C. P. Holmes, J. Casey and D. J. Cook

A Novel Low-temperature Mashing Schedule for Brewing with Unmalted Sorghum: Optimisation

Laboratory mashing experiments were used to investigate and optimise protocols for mashing with unmalted sorghum using a novel low-temperature (T_{max} = 78 °C) enzyme blend and mash schedule. Results are compared with equivalent data for a traditional high-temperature mash schedule (T_{max} = 95 °C). An experimental design approach was used to model wort quality (Extract, FAN content, filtration rate, fermentable sugars contents, colour and turbidity) produced by each mash schedule across a full factorial design space. The factors varied were 1. Mash-in pH (5.5 ± 0.25) 2. Calcium chloride addition (2 g/kg ± 1) 3. Potassium metabisulphite (KMS) addition (1 g/kg ± 1) and 4. The amount of enzyme blend added to each system (recommended dosage ± 50 %). Wort quality was particularly sensitive to mash-in pH with best results achieved close to pH 5.5 (hence selected as the design centre point). At pH values below 5.5 wort extract decreased with either schedule, whereas the impacts of elevated pH at mash-in were slightly higher wort FAN levels, but increased wort turbidity and colour and reduced filterability of the laboratory wort. The novel low-temperature mash schedule was able to produce wort of comparable quality with that from the high-temperature schedule. Design space models for the high- and low-temperature mash systems were used to suggest optimal conditions for each varied factor, with the objective of maximising wort extract and FAN, whilst minimising enzyme addition rates (hence cost). Optimised extracts of 10.6 and 10.8 °P were achieved for the low and high-temperature mash systems, with FAN levels of 60 and 57.2 mg/L respectively. Optimal conditions required a mash-in pH of 5.75 and around 2 g/kg KMS in each case. The high-temperature system required around 3 g/kg $CaCl_2$ addition for optimal performance whereas the low-temperature mash performed well with just 1 g/kg added $CaCl_2$. Use of the novel low-temperature mash schedule should facilitate substantial energy savings in the brewery, because it operates at lower maximum temperatures for a shorter overall process time and also because the mash schedule features only increases in process temperatures with time. This saves on the energy required to cool the mash in the traditional process from 95 °C (for starch gelatinisation) to the saccharification enzyme stand (60 °C).

Descriptors: sorghum, brewing, exogenous mash enzymes, mashing, starch, potassium metabisulphite

1 Introduction

Historically, the use of sorghum in the production of Western-style beers has been limited to use as an adjunct [2, 24]. However, interest in the use of sorghum as the primary grist component increased following a 1988 ban on the import of barley malt in Nigeria [9, 31]. Despite a repeal of the ban, interest remains for the use of sorghum as the primary cereal ingredient for brewing, particularly in Nigeria, and other African and Asian countries. The immediate benefit to brewers in the countries with warm climates where sorghum thrives is that grain can be sourced locally and the expense of transporting raw materials is reduced. Additionally, sorghum use in brewing is increasingly of interest to brewers in Western countries, due to an increased demand for gluten-free products (in response to coeliac disease) [5].

At present, much of the research regarding the use of sorghum in brewing has related to the use of malted sorghum. However, as compared to barley malt, malted sorghum is associated with relatively poor β-amylase activity [7, 33]. Although it has been argued that this comparatively reduced β-amylase activity is due to barley optimised malting conditions [2], current processes using sorghum malt usually require enzyme supplementation [10]. Other issues associated with sorghum malt include low free amino nitrogen (FAN), poor filtration, and poor breakdown of endosperm cell walls [25]. These issues, particularly the requirement for exogenous enzymes, make the use of unmalted sorghum a favourable option [10].

Sorghum starch has a high gelatinisation temperature (>72 °C) [25, 34], and consequently most current exogenous enzyme systems require long, high-temperature stands (>95 °C) to fully

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Table 1 Details of the exogenous mash enzymes used in the trial and their 'standard' addition rates for low and high-temperature mash schedules

Enzyme preparation	Principal Activities	Enzyme source	Temperature optimum (°C)	pH optimum	Low-temperature system (g/kg)	High-temperature system (g/kg)
Amylo 300	amyloglucosidase	*A. niger*	75	4.0	1.0	–
Promalt S-LTP	Amylolytic and proteolytic	GM and non-GM strains	50-70	5.0–7.0	1.0	–
Bioprotease P1	protease	*Bacillus spp.*	70	6.0	–	0.3
Hitempase STXL	α-amylase	*B. lichenformis*	90	6.0	–	1.0
MPA 5	α-amylase	*A. oryzae*	60	6.0	–	0.3

gelatinise starch for efficient saccharification. Recently, a novel, low-temperature mashing system has been developed which can achieve saccharification whilst at temperatures lower than the gelatinisation temperature of sorghum starch. Systems such as this may reduce the energy requirement of brewing with sorghum. In order for such savings to be realised, the technology must be capable of matching the performance of existing processes with regards to key brewing parameters such as extract, free amino nitrogen (FAN) and wort sugars profile. In the present study, the brewing performance of a reduced energy low-temperature mashing system and that of a typical high-temperature mashing system were compared, using a commercial sorghum brewing cultivar. An experimental design approach was used to identify optimal conditions for each system as influenced by factors such as mash pH, calcium or KMS concentrations.

2 Materials and methods

2.1 Raw materials and enzymes

Unmalted sorghum (Sorghum bicolor cv. Fara Fara) was sourced from Nigeria. Grain was stored in plastic bins at 4 °C until required. The gross composition of the sorghum grain was as previously published in *Holmes* et al. [14] (in g/100g db: ash (1.8); cellulose (11.7); hemicellulose (7.6); lignin (5.3); lipid (3.1); protein (9.4) and starch (61.7)). All exogenous enzymes used in the present research (Table 1) were sourced from Kerry Ingredients and Flavours (Naas, Co. Kildare, Ireland).

