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Comparison of Congress Mash with Final 65 °C Mash for Wort Production with Unmalted Barley, Tritordeum, and Quinoa, with or without Pregelatinization and/or Enzyme Addition

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ABSTRACT

Wort was prepared according to the Congress mash protocol with the addition of 40% unmalted barley, tritordeum, or quinoa. Mashes with quinoa filtered significantly slower than mashes with barley. In contrast, tritordeum filtered similarly as barley, even though tritordeum does not possess a husk. The lack of husk may have contributed to a higher extract yield of tritordeum compared to barley. The final 65 °C mash protocol resulted in shorter saccharification times, but slower wort filtration compared to the Congress mash protocol. When unmalted adjuncts were pregelatinized by heating in water for 20 min at 95 °C, the filtration following the Congress mash protocol was slower and the filtration following the final 65 °C mash protocol was faster than the same mashes prepared without pregelatinization. Pregelatinization also inactivated the endogenous enzymes in the (pseudo)cereals used. This was especially noticeable for quinoa, resulting in wort with markedly lower glucose concentrations, most likely due to the inactivation of endogenous amyloglucosidases. Wort filtration improved for both mashing protocols when an exogenous enzyme mix (Brewers Compass®) was added to each of the three different (pseudo)cereals. Furthermore, addition of the enzyme mix increased the extract yield of the final 65 °C mashes and the FAN levels of the Congress mashes. These findings confirmed the benefit of using the Brewers Compass® enzyme mix during mashing processes with high percentages of (up to 40%) unmalted (pseudo)cereals.

KEYWORDS

Barley; Congress mash; final 65 °C mash; quinoa; tritordeum

Introduction

In recent years, consumer demand for new beer styles and flavors is rising and brewers are increasingly interested in alternative brewing methods utilizing unmalted (pseudo) cereals.[1] Studies have demonstrated beer flavor diversification even with small amounts of unmalted (pseudo)cereals, for example unmalted oats,[2] corn, wheat, triticale, and rye.[3] These alternative brewing methods resulted in beers with novel flavors without the need for additional equipment or investments. Furthermore, there are indications that unmalted (pseudo)cereals can improve shelf life due to their reduced nitrogen compounds and aldehydes, compared to malted (pseudo)cereals-based beers.[4–9] However, unmalted and/or alternative cereals may contain more metal ions such as copper, iron and manganese, which may reduce beer shelf life by catalyzing Fenton-type oxidation reactions.[10–12]

An interesting novel cereal to explore in brewing applications is tritordeum (X *Tritordeum martinii* A. Pujadas *nothosp. Nov*). Tritordeum malt did not cause any technological difficulties for beer production in a study of Zdaniewicz, et al.[13] and therefore appears to have high potential for the brewing industry. However, the use of unmalted tritordeum in brewing applications has not been investigated in detail. Another raw material that has gained interest for brewing applications is quinoa (*Chenopodium quinoa* Willd.).[14,15] This pseudocereal has an excellent amino acid composition[16] and contains more minerals (such as potassium, phosphorus, magnesium, zinc, and calcium) than barley.[17,18] The Food and Agriculture Organization of the United Nations listed quinoa as 1 of 11 (pseudo)cereals of interest to brewers.[19] Unmalted quinoa was found to be suitable for brewing and had a positive effect on the overall sensory quality of the resulting beer.[14] The authors furthermore concluded that a 30% substitution

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with unmalted quinoa was possible even without exogenous enzyme addition, but the extract yield was 10% lower in comparison to the extract yield from barley malt.

These alternative (pseudo)cereals may have higher gelatinization temperatures than barley malt, which is around 56–62 °C.[20] The gelatinization temperature of tritordeum flour was around 60–63 °C[21], and the gelatinization temperature of quinoa flour or starch was in the range of 60–67 °C.[22–24] High starch gelatinization temperatures of added (pseudo)cereals could lead to insufficient starch degradation into fermentable sugars during mashing.[25] This problem can be solved by gelatinizing the unmalted (pseudo) cereal before or during mashing.

Most of the unmalted (pseudo)cereals possess lower levels of cytolytic, proteolytic, and amylolytic enzymes than barley malt, which could result in low extract yield, high wort viscosity, and consequently filtration problems and an unsatisfactory brewhouse efficiency.[1,26,27] To circumvent these problems, brewing enzymes can be added.[8]

Commercial brewers widely agree that the Congress mash protocol is not representative anymore for modern brewing practices and hence, its relevance could be questioned.[28–32] In a comprehensive study by Evans, et al.,[33] the mashing parameters of the Congress mash protocol were adapted to better emulate modern commercial brewing practices, with the newly designated protocol named the "final 65 °C" mash. The most important differences with the Congress mash are a larger grist size (0.7 mm), a lower initial grist water ratio (1:3), the addition of 0.3 mM Ca^{2+} to the mash and a higher mashing-in temperature (65 °C).

