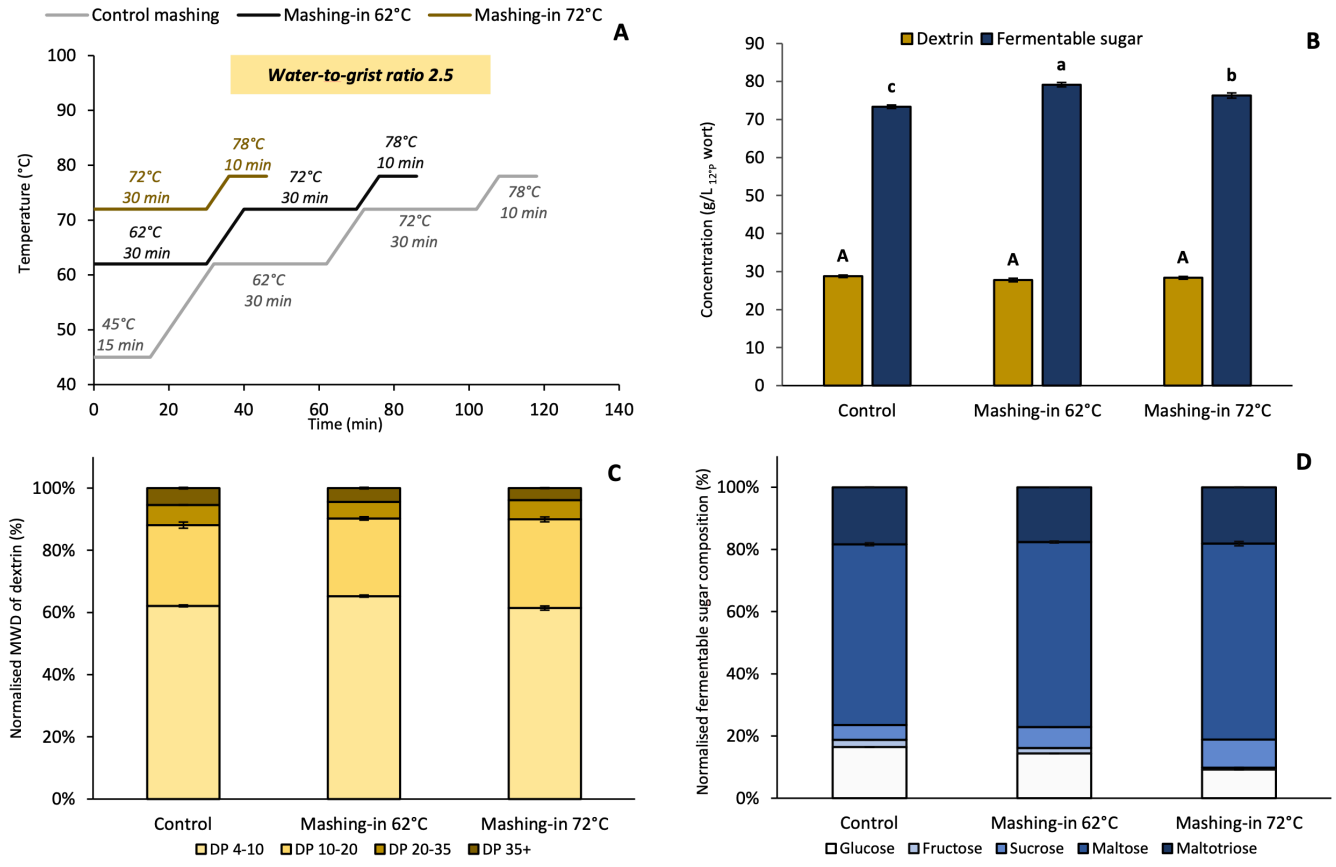
Impact of mashing-in temperature (T<72°C)

The influence of elevated mashing-in temperature on the content and composition of dextrin and fermentable sugars in the wort (Figure 6A) was examined by mashing-in at 62 and 72°C with a water-to-grist ratio of 2.5 (high gravity mashing).

Figure 6B shows the content of dextrin and fermentable sugar in first worts after control mashing and mashing-in at 62 and 72°C. It is notable that there was a significantly higher total fermentable sugar content in the wort after mashing in at 62°C (79.2 g/L) compared to the control mashing (73.4 g/L) ($p<0.05$). However, the only difference between control and mashing-in at 62°C was the incubation of the former at 45°C for 15 minutes before the

Figure 6.