2.2 Laboratory mashing protocols

The required amounts of potassium metabisulphite (KMS) and calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) were added to 300 mL brewing liquor (reverse osmosis water; RO) and atemperated to 50 °C in a heated water bath. Sorghum grain was milled using DFLU laboratory disc mill (Bühler Group, Uzwil, Switzerland) to EBC fine setting (0.2 mm gap setting). Atemperated liquor and grist were mixed with a glass stirring rod. Mash pH was adjusted to the required value by dropwise addition of 10 % (w/v) aqueous lactic acid. Enzymes were added at varying levels, according to the experimental design, and the mash beakers were placed in a bench top mash bath (1-cube R12, Havlickuv Brod, Czech Replublic) preheated to 50 °C. Temperature profile was selected according to the enzyme system in use (Fig. 1) and enzyme additions were varied according to the experimental design. The mash was stirred using the Hartong speed setting, and beakers were covered with foil to minimize evaporation. Immediately following mashing, samples were placed into a 20 °C cooled water bath for 20 min. Cooled samples were then made up to a weight of 700 g with RO water.

2.3 Experimental design

Experimental design and statistical analysis was achieved using Design Expert 7.0 (Stat-Ease Inc., Minneapolis, MN). A 2-level full factorial experiment (no blocks) was designed in order to evaluate the impacts of four mash additions on key mashing outputs. Four 'centre point' experiments were added to the full factorial design to add robustness in terms of covering the design space and better estimating the variability of replicate measurements. Thus the total number of mash experiments was 20 (= 2^4 + 4) for each mashing protocol (the full design was repeated using both high temperature and low temperature mash schedules). Significance ($p < 0.05$) of main effects on brewing parameters was assessed by Anova.

Mashing parameters and parameter ranges investigated were as follows (centre point, upper and lower levels of each factor): pH at mashing-in (pH 5.5 ± 0.25), potassium metabisulphite (KMS; 1 g/kg ± 1g/kg), calcium chloride dihydrate ($CaCl^2 \cdot 2H_2O$; 2 g/kg ± 1 g/kg),

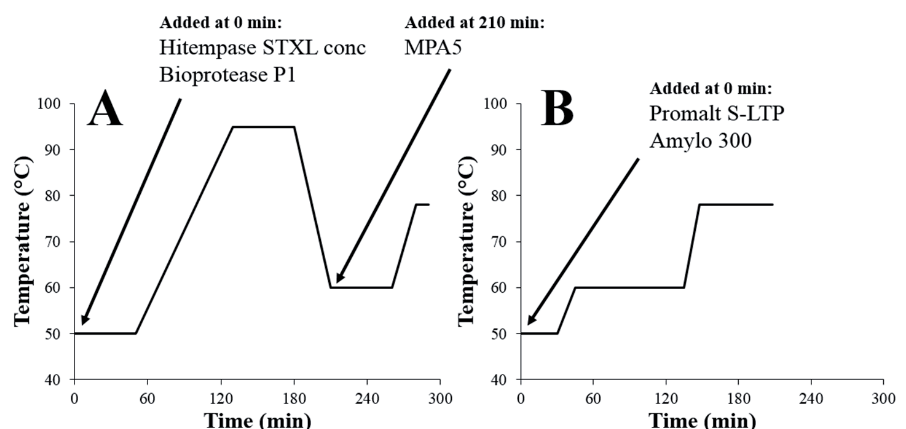

Fig. 1 Details of mashing schedules used for A) high-temperature mashing system and B) low-temperature mashing schedules