The aim of this study was to produce wort with 40% unmalted barley, tritordeum or quinoa to evaluate their suitability in a brewing process. Hereto, the Congress mash protocol and the final 65 °C mash protocol were used and compared with each other. Additionally, the impact of a pregelatinization step and/or the addition of brewing enzymes during these mashing processes was investigated.

Experimental

Raw materials

Commercial 2-row spring barley ("Planet") malt (pilsner) was acquired from Boortmalt (Zandvoort, Belgium), unmalted 6-row winter barley ("Etincel") from Albert Maltings (Ruisbroek, Belgium), white quinoa ("Vikinga") from Mill & Mix (Aartrijke, Belgium), and tritordeum was acquired from Agrasys (Barcelona, Spain). Milling of malt and cereals was carried out with a laboratory disk mill (DLFU, Bühler, Switzerland) at 0.2 mm (Congress mash protocol) or 0.7 mm (final 65 °C mash protocol). The barley malt was characterized by a total protein content of 10.6% (dry mass), β-glucan content in the wort of 137 mg/L (Analytica-EBC method 4.16.3), malt extract of 82.6% (Analytica-EBC method 4.5.1), fine/coarse extract difference of 1.3% (Analytica-EBC method 4.6), and a diastatic power of 285 WKU.

The starch and crude protein content of the unmalted (pseudo)cereals were determined using the polarimetric and Dumas methodology, respectively (Laboratory for Chemical Analysis, Ghent University, Belgium). The gelatinization temperatures of quinoa, tritordeum, and barley were measured using the MCR 302 rheometer (Anton Paar, Austria), as previously described.[34] Briefly, (pseudo)cereals were milled at 0.8 mm using the Hammertec-50HZ hammer mill (Foss Analytical, Denmark) and the moisture contents determined using the MA 150 moisture analyzer (Sartorius Weighing Technology GmbH, Germany) to calculate the mass needed to produce a 14% dry w/v suspension. The wort β-glucan concentration and diastatic power of the unmalted (pseudo) cereals were determined according to the Analytica-EBC method 4.16.3 and method 4.12, respectively.[35]

Sampling at the onset of the mashing process

Samples representative for the mash at the onset of the mashing process were created in triplicate. Hereto, a distinction was made between Congress mash and final 65 °C mash samples. For Congress mash samples, malt and unmalted (pseudo)cereals were milled at 0.2 mm, mixed at a 60:40 ratio, and 1 g of this grist mixture was brought into a 15 mL falcon tube together with 2 mL of 0.1 M sodium acetate buffer (pH 5.2) and 6 mL of distilled water.[35] The tubes were shaken thoroughly and heated in a warm water bath at 95 °C for 20 min to deactivate all endogenous enzymes. Samples were frequently shaken to prevent cake formation during the first 10 min of this step. After heating, samples were cooled to room temperature, centrifuged and supernatants were withdrawn for further analysis. The final 65 °C mash samples were prepared analogously to the Congress mash samples, but the malt and unmalted (pseudo)cereals were milled at 0.7 mm and 1 g of mixed grist was added to 5 mL of distilled water, 2 mL of 0.1 M sodium acetate buffer and 1 mL of 0.9 mM $CaCl_2$ solution in accordance with Evans et al.[33] Sugar profiles and FAN content were determined.

Mashing processes

All mashing processes were performed in triplicate in a mashing bath (Lochner Labor Technik GmbH, Germany) using 50 g of grist consisting of 30 g of malt and 20 g of unmalted tritordeum, quinoa or barley. All mashes were stirred at 200 rpm. To minimize pH variation between different mashing processes, 100 mL of distilled water was substituted for 100 mL of 0.1 M acetate buffer (pH 5.2) in every mashing process at the start of each mashing protocol. For each (pseudo)cereal, a Congress mash (C),[35] a Congress mash with pregelatinization (CP), a Congress mash with pregelatinization and additional exogenous enzymes (CPE), a final 65 °C mash (F),[33] a final 65 °C mash with pregelatinization (FP), and a final 65 °C mash with pregelatinization and additional exogenous enzymes (FPE) were carried out (Table 1).

Pregelatinization was performed by heating 20 g of the milled unmalted (pseudo)cereal in 100 mL 0.1 M sodium

Table 1. Overview of the different mashing procedures performed for 40% substitution with unmalted barley, tritordeum and quinoa.

Mashing protocol	Additional treatment	Abbreviation
Congress mash	None	C
	Pregelatinization	CP
	Pregelatinization and enzyme addition	CPE
Final 65 °C mash[33]	None	F
	Pregelatinization	FP
	Pregelatinization and enzyme addition	FPE

In all mashes, 40% of the malt was replaced by an unmalted (pseudo)cereal.

acetate buffer (pH 5.2) for 20 min at 95 °C in the mashing bath. Hereafter, this mixture was cooled to the appropriate mashing-in temperature and the appropriate malt and remaining water at the same temperature (with or without Ca^{2+} addition) were added. Exogenous enzymes were added as 30 µL of the Brewers Compass® enzyme mixture (Royal DSM, The Netherlands), containing α-amylase (225 Reference Amylase Units), cellulase, and protease activity.