Dextrin and fermentable sugar profile of wort from a control mash, with mashing-in at 62 and 72°C under high gravity (water-to-grist ratio 2.5) (A). Dextrin content (g/L) and total fermentable sugar content (g/L) of wort is expressed at 12°P (B). Standard error bars are based on triplicate measurements. The molecular weight distribution (MWD) of dextrin (C) and the fermentable sugar composition in the wort (D) are presented. Standard error bars are based on duplicate and triplicate measurements for the molecular weight distribution of dextrin and fermentable sugar profile, respectively. A different capital and lowercase letter indicates a significant difference (α=0.05) in the content of dextrin and fermentable sugar.



temperature increased to 62°C. Given the range of gelatinisation temperatures under high gravity mashing conditions (Figure 4), it can be argued that during the heating-up from 45 to 62°C during control mashing, a portion of the starch underwent gelatinisation and conversion into fermentable sugars (Langenaeken et al. 2019). Consequently, during this time the extract content in the wort increased, resulting in a leverage effect on the gelatinisation temperature of the remaining ungelatinised starch granules (De Schepper and Courtin 2022b). Subsequently, the gelatinisation temperature of these ungelatinised starch granules rose to temperatures at which beta-amylase and limit dextrinase inactivation occurred (>65°C) (Stenholm and Home 1999; Evans and Fox 2017; De Schepper et al. 2022b). Conversely, when mashing in at 62°C, a larger starch population underwent gelatinisation and became accessible for degradation by beta-amylase and limit dextrinase. This effect was also reflected in the significantly higher maltose concentration observed in the wort following mashing-in at 62°C (47.1 g/L) compared to control mashing (42.7 g/L) (Figure 6D).

Following mashing-in at 72°C, a significantly lower fermentable sugar content (76.3 g/L), was obtained compared to mashing-in at 62°C (79.2 g/L). At 72°C, a greater proportion of the starch population underwent instant gelatinisation compared to 62°C. However, beta-amylase and limit dextrinase were also more rapidly inactivated. Consequently, the balance between starch gelatinisation and enzyme inactivation was compromised at 72°C, and beta-amylase and limit dextrinase activity became limiting. This observation aligns with the findings of Evans et al (2005), who reported that 65°C was the optimal mashing-in temperature for achieving maximum wort fermentability by balancing starch gelatinisation and enzyme activity. Furthermore, invertase and α-glucosidase are promptly inactivated at 72°C. Im and Henson (2021) found that α-glucosidase is rapidly inactivated above 72°C, with invertase rapidly inactivated at temperatures >55°C (Laus et al. 2022). This may explain the higher sucrose content in wort after mashing in at 72°C (6.9 g/L) compared to 62°C (5.3 g/L) (Figure 6D).

The dextrin content of the wort samples obtained from control mashing (28.8 g/L), 62°C mashing-in (27.8 g/ L), and 72°C mashing-in (28.4 g/L) followed an opposing trend to that of the fermentable sugar yet remained statistically insignificant (Figure 6B). Similarly, the molecular weight distribution of dextrin (Figure 6C) was comparable between the wort samples from all three temperatures. Intriguingly, mashing in at 45, 62, and 72°C yielded no discernible differences in dextrin content and structure, despite the known effects on starch gelatinisation, enzyme activity, and enzyme inactivation. Accordingly, increasing the mashing-in temperatures (< 72°C) at high gravity using 100% barley malt is not a suitable strategy to increase dextrin formation.

The impact of high mashing-in temperature (T>78°C) in combination with a thermostable α-amylase was investigated with regard to the content and composition of dextrin and fermentable sugars in the wort. Such conditions are a common approach in the production of non-alcoholic beers, aimed at minimising the formation of fermentable sugars during mashing (Vanderhaegen 2013). At temperatures >78°C, endogenous enzymes in the malt are rapidly inactivated, limiting the production of fermentable sugars (Muller 1991; De Schepper et al. 2022a). Additionally, supplementation with thermostable α-amylase is often necessary to ensure complete starch hydrolysis. Given the main objective of this study was to enhance dextrin formation during mashing, isothermal mashing-in temperatures of 78, 85 and 92°C - in combination with thermostable α-amylase supplementation - were investigated (Figure 7A).