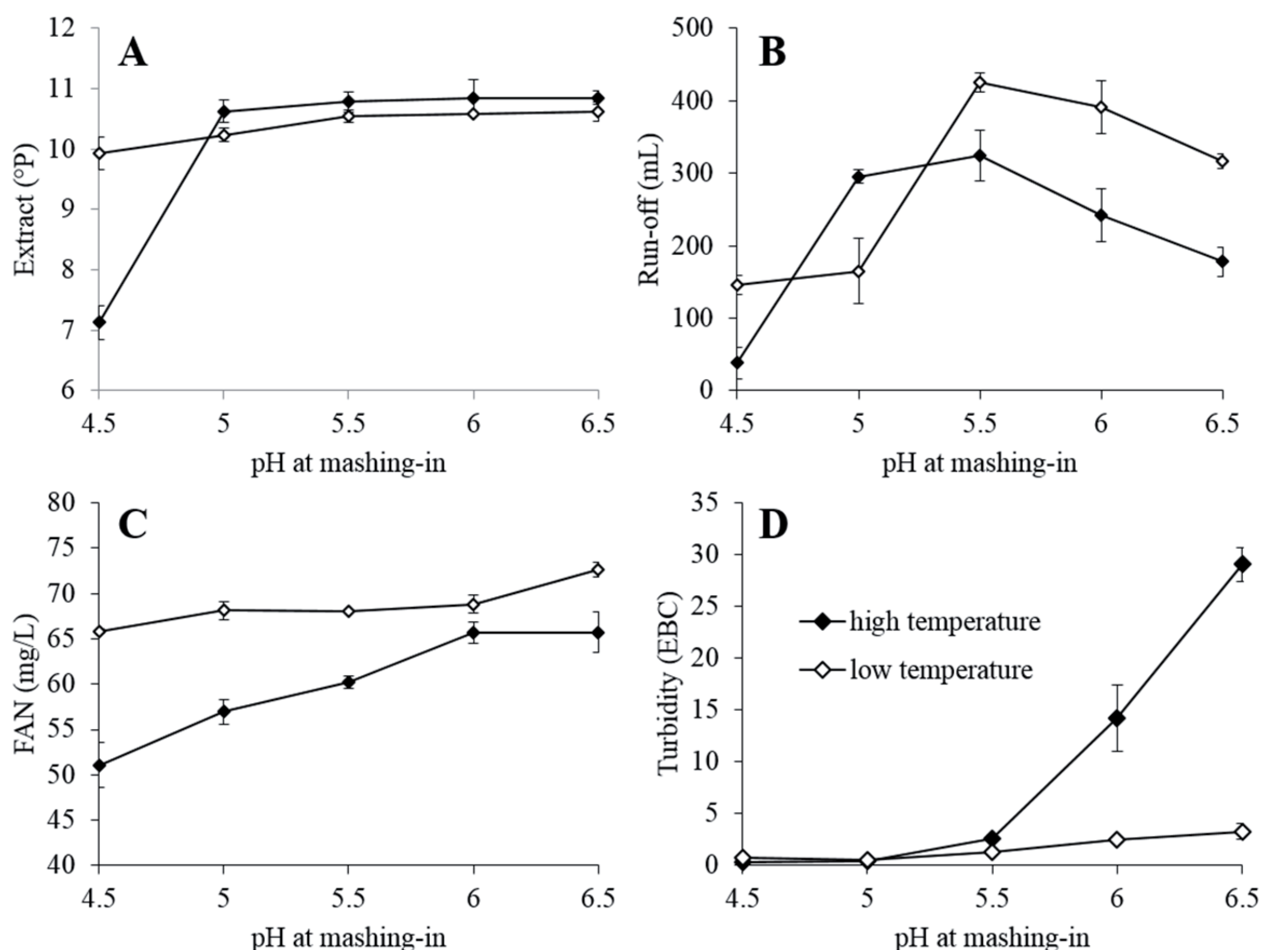

Fig. 2 Impacts of varying mash-in pH on A) wort extract, B) filtration rate, C) FAN and D) turbidity using both the high and low-temperature mashing regimes. Results are the mean of triplicate analyses ± standard deviation. All mashes with constant addition rates of: $CaCl_2 \cdot 2H_2O$ (2 g/kg), KMS (1g/kg), and enzyme (100 % of standard dose)

and enzyme concentration (standard addition ± 50 %; Table 1). The high-temperature enzyme system consisted of Hitempase STXL conc, Bioprotease P1 and MPA5. The low-temperature enzyme system consisted of Promalt S-LTP and Amylo300.

Further factorial designed experiments were used to investigate the impact of varying individual enzyme components within each system (low-temperature system: 2^2 factorial design, 3 centre points, 12 additional data points, no blocks; high-temperature system: 2^3 factorial design, 4 centre points, 12 additional data points, no blocks). Standard mash parameters of $CaCl_2$ (2 g/kg), KMS (1 g/kg), and pH (5.5) were used. The enzymes of the high-temperature system and low-temperature system were each adjusted ± 50 % from their standard dose rates to better discern their individual impacts on mash performance (Table 1).

2.4 Measurement of wort run-off volume

Samples were filtered through pleated filter papers (Whatman 2555 1/2 320 mm) into 500 mL Erlenmeyer flasks. The first 100 mL filtrate was returned to the filter and the samples were allowed to filter for 10 min before the funnel was moved to a clean 1 L Erlenmeyer flask to drain. The volume of wort collected during those 10 min of filtration was measured as an index of speed of filtration.

2.5 Analysis of wort turbidity

Wort haze was measured using a Vos Rota turbidity meter (Haffmans, Venlo, Netherlands). Glass cuvettes (60 mm diameter) were filled with filtered wort sample, capped and placed into the Vos Rota chamber (previously rinsed and cleaned with RO water). Scattered light was measured at angles of 90° and 25° using a wavelength of 650 nm. The turbidity meter was calibrated up to 20 EBC units; samples exceeding this value were diluted to fit within the calibrated range of the device.

2.6 Wort colour measurement

Wort colour was determined according to Analytica-EBC method 4.7.1. Wort samples were filtered through a 0.45 µm syringe filter (Sartorius, Göettingen, Germany) into a 2 mL plastic cuvette. Sample absorbance was measured with a Jenway 7315 spectrophotometer (Bibby Scientific, Staffordshire, UK) at 430 nm.

2.7 Analysis of wort density and specific gravity

Samples were passed through a Minisart cellulose acetate 0.45 µm syringe filter (Sartorius, Göttingen, Germany) and 30 mL filtrate was passed through an Anton Paar DMA 4500 (Anton

Paar, Graz, Austria). Samples were equilibrated to 20.0 °C before measurement of density and specific gravity (SG). Samples were measured in triplicate.

2.8 Free amino nitrogen determination

The free amino nitrogen (FAN) content of samples was determined according to Analytica-EBC method 8.10.1 (ninhydrin method). Samples absorbance values (570 nm) were compared against a glycine standard solution (2 mg/L). Samples were analysed in triplicate.

2.9 HPLC analysis of brewing sugars

Prior to analysis, wort was passed through a 0.45 µm filter. Sample (5 µl) was injected onto a Phenomenex Luna NH2 column (250 x 2 mm ID with a 5 µm particle size; 40 °C; Phenomenex, Torrence, CA) using a Jasco liquid chromatograph (Jasco, Oklahoma City, OK). Analytes were separated isocratically using 80 % aqueous acetonitrile (degassed by sonicating in a waterbath) with a flow rate of 0.7 mL/min and a maximum operating pressure of 400 psi. Compounds were detected using refractive index detection (Jasco RI-2030 plus) and quantified against a standard curve of fructose, glucose, sucrose, maltose, and maltotriose. Samples were analysed in triplicate.

3 Results and discussion

By way of a scoping experiment, the impact of varying mash pH (4.5–6.5) on the performance of each enzyme system was investigated (Fig. 2). Low pH values (pH < 5) were associated with reduced extract (Fig. 2A) and longer wort filtration times (Fig. 2B), whilst higher pH values (pH > 6) although resulting in slightly improved FAN yields (Fig. 2C) gave rise to issues with turbidity and reduced run-off rates. The results from these trials were used to establish the pH range used in subsequent experiments (pH 5.5 ± 0.25).