Analyses

During mashing, the saccharification time was determined at the final mashing temperature (70 °C for the Congress mash protocol and 74 °C for the final 65 °C mash protocol). Hereafter, the mash was cooled to ambient temperature and filtered as described in Analytica-EBC method 4.5.1.[35], and the filtrate mass was measured as a function of time. Samples of the filtered wort were analyzed to determine the extract content, pH, FAN, and sugar profile.

Saccharification time

Saccharification time was determined according to the standard Analytica-EBC method 4.5.1.[35]

Filtration

Filtration was assessed by vigorously stirring and pouring the content of the mash beakers into a funnel inside a tared Erlenmeyer flask containing a grade 597 ½ filter paper with 4–7 µm pore size (Whatman, The United Kingdom). The mass of the seeped through wort was measured after 5, 10, 20, 40, 60, and 90 min.

Gravity and pH

The gravity and pH of the wort samples and of a control sample (1:3 solution of 0.1 M sodium acetate buffer/demineralized water) were measured with a DMA 4500 and Alcoholyzer Plus (Anton Paar, Austria). The control sample gravity was subtracted from the values of the wort samples.

FAN concentration

The FAN in the wort was measured according to the Analytica-EBC method 8.10.[35]

Wort sugars

Wort sugars were analyzed using high-performance anion exchange chromatography coupled with a pulsed amperometric detector (HPAEC-PAD) using a Dionex ICS-3000 system (Thermo Fisher Scientific, United States). Hereto, 35 µL of sample was added to 965 µL of a deproteinization solution (25% acetonitrile) containing rhamnose as the internal standard. After centrifugation (10 min and 16000 rpm at 4 °C), 35 µL of supernatant was added to 965 µL of MQ water. Then, 10 µL of this dilution was injected and separated in a PA100 guard column (50 mm x 4.6 mm) followed by a PA100 analytical column (250 mm x 4.6 mm) at 30 °C. The eluent consisted of a gradient of 84 mM NaOH in ultrapure water (eluent A), and 84 mM NaOH with 250 mM sodium acetate (eluent B). An external standard curve was used for quantification.

Statistical analysis

The results are presented as the mean ± standard deviation of three independent biological replicates. Normal distribution and homoscedasticity of the samples was assumed. All statistical tests were performed using Excel 2013. Differences in mean between two or multiple treatments were determined using two-sample t-test or one-way ANOVA, respectively. The significance level was set at 0.05.

Results and discussion

Starch and protein content, β-glucan content, gelatinization temperature, and diastatic power of barley, tritordeum, and quinoa

The starch content,[36–38] protein content,[38–40] and gelatinization temperature[20,21,23] of the different (pseudo)cereals used in this experiment (Table 2) were in line with literature values. The protein content of barley was rather low, which might have been reflected in the wort characteristics.[41] The diastatic power of barley malt and unmalted barley was also in line with the literature.[42,43] The diastatic power of unmalted tritordeum was similar to unmalted barley, whereas the diastatic power of quinoa was markedly lower, but values for these two unmalted (pseudo)cereals could not be found in the literature.

Production of Congress wort (C) with unmalted barley, tritordeum, or quinoa

During the Congress mashing processes (C), the saccharification was completed first for barley, followed by tritordeum, and lastly quinoa (Table 3). Unmalted barley and

Table 2. Starch content, protein content, gelatinization temperature, wort β-glucan concentration and diastatic power of unmalted barley, tritordeum and quinoa.

	Barley	Tritordeum	Quinoa
Starch content (% dry mass)	55.9 ± 0.8	57.0 ± 0.8	54.3 ± 0.8
Protein content (% dry mass)	9.1 ± 0.2	13.7 ± 0.2	12.0 ± 0.2
Gelatinization temperature (°C)	56	58	63
Wort β-glucan concentration (40% substitution) (mg/L)	449 ± 46	51 ± 37	29 ± 5
Diastatic power (°WK)	251 ± 28	260 ± 9	52 ± 4

Table 3. Saccharification time (min) of the different mashes with 40% unmalted (pseudo)cereal substitution (C: Congress mash, F: Final 65 °C mash, P: Pregelatinization and E: addition of exogenous enzymes).

Unmalted cereal	C	CP	CPE	F	FP	FPE
Barley	15	15	10	5	10	10
Tritordeum	20	15	15	10	15	15
Quinoa	30	20	15	20	>20	>20

tritordeum had a similar diastatic power and gelatinization temperature, which could explain the small difference in saccharification time. Quinoa had a markedly lower diastatic power than barley and tritordeum, which could have contributed to the longer saccharification time.