Figure 7B reports the content of fermentable sugar and dextrin in wort after control and isothermal mashing at 78, 85, and 92°C. An increase in mashing-in temperature corresponded to a higher dextrin and lower fermentable sugar content in the wort. The content of dextrin in the control wort (29 g/L) was significantly lower than that obtained from isothermal mashing at 78°C (62 g/L), 85°C (78 g/L), and 92°C (88 g/L). The higher dextrin content at higher mashing-in temperatures was at the expense of total fermentable sugars, which were respectively 46, 29 and 20 g/L for the isothermal mashing program at 78, 85, and 92°C. Interestingly, the cumulative content of dextrin and fermentable sugars (107-108 g/L) was the same for the three isothermal mashing schemes. Moreover, isothermal mashing yielded a higher cumulative fermentable

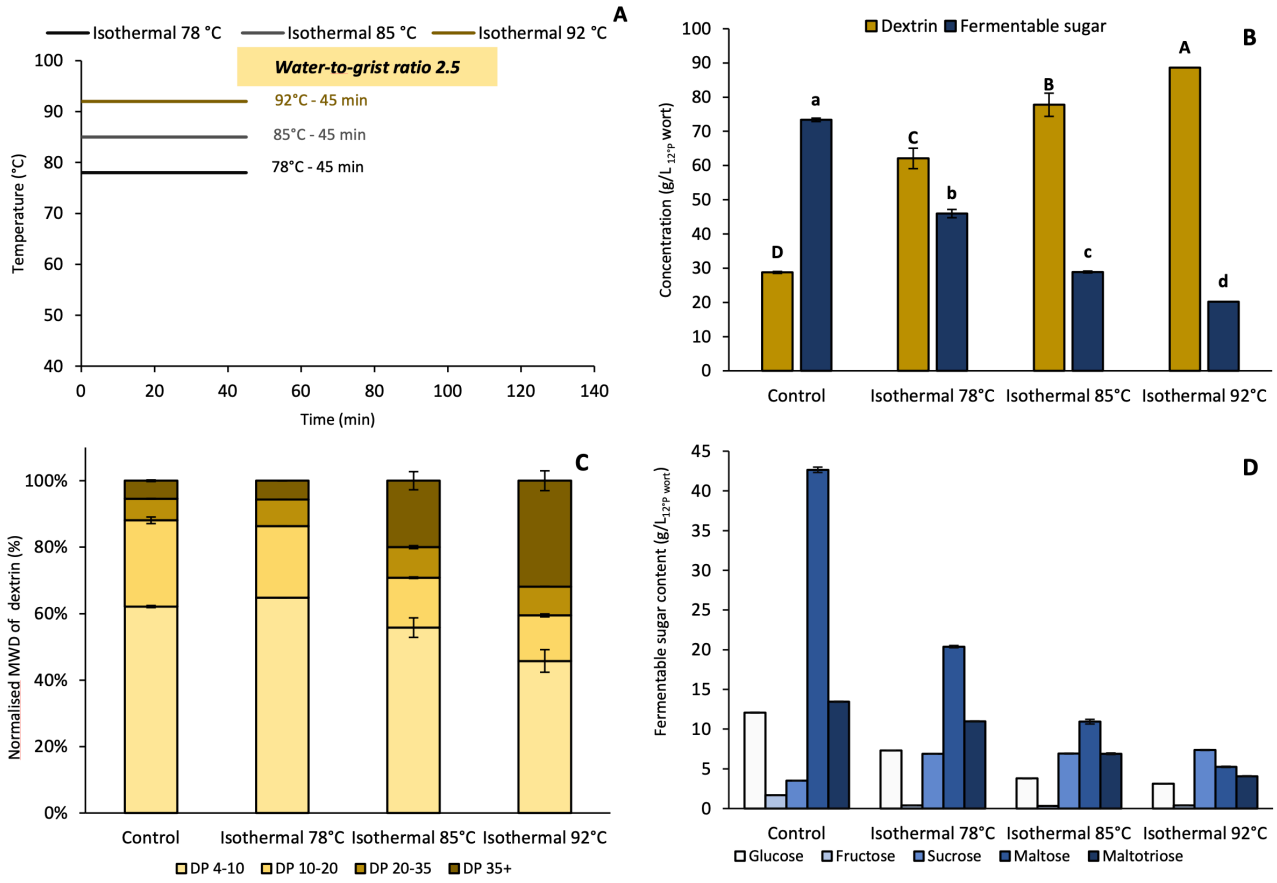
sugar and dextrin content (5-6 g/L) compared to control mashing. This can be explained by the instant gelatinisation of the total starch population (Figure 4) and the improved starch hydrolysis by thermostable α-amylase. This suggests that during mashing, with the temperature not exceeding 78°C, a portion of starch remains ungelatinised.