Despite a comparative reduction in extract, the low-temperature system appeared more resilient to changes to mashing-in pH.

Table 2 Impacts of increasing selected mash parameters on mashing and wort characteristics of high and low-temperature mashing schedules

	p-value					
	$CaCl_2$	enzyme concentration	KMS	mashing-in pH	model R^2	model prob > F
High-temperature mash schedule						
run-off volume	0.0014 (+)	0.0017 (+)	–	–	0.64	0.0003
colour	0.0036 (–)	<0.0001 (+)	<0.0001 (–)	<0.0001 (+)	0.93	<0.0001
FAN	0.0165 (–)	0.0015 (+)	<0.0001 (+)	–	0.82	0.0001
fructose	–	0.0055 (+)	–	–	0.37	0.0055
glucose	–	<0.0001 (+)	0.0117 (+)	0.0135 (–)	0.96	<0.0001
maltose	–	0.0001 (+)	–	–	0.77	0.0002
maltotriose	–	<0.0001 (–)	0.0104 (+)	–	0.75	<0.0001
extract (°P)	–	0.0001 (+)	0.0013 (+)	–	0.74	<0.0001
haze 25°	–	–	0.0023 (+)	0.0145 (+)	0.56	0.0013
haze 90°	0.0274 (–)	–	<0.0001 (+)	–	0.80	<0.0001
wort pH	–	–	–	0.0001 (+)	0.84	<0.0001
Low-temperature mash schedule						
run-off volume	0.0013 (+)	<0.0001 (+)	–	–	0.97	<0.0001
colour	–	<0.0001 (+)	–	<0.0001 (+)	0.88	<0.0001
FAN	–	0.0011 (+)	0.0039 (+)	–	0.99	0.0068
fructose	–	0.0077 (–)	–	–	0.54	0.0076
glucose	0.0049 (+)	0.0002 (+)	0.0068 (+)	–	0.75	<0.0001
maltose	<0.0001 (+)	<0.0001 (+)	–	0.0215 (+)	0.93	<0.0001
maltotriose	–	–	–	–	–	–
extract (°P)	0.0001 (+)	<0.0001 (+)	<0.0001 (+)	0.0069 (+)	0.93	<0.0001
haze 25°	–	0.0032 (+)	–	0.0154 (+)	0.55	0.0018
haze 90°	–	0.0004 (+)	–	0.0027 (+)	0.75	<0.0001
wort pH	–	–	–	<0.0001 (+)	0.92	<0.0001

Results are displayed as *p*-values showing the significance of each factor ($CaCl_2$, enzyme concentration….) in the predictive models for each parameter (run-off volume, colour etc.). The impact of increasing each factor on a particular measured parameter is indicated by either (+), increases the parameter value, or (–) decreases the measured parameter value. For example, in the high temperature mash system wort run-off volume was significantly impacted by both $CaCl_2$ and overall enzyme dosage, each of which increased run off volume (+) as their concentration increased across the design space

Mashing-in at pH 4.5 resulted in a set mash when using the high-temperature system (Fig. 2B); this was not observed with the low-temperature mash. In addition, at pH 5.5–6.5 the run-off volume achieved with the low-temperature enzymes was increased as compared to the high-temperature system (Fig. 2B).

3.1 Full factorial experimental design

Performance of the existing high temperature and the novel low temperature mashing systems were evaluated across a full factorial design space, using the following factors: mash in pH (5.25–5.75), $CaCl_2$ dihydrate (1–3 g/kg), KMS (0–2 g/kg) and enzyme dose rate (standard (Table 1) ± 50%). Experimental data were modelled using Design Expert software to produce a model for each measured wort parameter. The significance of each of the varied factors in these models is reported in table 2 on page 103 ($p < 0.05$); non-significant factors were removed from models. The directionality of the impact of a factor is shown in brackets in table 2, following the *p*-value. Thus, reading across the first line, in the high temperature mash regime, run off volume (mash filtration rate) was increased significantly by increasing calcium and enzyme concentrations, but there was no significant effect of KMS or mash-in pH on this parameter over the ranges investigated in the factorial design.

Two other modelling criteria are reported in table 2 to demonstrate the significance (model prob > F) and predictive power (model R^2) of each model. All of the reported models are highly significant (model prob > F is << 0.05) and have acceptable predictive power, with values closer to 1.00 indicating better fit between the model and experimental data.

3.2 Impact of pH on mashing systems

Increasing mashing-in pH when using the high-temperature enzyme system resulted in significant increases in wort colour, wort pH, and haze (Fig. 2D; Table 2). However, an increase in mashing-in pH also correlated with a significant decrease in glucose concentration (Table 2). This suggests that α-glucosidase activity present within the enzyme blend was not optimally active at higher mashing-in pH values; this observation agrees with earlier research indicating that α-glucosidases are thought to be optimally active in more acidic mash conditions (e.g. pH 3.75) [32]. Similarly, for the low-temperature system, increasing mash-in pH also resulted in significant increases in the colour, pH, and haze of the wort, however, there was also a significant increase in extract and wort maltose concentration. Previous generations of the Promalt enzyme cocktail have been characterised as having a pH optimum close to 6.0 [8], the results here are in agreement with these findings. As the pH optimum of Amylo300 is closer to 4.0 (Table 1) [26], the improved maltose yield observed here is probably due to the combined effect of improved Promalt S-LTP activity combined with inhibition of amyloglucosidase activity from