The filtrate mass after 40 min was highest for the barley (322 ± 5 g) and tritordeum (336 ± 7 g) C mashes, followed by the quinoa C mash (251 ± 13 g; Figure 1). Despite the absence of husk in tritordeum, the filtrate mass was similar to barley. The latter contains a husk, which is known to facilitate filtration.[19] Quinoa substantially impaired the filtration. This might be caused by lower starch and/or protein degradation than in the other (pseudo)cereals, as this is known to increase wort viscosity, especially at 20 °C.[14,44] The β-glucan concentration in the wort was substantially lower for quinoa than for unmalted barley or tritordeum and could not explain the impaired filtration (Table 2).

The gravity of tritordeum wort (8.33 ± 0.05°P) was similar ($p = 0.05$) to the gravity of barley wort (8.26 ± 0.03°P), which were both significantly higher than the gravity of quinoa wort (8.05 ± 0.03°P; $p < 0.01$ for both; Figure 2). The pH of barley, tritordeum and quinoa wort samples were 5.40 ± 0.01, 5.65 ± 0.01 and 5.81 ± 0.03, respectively. These results indicate that quinoa and tritordeum increase the wort pH in

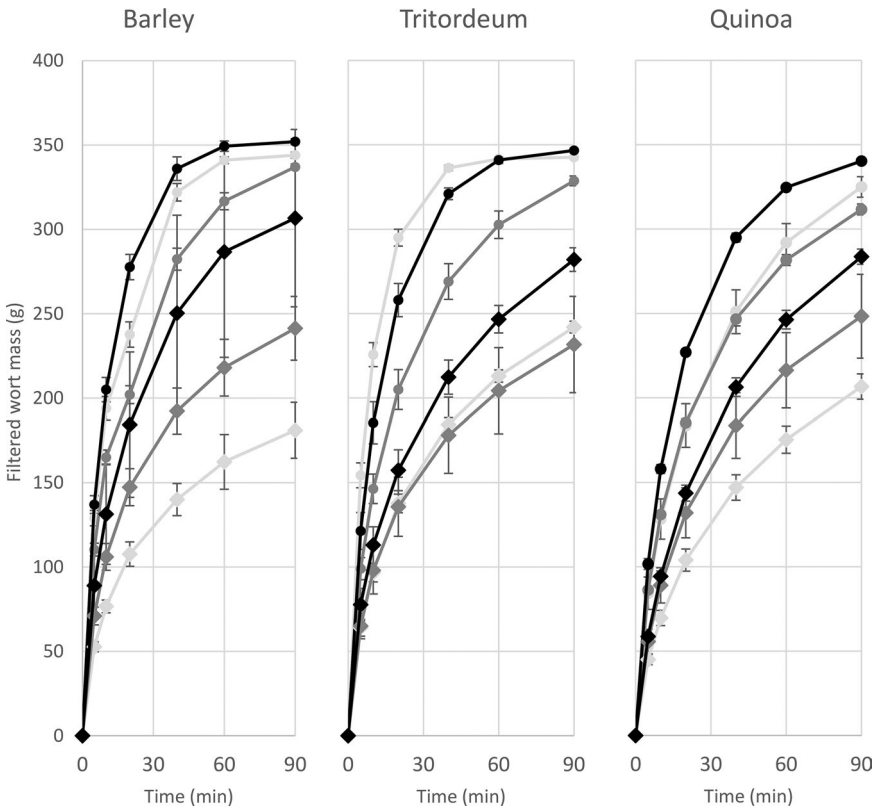

Figure 1. Filtrate mass in grams (mean ± standard deviation) of malt substituted with resp. 40% unmalted barley, tritordeum, and quinoa for different mashing procedures (● CPE, ● C, ● CP, ◆ FPE, ◆ FP, ◆ F and C: Congress mash, F: final 65 °C mash, P: pregelatinization and E: addition of exogenous enzymes).

