Mashing with unmalted sorghum using a novel low temperature enzyme system: impacts of sorghum grain composition and microstructure

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TITLE RUNNING HEAD: A low temperature mash schedule for unmalted sorghum

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Abstract

Brewing lager beers from unmalted sorghum traditionally requires the use of high temperature mashing and exogenous enzymes to ensure adequate starch conversion. Here, a novel low-temperature mashing system is compared to a more traditional mash in terms of the wort quality produced (laboratory scale) from five unmalted sorghums (2 brewing and 3 non-brewing varieties). The low temperature mash generated worts of comparable quality to those resulting from a traditional energy intensive mash protocol. Furthermore, its performance was less dependant on sorghum raw material quality, such that it may facilitate the use of what were previously considered non-brewing varieties. Whilst brewing sorghums were of lower protein content, protein *per se* did not correlate with mashing performance. Rather, it was the way in which protein was structured (particularly the strength of protein-starch interactions) which most influenced brewing performance. RVA profile was the easiest way of identifying this characteristic as potentially problematic.

Keywords

Sorghum brewing, exogenous mash enzymes, mashing, sorghum starch

1. Introduction

Brewing lager style beers using predominantly unmalted sorghum requires the use of exogenous mash enymes and specific mashing schedules tailored to the conversion of sorghum starch. This is in part due to the high gelatinisation temperatures of sorghum starch (Espinosa-Ramírez, Pérez-Carillo & Serna-Salvídar, 2014) which typically require that mash is first heated to a high temperature (e.g. 95 °C) in order to fully gelatinise the starch, followed by cooling and addition of exogenous mash enzymes to assure breakdown of starch to sugars. Production of Western-style beers with sorghum is currently limited to the use of light-skinned, low polyphenol sorghum cultivars. Traditional sorghum beers are usually produced using brown or red skinned sorghum cultivars (Lyumugabe, Gros, Nzungize, Bajyana, & Thonart, 2012). Use of darker skinned, high tannin cultivars in brewing is thought to result in inhibition of mash enzymes and an objectionable increase in product bitterness (Kobue-Lekalake, Taylor, & de Kock, 2007; Novellie, 1981). However, some workers have suggested that the use of high-tannin sorghum cultivars is responsible for only a minor increase in bitterness, not detectable by all panellists (Daiber, 1975). In addition, the impact of polyphenols on saccharification has also been disputed in mashing using sorghum malts (Dufour, Melotte, & Srebrnik, 1992). It has been suggested that the reduced saccharification of some sorghum malts is due not to polyphenols, but to starch characteristics and poor diastatic potential (Dufour, Melotte, & Srebrnik, 1992).

As new enzyme blends become available which enable lower temperature mashing conditions to be employed, it is of interest to study how this impacts on the brewing performance of different sorghum cultivars. Furthermore, in some regions the objective to brew with locally produced raw materials can make it of interest to use varieties previously considered as sub-optimal for brewing, but which show good

agronomic performance. In the present study, five sorghum samples were sourced: two brewing cultivars (yellow (Nigeria) and yellow (Cameroon)) and three forage cultivars (red (Mexico), white (Nigeria) and white (Ghana)). Laboratory scale brewing trials were conducted with each of the cultivars, comparing the performance of a traditional (high temperature) sorghum mashing schedule with a novel low temperature schedule utilising an exogenous enzyme blend (Figure 1). The latter was developed to enable the digestion of sorghum starch without a high temperature gelatinisation stand prior to saccharification. One objective of the trials was to determine whether the low temperature mashing system could produce worts of comparable brewing quality to those brewed using the traditional mash schedule. A further objective was to study the impacts of cultivar on mashing performance and to try to better understand the interactions between kernel structure and composition and mashing performance.

2. Materials and Methods.

2.1 Sorghum grain samples

Five samples of sorghum grain were sourced by Diageo and Kerry Enzymes. As cultivar identities were unavailable each sorghum variety was identified by colour and country of origin. Two brewing cultivars were received: yellow (Nigeria) and yellow (Cameroon). Three forage cultivars were received: red (Mexico), white (Nigeria) and white (Ghana). Upon arrival, samples were stored at 4°C in plastic bins (as advised by Kerry Enzymes).

2.2 Sorghum grain compositional analysis

2.2.1 Moisture content

Milled samples (5 g; 0.2 mm EBC fine grind) were weighed into pre-weighed foil trays and placed into a convection oven at 130°C for 90 min. Samples were removed into a desiccator, allowed to cool for 30 min and re-weighed to calculate moisture content through weight loss. Five replicate measurements were taken.

2.2.2 Starch content

Starch content was determined using a Starch (GO/P) Assay Kit (Sigma STA20). Whole grain samples (10 mg and 50 mg respectively) were finely milled in a coffee grinder and used with the kit alongside a wheat starch standard (10 mg). To remove non-starch sugars from the sample before analysis the samples were incubated at 85°C for 5 min then washed twice in 80 % (v/v) aqueous ethanol solution. To allow the kit to act effectively upon sorghum's resistant starch, 2 mL dimethyl sulphoxide (DMSO) was added to each sample prior to analysis; these were then incubated in boiling water for 5 min.