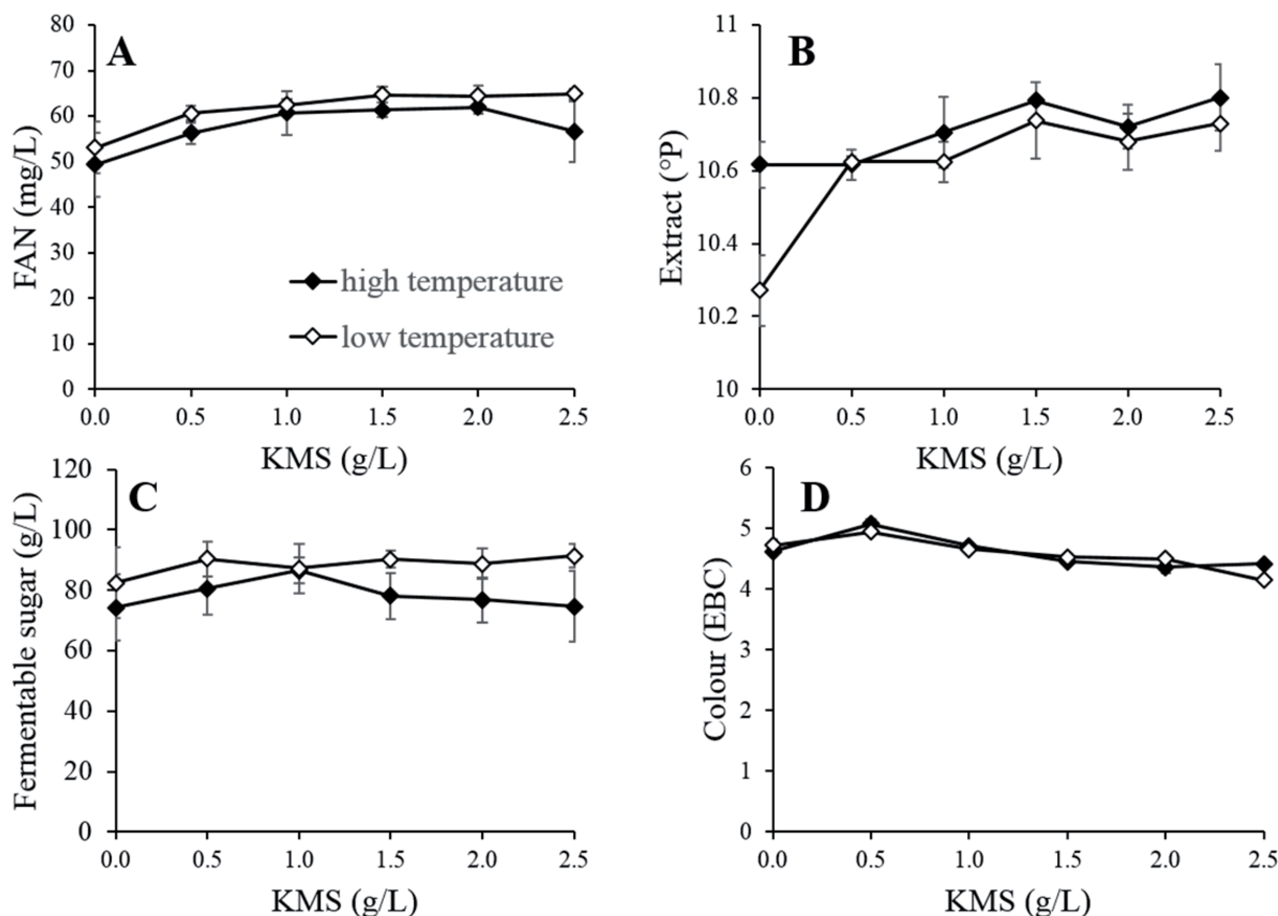

Fig. 3 Impacts of varying mash potassium metabisulphite (KMS) on A) wort FAN, B) extract, C) fermentable sugars and D) wort colour using both the high and low-temperature mashing regimes. Results are the mean of triplicate analyses ± standard deviation. Mashing-in achieved at pH 5.5 with constant addition rates of: $CaCl_2 \cdot 2H_2O$ (2 g/kg), and enzyme (100 % of standard dose)

Table 3 **A summary of experimental data used to develop statistical models displayed as wort characteristics at minimum, centre point and maximum variable values for high- and low-temperature models**

	High-temperature mash schedule			Low-temperature mash schedule		
	minimum	centre point[a]	maximum	minimum	centre point	maximum
run-off volume (mL)	144	248 ± 130	385	247	423±21	498
colour (EBC)	4	4.7 ± 0.7	6	4.2	4.3 ± 0.2	4.6
FAN (mg/L)	48	55 ± 8.7	75	50	68 ± 1.7	59
fructose (g/L)	0.8	0.7 ± 0.1	0.8	0.6	0.6 ± 0.1	0.8
glucose (g/L)	7.1	6.5 ± 0.7	11.2	24.4	35.7 ± 1.9	49.7
maltose (g/L)	15.4	23.2 ± 7	55.4	15.5	21.9 ± 1.0	21.5
maltotriose (g/L)	25.7	22.5 ± 5.5	29.7	13	13.7 ± 1.2	12
extract (°P)	10.01	10.38 ± 0.32	11	9.87	11.03 ± 0.43	10.91
haze 25° (EBC)	0.21	1.68 ± 2.56	14.97	0.3	1.48 ± 0.21	1.55
haze 90° (EBC)	0.41	0.84 ± 1.13	8.35	0.35	1.21 ± 0.08	1.07
wort pH	5.46	5.66 ± 0.26	5.79	5.52	5.64 ± 0.04	5.71

[a] Centre point values are the mean of four replicate mashes ± standard deviation. Centre point conditions were: $CaCl_2$ 2 g/kg, KMS 1 g/kg, enzyme addition 100 % of standard dose rate, mashing-in pH 5.5

Amylo300. In both systems, the impact of mash-in pH (Table 2) on wort colour is probably due to improved extraction of pigments from the grist [17]. The link between pH and haze formation (Table 2) has been also been reported previously [29] and is corroborated by the present research. In addition to particulate matter and haze-active proteins, it is also possible that polyphenols may have played a role in the observed formation of haze material at increased pH values [30].