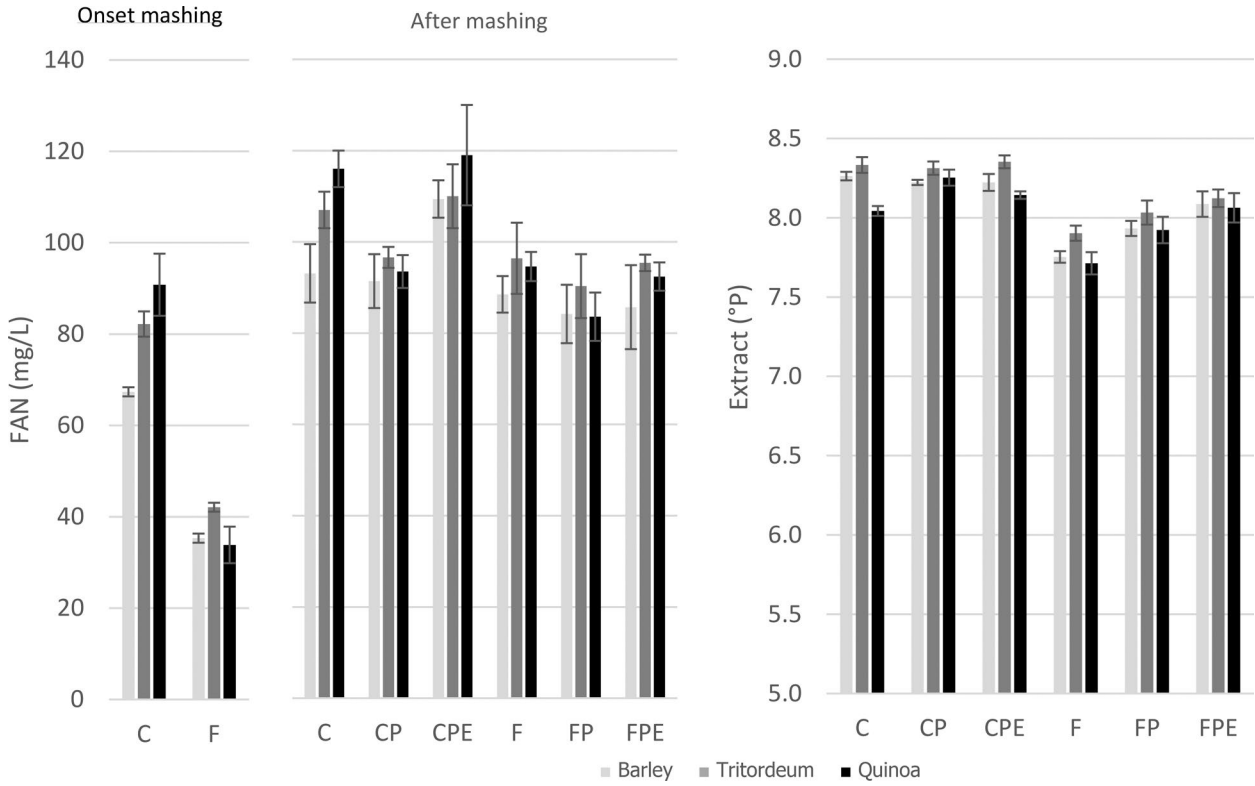

Figure 2. FAN levels (left) and wort gravity (right) (mean ± standard deviation) of the different samples (C: Congress mash, F: final 65 °C mash, P: pregelatinization and E: addition of exogenous enzymes).

comparison to barley ($p = 0.01$) despite the inclusion of the sodium acetate buffer (pH 5.2). An increase in wort pH has also been observed when barley malt was replaced with increasing amounts of unmalted oats or sorghum.[45,46]

The highest FAN was observed for quinoa, followed by tritordeum and lastly barley (Figure 2). A similar trend was found at the onset of mashing. The unmalted barley used in this study was a low-protein variety, and barley varieties with higher protein content may result in higher FAN levels.[41] The difference in FAN will indeed be a consequence of the protein content, availability and/or degradability of the (pseudo)cereal.[47]

Wort produced with unmalted barley contained approximately 15% glucose, 49% maltose, and 27% maltotriose relatively to all measured sugars (Figure 3). This wort was somewhat lower in maltose and slightly higher in maltotriose in comparison to a standard 100% barley malt wort, which usually contains 10–15% glucose, 50–60% of maltose, and 15–20% maltotriose.[48–50] Wort produced with tritordeum contained 10% glucose, 67% maltose, and 13% maltotriose. Maltotriose is often not consumed during fermentation,[49] and thus the use of unmalted tritordeum can result in higher fermentability in wort and beer. The maltose concentration in tritordeum wort (45.2 ± 1.3 g/L) was approximately two times the concentration in barley wort (21.7 ± 2.0 g/L) and four times the concentration in quinoa wort (12.4 ± 2.1 g/L). The quinoa wort contained almost six times more glucose (33.4 ± 3.7 g/L) than the barley (6.79 ± 0.58 g/L) and tritordeum wort (6.69 ± 0.14 g/L), which showed similar glucose concentrations. Deželak, et al.[51] also found that wort produced with quinoa malt contained a five times higher glucose concentration than wort produced with barley malt and a low maltose concentration as well. The concentrations of maltopentaose and maltohexaose, as well as sucrose, decreased during the mashing processes for all three (pseudo)cereals, which is in accordance with the literature.[52] The decrease in sucrose is caused by invertase, which is not located in the endosperm of germinated barley but is only found in the developing seedling organs.[53] Malted barley is deculmed and hence, any residual invertase after deculming would have to originate from the axis of the seedling.[52]

Congress mashing process (C) versus final 65 °C mashing process (F)

The saccharification times of the final 65 °C mashes (F) were always shorter than or equal to the Congress mashes (C; Table 3). The higher mashing temperatures of the F mashing process most probably caused faster amylolysis. The C mashing process led to a delay in starch liquefaction because of the suboptimal temperatures for starch gelatinization during mashing-in. Furthermore, the addition of calcium in the F mashes and the higher grist/water ratio may have stabilized the α-amylase enzymes.[54,55]