Figure 7D shows a significant difference in the sugar profile of the wort samples. The content of glucose, maltose, and maltotriose decreased with increasing mash temperature. A comparison between the fermentable sugar composition of the wort from isothermal mashing at 78°C and 92°C shows a decrease in content of glucose from 7.3 to 3.1 g/L, maltose from 20.4 to 5.3 g/L, and maltotriose from 11.0 to 4.1 g/L. No significant differences were observed between sucrose and fructose. The reduced formation of maltose and maltotriose at higher mashing-in temperatures was probably due to the faster inactivation of beta-amylase and limit dextrinase (Stenholm and Home 1999; De Schepper et al. 2022a, 2021). As a result, more starch was available for the thermostable α-amylase, resulting in increased dextrin levels.

Interestingly, the fermentable sugars in wort after isothermal mashing at 92°C appeared to originate from the extraction of the fermentable sugars already present in the barley malt (data not shown), suggesting that starch degradation did not contribute to additional fermentable sugar formation. This implies that endogenous beta-amylase and limit dextrinase had a negligible role in starch degradation when mashing-in at 92°C.

Figure 7.

Dextrin and fermentable sugar profile of wort with isothermal mashing at 78, 85 and 92°C and supplementation with thermostable α-amylase (A) compared to the control mashing scheme at high gravity (water-to-grist ratio 2.5). Dextrin content (g/L) and total fermentable sugar content (g/L) of wort are expressed at 12°P (B). Standard error bars are from triplicate measurements. The molecular weight distribution (MWD) of dextrin in the wort (C) and the fermentable sugar composition in the wort (D) are shown. Standard error bars are based on duplicate and triplicate measurements for the molecular weight distribution of dextrin and fermentable sugar profile. A different capital and lowercase letter indicates a significant difference (α=0.05) in the content of dextrin and fermentable sugar.



As shown in Figure 7C, the molecular weight distribution of dextrin in wort obtained after isothermal mashing at 78°C was similar to the control mash. However, wort samples obtained from mashing at 85 and 92°C, exhibited larger dextrin structures with the proportion of the dextrin population with a DP 35+ was 5% for control mashing, 20% for isothermal mashing at 85°C, and 32% for isothermal mashing at 92°C. Given that thermostable α-amylase originating from *Bacillus licheniformis* has an optimal temperature of about 100°C in the presence of calcium ions and at the pH of the mash, it is unlikely that α-amylase activity became limiting at 85 and 92°C (Takasaki et al. 1994; Samanta et al. 2014). Therefore, it is plausible to consider that the formation of larger dextrin structures resulted from the reduced limit dextrinase and beta-amylase activity at the high temperatures or differences in substrate specificity between the malt α-amylase and the microbial thermostable α-amylase. All in all, it can be concluded that isothermal mashing at temperatures exceeding 78°C leads to a higher dextrin content in the wort, making it an effective strategy to increase the dextrin levels in beers.

Conclusions

This study explored different strategies to enhance dextrin formation during mashing, in particular in the production of non-alcoholic and low-alcohol beers. The formation of dextrin, at the expense of fermentable sugar, is sought to enhance palate fullness. Our findings show that changing the mashing profile has a limited impact on dextrin and fermentable sugar levels once a mashing thickness and barley malt have been established. However, isothermal mashing at 78°C, supplemented with a thermostable α-amylase, proved an effective approach to increase the dextrin content of wort to about 60 g/L. By applying these conditions, the molecular weight distribution of dextrin closely resembled that of a commercial pilsner-type and non-alcoholic pilsner beer. However, the potential adverse effects of high mashing-in temperatures require attention. Although this study focused on mashing one variety of malted barley, it is anticipated that other varieties or cereal adjuncts, with different starch content, properties, and enzyme composition may also affect the content and composition of fermentable sugars and dextrin. This study can serve as a framework for further research and provide practical solutions to increase dextrin levels during brewing, leading to the improved palate fullness of non-alcoholic and low-alcohol beers.

Author Contributions

Pieter Michiels: conceptualisation, methodology, formal analysis, investigation, data curation, writing (original draft), visualisation, project administration, funding acquisition.
Dries Croonen: conceptualisation, validation, formal analysis, investigation, data curation, writing (review and editing)
Charlotte De Schepper: conceptualisation, methodology, writing (review and editing)
Winok Debyser: supervision, writing (review and editing), project administration.
Niels Langenaeken: conceptualisation, methodology, supervision, writing (review and editing), funding acquisition.
Christophe Courtin: conceptualisation, methodology, resources, supervision, funding acquisition, writing (review and editing).

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Conflict 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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