Extract yield was slightly lower across the design space for the low-temperature mash, relative to the traditional high-temperature process. Within the pH range investigated in the factorial designed experiment (pH 5.5 ± 0.25), pH did not play a significant role with regards to FAN yield (Table 2). The low-temperature system provided a higher concentration of FAN compared to the high-temperature system at all pH values, although still less than the 100–150 mg/L (Fig. 2C) commonly quoted as required for efficient yeast activity [3, 20].

3.3 Impact of calcium chloride addition to mash

The stabilising impact of Ca^{2+} on amylase enzyme activity has been well established. The mode of action (for α-amylases) is thought to involve the interaction of a Ca^{2+} cation with negatively charged amino acids, which acts to stabilise the three dimensional structure of the enzyme at higher temperatures [19, 21]. Interestingly, whilst the low-temperature system adhered to current thinking with increased extract resulting from $CaCl_2$ addition (Table 2), the high-temperature system did not (no statistically significant link). There was however a positive influence of increased $CaCl_2$ on wort run-off volume (Table 2). It has been previously suggested that calcium chloride impacts run-off volume through the stabilising effect of a lower mash pH on enzyme activity [13]. In the high-temperature system, this appears to have not been the case. Mashing-in pH was manipulated independently of $CaCl_2$ and did not have a significant impact on wort-run-off volume or extract. These results indicate that $CaCl_2$ can affect run-off volume independently from its impact on wort pH. It has previously been reported that Ca^{2+} may disrupt gel forming mash components and increase run-off [27], similar interactions may have been at work here.

In the high-temperature system, increased $CaCl_2$ correlated with a decrease in wort FAN, this is contrary to prior reports, where increased Ca^{2+} has been associated with increased FAN [27]. These results suggest that an excess of Ca^{2+} could have a potentially detrimental impact on FAN content, perhaps due to chelation of mash components required for efficient proteolysis. Interestingly, this inhibition was less evident at increased KMS addition rates (data not shown).

3.4 The influence of potassium metabisulphite addition

Free amino nitrogen (FAN) yield is probably the parameter of most interest with regards to potassium metabisulphite (KMS) addition in sorghum brewing. It has previously been asserted that KMS addition helps to improve FAN yield [22] and this is supported by the present data (Fig. 3A, Table 2). This improved FAN yield is thought to result from the reduction of intermolecular disulphide bonds and subsequent improved access for protease activity [22]. Despite the increase in FAN content associated here with KMS addition, the maximum concentration of FAN achieved using model conditions was 75 mg/L in the high-temperature system and 59 mg/L in the low-temperature system (Table 3). The FAN content of the worts in the selected model were all below the concentration found in barley malt worts (e.g. 200 mg/L) [16] and the concentration required for rapid yeast activity during fermentation (130–150 mg/L) [20]. Even with increased use of KMS (within the range used in this research), nitrogen supplementation of wort would probably still be required for adequate fermentation.

In both enzyme systems, increasing KMS content of the mash was associated with significantly increased extract (Table 2; Fig. 3B). Although increased KMS was also associated with significant increases to wort glucose (Table 2), the overall impact upon fermentable wort sugar seems quite minor (Fig. 3C). It is likely that the majority

Table 4 Impacts on mashing performance of increasing selected individual enzyme dose rates as part of the high-temperature mashing system

	p-value			Model R^2	Model prob>F	Model type
	Hitempase STXL conc	Bioprotease P1	MPA5			
Run-off Volume	0.0017 (+)	–	<0.0001 (+)	0.91	<0.0001	2FI
Colour	0.0005 (+)	<0.0001 (+)	–	0.81	<0.0001	Linear
FAN	–	<0.0001 (+)	–	0.86	<0.0001	Linear
Fructose	–	–	–	–	–	–
Glucose	<0.0001 (+)	–	–	0.73	<0.0001	Linear
Maltose	–	–	<0.0001 (+)	0.9	<0.0001	Linear
Maltotriose	0.0331 (+)	–	0.0091 (+)	0.4	0.006	Linear
Extract (°P)	<0.0001 (+)	0.0012 (+)	–	0.76	<0.0001	Linear
Haze 25°	<0.0001 (+)	0.0420 (+)	<0.0001 (+)	0.9	<0.0001	3FI
Haze 90°	<0.0001 (+)	0.0052 (+)	<0.0001 (+)	0.93	<0.0001	3FI
Wort pH	–	–	–	–	–	–

Results are displayed as *p*-values showing the significance of each factor (individual enzyme concentrations) in the predictive models for each parameter (run-off volume, colour etc.). The impact of increasing each factor on a particular measured parameter is indicated by either (+), increases the parameter value, or (–) decreases the measured parameter value. For example, wort FAN was only significantly impacted by concentrations of Bioprotease P1, which increased wort FAN significantly as concentration of the enzyme increased
2FI = 2 factor interaction model. 3FI = 3 factor interaction model