The filtrate mass after 40 min was always substantially and significantly higher for the C mashes than for the F mashes ($p < 0.01$; Figure 1) and these observations were in line with findings of Evans, et al.[33] The C mashes resulted in faster filtrations, probably due to lower temperatures during the

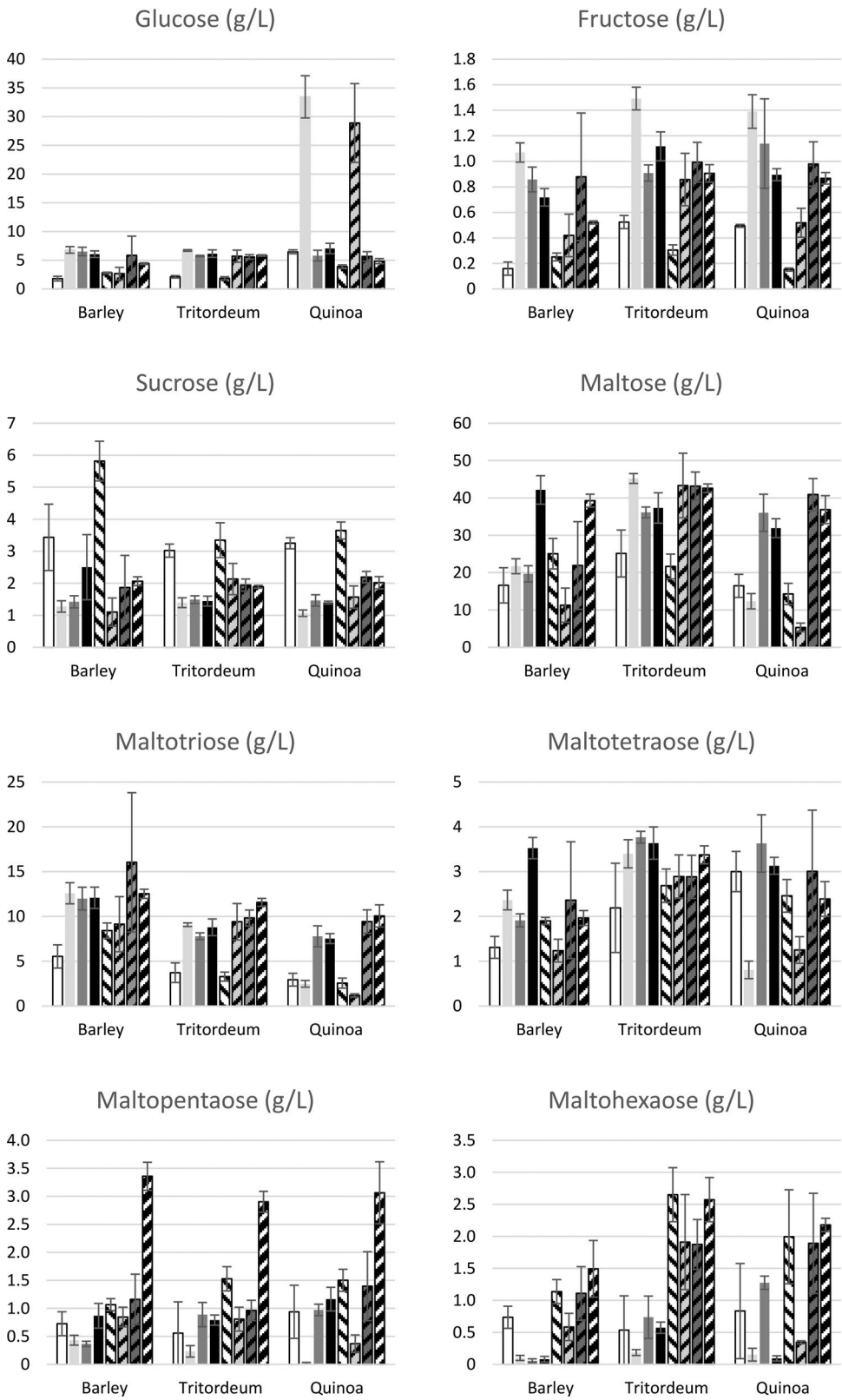

Figure 3. Sugar concentrations in g/L (mean ± standard deviation) in the different wort samples (☐ Congress mash sample at the onset of mashing, ▒ C, ▒ CP, ■ CPE, final 65 °C mash sample at the onset of mashing, ▨ F, ▨ FP, ▨ FPE and C: Congress mash, F: final 65 °C mash, P: pregelatinization and E: addition of exogenous enzymes).

first stages of the C mashing protocol, which might have led to a more extensive breakdown of proteins, β-glucans, and/or other polysaccharides.[56] The C mashing process includes the β-glucanase rest at 45 °C and encompasses the protein degradation temperature window around 54 °C, whereas the F mashing process immediately starts with mashing-in at 65 °C. At temperatures above 50 °C, solubilization of β-glucans from intact cell walls continues,[57,58] whereas β-glucanase

activity is rapidly lost.[59] The pH of the filtered wort was not influenced by the mashing process.