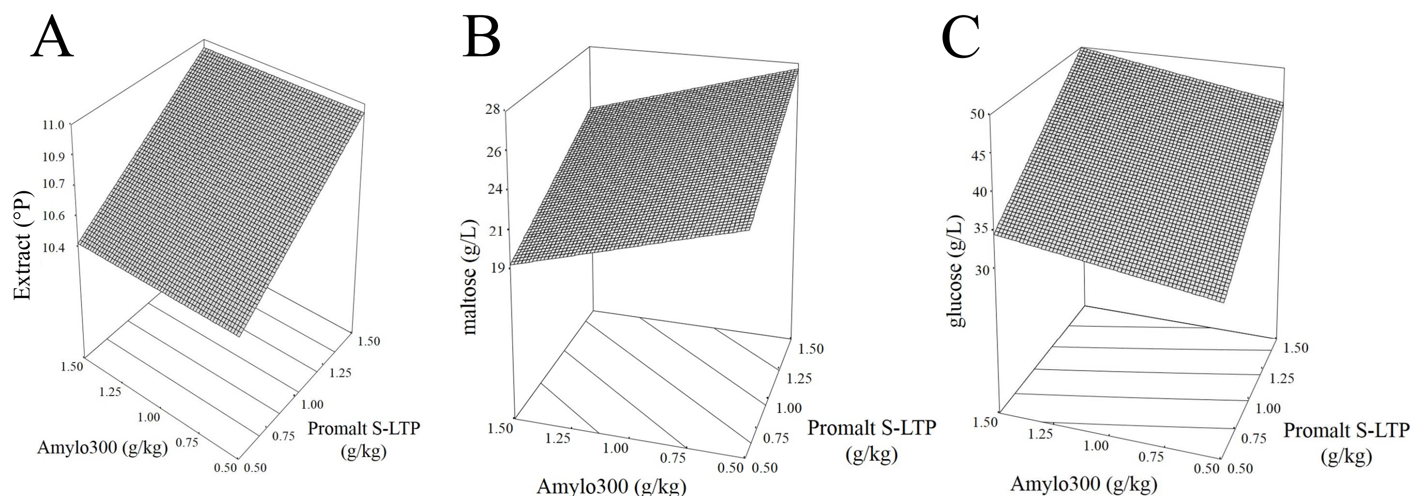

Fig. 4 Response surface models showing the impacts of varying enzyme addition rates to the low-temperature mashing system on A) wort extract, B) wort maltose content, and C) wort glucose content

of the increased extract associated with KMS addition results from its impact on the protein matrix surrounding starch granules, thus facilitating more complete liquefaction of starch on mashing.

The other key impact of KMS addition was on wort colour (Fig. 3D). It would be expected that the high-temperature mashing profile (reaching 95 °C during gelatinization stand), would result in high wort colour due to non-enzymatic browning through Maillard chemistry. The addition of KMS to the mash significantly reduced wort colour in high-temperature mashing. The addition of KMS to mashing is known to result in elevated sulphur dioxide (SO_2) concentration in wort [6, 17]. Non-enzymatic browning has been shown by previous researchers to be inhibited by SO_2 [4, 12], this perhaps offers an explanation as to the findings in this research.

In the low-temperature mashing system there was no statistically significant response of colour formation to KMS addition, perhaps due to overall reduced non-enzymatic browning modulated by lower mashing temperature (60 °C).

3.5 Activities and impacts of mashing enzyme content

The variable that had the greatest impact upon wort quality and characteristics when using either enzyme system was enzyme concentration. In both systems, increased enzyme concentration significantly increased wort run-off volume, wort colour, FAN and wort extract (Table 2). Additionally, in the high-temperature system a significant decrease in maltotriose was observed (with a

corresponding increase of glucose and maltose), suggesting an improved breakdown of small polysaccharides in the wort with increased enzyme concentration. Increased enzyme use in low-temperature mashing was associated with significantly increased wort turbidity (measured at 25° and 90°). Most likely the increased proteolytic activity due to higher additions of Promalt S-LTP resulted in increased solubilisation of turbidity-inducing components, or there may even have been haze-active components in the enzyme preparations themselves.

Analysis of the individual enzymatic components provided some clues as to the action of each enzyme. Hitempase STXL conc (known to possess α-amylase activity; Table 1) resulted in significant increases to run-off volume, wort colour, haze, glucose and maltotriose. The significant increase in extract (without subsequent maltose increase) associated with Hitempase addition suggests the formation of soluble dextrins (Table 4). In addition, there was an observed increase in glucose content when Hitempase concentration was increased; the presence of α-glucosidase activity in the commercial enzyme preparation could potentially explain this observation [1].

The MPA5 enzyme preparation primarily increased run-off and maltose/maltotriose content. These findings are probably attributable to β-amylase activity, which acts by hydrolysing the penultimate α-(1-4)-glycosidic bond on a polysaccharide, yielding individual maltose units [33].

The primary wort characteristics impacted by increased Bioprotease P1 were FAN content and colour. The model identifies Bioprotease P1 as having a significant impact on wort FAN yield (Table 5). However it should be noted that the maximum FAN content achieved was still suboptimal (71 ± 0.6 mg/L; fixed mash additions with maximum protease addition). Whilst one could consider investigating the use of higher levels of Bioprotease P1 to elevate the wort FAN content further, the data reported here suggest that such an approach would not be cost effective.

The low-temperature enzyme system exceeded the high-temperature enzymes in terms of extract yield (°P; at centre-point values), however it also resulted in a different sugar composition. In the high-temperature system (at centre-point values; Table 3) maltose and maltotriose were the major sugars (>20 g/L) with lower amounts of glucose (6.5 g/L). In the low-temperature system, maltose content was similar to that seen in the high-temperature system (21.9 g/L). However, glucose was present at a comparatively elevated concentration (35.7 g/L) whilst maltotriose was decreased (13.7 g/L). Both enzyme components of the low-temperature system were associated with an increase in wort glucose, however, Amylo300 content was also correlated with a significant decrease in maltose (Fig. 4; Table 5). Consideration of the individual enzyme components of the low-temperature system revealed that whilst Promalt S-LTP was the major driving force behind the enzyme system (in terms of extract; Table 5), Amylo300 played a substantial role in sugar composition. The difference in sugar composition could have consequences for fermentation; worts high in glucose have been previously reported to result in slow fermentation and overproduction of off-flavours [18]. In future work it would be interesting to study Promalt S-LTP in isolation as the results here suggest that the impact of Amylo300 on finished wort extract (°P) was relatively subtle, impacting primarily sugar composition (Table 5; Fig. 4).

As the gelatinisation temperature of sorghum is usually higher than 70 °C [28] it is hypothesized that Promalt S-LTP contains an α-amylase capable of digesting raw starch. In recent years, a number of microorganisms (bacterial and fungal) have been found to produce amylases capable of digesting raw starch [11, 15, 23]. Without the necessity to gelatinise the starch of sorghum, there is the potential to reduce substantially the energy requirements of

Table 5 Impacts on mashing performance of increasing selected individual enzyme dose rates as part of the low-temperature mashing system

	p-value				
	Promalt S-LTP	Amylo 300	Model R2	Model prob > F	Model Type
Run-off volume	<0.0001 (+)	–	0.63	<0.0001	Linear
Colour	<0.0001 (+)	–	0.90	<0.0001	Linear
FAN	<0.0001 (+)	–	0.96	<0.0001	Linear
Fructose	–	–	–	–	
Glucose	<0.0001 (+)	<0.0001 (+)	0.97	<0.0001	2FI
Maltose	<0.0001 (+)	<0.0001 (–)	0.90	<0.0001	Linear
Maltotriose	<0.0001 (–)	–	0.88	<0.0001	Linear
Extract (°P)	<0.0001 (+)	–	0.84	<0.0001	Linear
Haze 25°	0.0163 (+)	–	0.31	0.0163	Linear
Haze 90°	0.0099 (+)	–	0.35	0.0099	Linear
Wort pH	–	–	–	–	

Results are displayed as p-values showing the significance of each factor (individual enzyme concentrations) in the predictive models for each parameter (run-off volume, colour etc.). The impact of increasing each factor on a particular measured parameter is indicated by either (+), increases the parameter value, or (–) decreases the measured parameter value. For example, wort FAN was only significantly impacted by concentrations of Promalt S-LTP, which increased wort FAN significantly as concentration of the enzyme increased
2FI = 2 factor interaction model

Table 6 Optimised mashing additions for the high and low-temperature mashing systems together with predicted outputs of wort extract and FAN

	Model optimal conditions				Predicted output	
	$CaCl_2$ (g/kg)	enzyme concentration (%)	KMS (g/kg)	mashing-in pH	extract (°P)	FAN (mg/L)
High-temperature system	2.98	63.67	1.99	5.75	10.77	57.18
Low-temperature system	1.00	77.34	2.00	5.75	10.56	60

The optimised conditions are derived from the modelled design spaces (Design Expert v 7.0, Statease) with the input criteria to maximise extract and FAN yield whilst minimising the required exogenous enzyme addition

brewing by using low-temperature mash conditions such as those employed here.

3.6 Optimisation of sorghum mashing, based on predictive modelling

The model design space developed for each sorghum mashing system (using Design Expert 7.0 software) allowed for the prediction of output values based on optimised mashing parameters. The criteria used as a basis for optimisation were that enzyme blend concentration (the most expensive parameter) should be minimised, whilst attempting to maximise wort extract and FAN. The optimised conditions (Table 6) selected by the modelling software predicted comparable extract values for each system, 10.77 °P and 10.56 °P for high- and low-temperature systems respectively. However, the optimised condition suggests that the high-temperature process can achieve the predicted extract with only 63.67 % of the standard enzyme requirement whilst low-temperature mashing required 77.34 % (note that these are different enzyme blends and each is being expressed relative to the recommended standard dose rate used in these trials, so this does not represent a cost comparison). Furthermore, whilst the optimised extract for the high-temperature system (10.77 °P) was higher than those achieved at standard mashing parameters (10.38 °P; Table 3), this was not the case for the low-temperature system (predicted: 10.56 °P, standard: 11.03 °P), hence minimising the enzyme usage in this mash system had a negative consequence on wort extract. Despite optimising for maximum FAN yield, neither optimised regime predicted FAN > 60 mg/L (Table 6).

4 Conclusion/Summary

One of the primary difficulties involved in the brewing of Western-style beers with sorghum grain is the high gelatinisation temperature of sorghum starch (often higher than 70 °C [25, 28]) which presents a problem for enzyme access and efficient saccharification. The two enzyme systems studied in this research represent different methods of circumventing this issue. The traditional approach uses a high-temperature stand (95 °C) capable of gelatinising sorghum starch in conjunction with a heat-stable dextrinising enzyme (Hitempase STXL conc). Following the initial gelatinisation at high temperature, the mash must be cooled to around 60 °C, which uses additional process time and energy, prior to the addition of the heat-labile saccharifying enzyme (MPA5). The enzymes of the low-temperature enzyme system act at 60 °C, below the gelatinisation temperature of most sorghum grain cultivars. The low-temperature mashing process takes 210 min as compared to the 290 min of the high-temperature system. Considering these differences it is clear that the low-temperature system offers some key advantages over the high-temperature method in terms of energy consumption. In addition, the low-temperature system provided a wort of 11.03 °P and FAN 68 mg/L as compared to 10.38 °P and FAN 55 mg/L in the high-temperature system (at model centre point values). A key advantage of the low-temperature system was its robustness over a wider range of pH values, it will be interesting to evaluate the impact of individual enzyme components on this characteristic.

The sugar composition of the worts produced by each system varied considerably, but this was primarily because of the different diastatic enzyme activities utilised in each case. The use of Amylo300 in the low-temperature system resulted in a significant increase in wort glucose and a corresponding decrease in maltose. However, the low temperature performance of the system is not dependent on Amylo300, thus other blends, or even the use of Promalt S-LTP in isolation (since it is designed to fulfil all of the enzyme requirements of mashing) could be evaluated in order to vary the wort sugars spectrum, as desired.

KMS addition correlated with an increase in wort extract and FAN content. It also caused a reduction in wort colour for high-temperature processed worts, perhaps due to the known inhibitory effects of sulphite on non-enzymatic browning.

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