The extract levels of the C wort samples were always higher than the F wort samples (Figure 2). These observations are in line with the findings of Evans et al.[60] The higher extract levels in the C wort samples might be caused by longer and more extensive breakdown of β-glucans, proteins, and/or starch. The β-glucans are indeed known to reduce extract yields in the brewhouse, since poor β-glucan degradation increases unconverted starch and protein in the spent grains.[61]

The FAN levels at the onset of mashing were almost twice as high for the C mashes compared to the F mashes (Figure 2). This can only be explained by the milling size, which was smaller for the C mashing process. At the end of the mashing process, the differences in FAN largely disappeared, but the FAN remained slightly higher for the C mashes than for the F mashes (Figure 2). The higher FAN levels in wort produced with the C mashing process could also be attributed to the lower mashing-in temperatures than applied during the F mashing process, allowing activity of proteases.[62]

The concentrations of mono-, di-, tri- and tetrasaccharides were higher in wort after C mashing processes than after F mashing processes, whereas the concentrations of maltopentaose and maltohexaose were higher in the F wort samples than in the C wort samples. This indicates that the starch degradation was more extensive during the C mashing processes than during the F mashing processes. This is in accordance with a lower wort fermentability produced at higher mashing temperatures.[55,60]

Pregelatinizing unmalted (pseudo)cereals during congress (CP) and final 65 °C (FP) mashing processes

Pregelatinizing the starch of the unmalted (pseudo)cereals shortened the saccharification time during the Congress mashing processes (CP) but increased the saccharification time during the final 65 °C mashing processes (FP; Table 3). These findings may be the result of the interplay between the activity of endogenous enzymes and the starch availability. Pregelatinization will render the starch more available for enzymatic degradation but will also inactivate the endogenous enzymes in the (pseudo)cereal. For the CP mashes, the increased availability of pregelatinized starch in combination with the low mashing-in temperature may have outweighed the inactivation of the endogenous enzymes of the unmalted barley and tritordeum, resulting in a reduced saccharification time. However, this does not explain the longer saccharification time of the CP mashing processes with quinoa, the (pseudo)cereal with smaller endogenous enzyme activity. For the FP mashes, pregelatinizing the starch of the (pseudo)cereal did not substantially increase starch availability compared to the F mashes, because starch already gelatinizes at the higher mashing-in temperature used in the final 65 °C mashing protocol. In addition, pregelatinization leads to inactivation of the enzyme potential of the (pseudo)cereal used, which subsequently increases saccharification time.

For the Congress mash processes, the filtrate mass after 40 min was significantly lower when the (pseudo)cereals were pregelatinized (Figure 1). This was especially the case for barley ($p < 0.01$) and tritordeum ($p < 0.01$), but only to a lesser extent for quinoa ($p = 0.31$). These results may be caused by the inactivation of the endogenous (pseudo)cereal enzymes, which are substantial in barley and tritordeum, but negligible in quinoa (see Section 4.1.). For the final 65 °C mash processes, the filtrate mass after 40 min of filtration was significantly higher for barley ($p < 0.01$) and quinoa ($p = 0.02$) mashes when the (pseudo)cereals were pregelatinized. This effect was not seen for tritordeum ($p = 0.33$). This might be explained by a better starch availability and breakdown due to the pregelatinization procedure, resulting in lower viscosity, better filtration, and a higher extract content.

The extract levels of the CP mashes with barley and tritordeum were similar to the corresponding C mashes (Figure 2). For quinoa, the extract level of the CP wort was significantly higher than the extract level after the C mashing process ($p < 0.01$). The extract levels of the FP mashes were always higher than the extract levels of the corresponding F mashes. Furthermore, a higher gravity was related with a higher filtrate mass after 40 min, which could be explained by a more extensive carbohydrate breakdown. Pregelatinization had no remarkable effect on wort pH.

Overall, a slightly lower FAN level was found in the CP and FP wort samples than in the C and F wort samples (Figure 2), which could be due to inactivation of endogenous proteases in the unmalted (pseudo)cereals during the pregelatinization step. Furthermore, the difference in FAN level was larger between the C and CP wort samples than between the F and FP wort samples. Low mashing-in temperatures during the Congress mashing protocol are indeed suitable for protein degradation by endogenous proteases in the unmalted (pseudo)cereals, whereas high mashing-in temperatures during the final 65 °C mashing protocol are not, due to heat inactivation of these proteases.

The concentrations of glucose and maltose remained similar with or without pregelatinization of the barley or tritordeum (Figure 3). For quinoa, the concentrations of glucose were significantly higher for C and F mashes than for CP ($p < 0.01$) and FP ($p < 0.01$) mashes, whereas the concentrations of maltose were significantly lower for the C and F mashes than for the CP ($p < 0.01$) and FP ($p < 0.01$) mashes. This was caused by endogenous amyloglucosidases in unmalted quinoa, which release glucose from the non-reducing ends of starch.[63,64] A pregelatinization step inactivated these amyloglucosidases and may therefore be applied to lower glucose and increase maltose concentrations in wort produced with quinoa.

Enzyme addition during congress (CPE) and final 65 °C (FPE) mashing processes with pregelatinized unmalted (pseudo)cereals

Overall, the addition of the exogenous enzyme mix Brewers Compass® (containing amylases, endo-protease, β-glucanases, and hemicellulases) had only a limited influence on the

saccharification time during mashing processes with pregelatinized unmalted (pseudo)cereals (Table 3). Compared to the CP mashes with barley or quinoa, enzyme addition (CPE) shortened the saccharification time by approximately 5 min, similar to the results reported by Kordialik-Bogacka, et al.[14]

For quinoa, the saccharification time of the CPE mash (15 min) was shorter than the FPE mash (>20 min), while for barley and tritordeum, the saccharification times of the CPE and FPE mashes were similar. A lower mashing-in temperature in the CPE mashing protocol than in the FPE mashing protocol is better suited for protein hydrolysis by proteases from barley malt and Brewers Compass®. This probably resulted in more extensive protein degradation, which increased availability of the protein-embedded starch granules of quinoa,[65] leading to a faster starch hydrolysis and a shorter saccharification time.

Compared to the CP and FP mashes with barley, tritordeum, or quinoa, the CPE and FPE mashes resulted in higher filtrate masses after 40 min ($p < 0.05$ for both CP vs CPE mashes and FP vs FPE mashes with tritordeum; Figure 1). This confirms the beneficial effect of the added enzyme mix on the filtration process, due to a more extensive breakdown of macromolecules such as β-glucan, protein, and starch.[44,56] The CPE and FPE mashes with barley or quinoa resulted in higher filtrate masses after 40 min compared to their respective C and F mashes. However, for tritordeum, the CPE and FPE mashes resulted in lower filtrate masses after 40 min compared to their respective C and F mashes, which can be explained by the inactivation of high amounts of endogenous enzymes present in tritordeum during the pregelatinization step.[39] The endogenous enzymes in tritordeum thus conferred more benefit to the mashing process than the endogenous enzymes in barley and quinoa.

Adding exogenous enzymes during the CPE mashes with unmalted barley, tritordeum or quinoa did not increase the extract content of the wort compared to the CP mashes (Figure 2). However, adding exogenous enzymes to the FPE mashes resulted in a significantly higher extract content than the FP mashes for barley ($p = 0.02$) and quinoa ($p = 0.02$) but not for tritordeum ($p = 0.07$). The extract content of the CP wort samples was already high and presumably close to the maximum extractability, whereby the addition of the exogenous enzymes could not have a substantial effect on the extract content. However, the extract content of the FP wort samples was substantially lower and therefore extractability could still be increased by addition of the exogenous enzymes, leading to a higher extract content in the FPE wort samples.

Overall, the addition of the exogenous enzymes increased the FAN levels (Figure 2), whereby the increase was larger for the Congress mashes than for the final 65 °C mashes, as the former has a lower mashing-in temperature, which is more suitable for protein degradation.[62]

The addition of the exogenous enzymes resulted in higher maltopentaose concentrations for the FPE mashes with each of the three (pseudo)cereals compared to their FP mashes (Figure 3). The maltohexaose concentrations showed a similar, though less pronounced, pattern. No general trends could be discerned for the C, CP, and CPE mashes.

Conclusion

In this study, unmalted barley, tritordeum, and quinoa were used in a 40% barley malt substitution to produce wort samples according to the Congress mash protocol and final 65 °C mash protocol described by Evans et al.[33] Addition of 40% unmalted tritordeum instead of unmalted barley did not impact wort filtration, despite the absence of a husk in tritordeum, but the filtration rate decreased markedly when 40% unmalted quinoa was added. Adding quinoa also resulted in substantially lower extract yields compared to unmalted barley with remarkably high glucose concentrations, while adding 40% unmalted tritordeum gave rise to a slightly higher extract yield. Overall, the use of the Congress mash protocol resulted in better wort filtration, better extract yield, and higher FAN levels. However, shorter saccharification times were obtained during the final 65 °C mash protocol. Pregelatinizing the unmalted adjunct had a positive impact on the filtration of Congress mashes, but a negative impact on the filtration of final 65 °C mashes. Pregelatinization also slightly increased the extract yield of the final 65 °C mashing processes, despite the inactivation of endogenous enzymes in the unmalted adjuncts. This inactivation was especially noticeable in the sugar profile of quinoa wort samples, where pregelatinization resulted in substantially lower glucose concentrations. Addition of the exogenous enzyme mix Brewers Compass® improved the filtration for both Congress and final 65 °C mashes, improved the extract yield for the final 65 °C mashes, and increased the FAN during Congress mashes, supporting the claims of the producer.

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