104 *2.2.3 Cellulose and hemicellulose*

105 Ion chromatography was used to determine monomeric sugars in acid hydrolysed
106 samples, whilst HPLC was used to determine sugar degradation products produced.
107 Cellulose was estimated as the sum of glucose and hydroxymethylfurfural (HMF)
108 minus determined starch. Hemicellulose was estimated as the summed concentrations
109 of xylose, arabinose and furfural. Samples (60 mg) were weighed into heat resistant
110 screw-capped (with PTFE seal) glass tubes. 12 M H_2SO_4 (2 mL) was added and the
111 contents incubated at 37°C for 1 h. Water (22 mL) was added and the sample was
112 further incubated at 100°C for 2 h. The samples were filtered over glass microfibre
113 syringe filters (Whatman 25 mm 0.45 μm GD/X glass microfibre) and 1 mL was
114 transferred to HPLC vials and analysed via ion chromatography and HPLC.

115 *2.2.3.1 Ion Chromatography Analysis*

116 Sample (10μL) was injected onto a Dionex CarboPac20 column (3 mm x 150 mm)
117 coupled to a Dionex ICS 3000 with an electrochemical detector (Dionex, California,
118 USA). The samples were eluted isocratically with degassed 10 mM NaOH at a flow
119 rate of 0.5 mL/min running at around 3000 psi. Compounds were detected using an
120 electrochemical cell over a 30 min run time. The column was regenerated after each
121 sample run by flushing with 200 mM NaOH at 0.5 mL/min for 10 min.

122 *2.2.3.2 HPLC Analysis*

123 10 μL was injected onto a C18 Techsphere column (250 x 4.6 mm ID; HPLC
124 Technology, Macclesfield, UK) using a Waters 2695 liquid chromatograph (Waters,
125 Massachusetts, USA). Gradient elution was used to separate the analytes, using a
126 solvent mixture of 1 % (v/v) acetic acid (aq): methanol (80:20) ramped to 50:50 over
127 30 min period with a total flow rate of 1 mL/min at a pressure of approx. 2950 psi.
128 Compounds were detected using a Waters 996 Photodiode-Array detector using UV

detection at 270 nm. After 30 min the methanol was increased to 100 % over 1 min, held for 2 min before returning to initial solvent conditions for the next run. External standards of hydroxymethylfurfural (HMF) and furfural (0.1 g/L) were used for calibration. Samples were analysed in triplicate.

2.2.4 Lipid

Solvent-extractable lipid was determined via an adapted Folch determination (Cequier-Sanchez, Rodriguez, Ravelo, & Zarate, 2008). Sample (400 mg) was added to a capped glass test tube with 12 mL dichloromethane/methanol (2:1; v/v). The samples were left for 2 h at room temperature with occasional hand agitation before filtering through a Whatman GD/X glass microfiber filter (0.45 μm pore size). To the filtrate 2.5 mL KCl (0.88 %; v/v) was added and after vigorous agitation the samples were centrifuged at 380 x g at 4°C for 5 min. The aqueous upper layer was discarded and the lower phase was dried over nitrogen gas. The remaining lipid was weighed. Four replicate analyses were performed.

2.2.5 Protein

A Thermo Flash Nitrogen Analyser (ThermoFisher Scientific, Waltham, Massachusetts, USA) was used to determine protein content of the samples. Sample (50 mg) was sealed in a tin capsule and combusted at approximately 1800°C. Quantitation was achieved with Eager 300 software using an L-aspartic acid standard. Protein was determined using the N x 6.25 conversion factor. Samples were analysed in triplicate.

2.2.6 Ash

Ash content was determined according to the method proposed by Santos, Jimemez, Bartolome, Gomez-Cordoves, & del Nozal (2003). Sorghum, wheat or spent grain sample (1 g) was accurately weighed into crucibles of known mass (ashed to constant

mass); these were placed into a muffle furnace at 580°C for 24 h. After ashing the samples were placed directly into a desiccator for 30 min. The sample was then accurately weighed to 3 decimal places. Samples were analysed in triplicate.

2.2.7 Lignin

Determination of sorghum lignin was achieved via an adapted version of the acetyl bromide method (Iiyama & Wallis, 1990), as it was necessary to firstly remove tannin from the grain. Tannin was washed from the milled sorghum grain using a method adapted from Morrison, Asiedu, Stuchbury, & Powell (1995). Milled sorghum (100 mg) was weighed into a polypropylene tube and mixed with 10 mL acetone:water (70:30; v/v). Samples were incubated at 30°C in a water bath for 30 min. After incubation, samples were centrifuged at 500 *g* for 5 min. Extraction was repeated twice with acetone:water (70:30; v/v) before a final wash with acetone. Water was used to quantitatively transfer the samples into thick-walled glass tubes. The samples were dried at 50°C for 48 h. To the dried samples 4 ml acetyl bromide reagent (25 % acetyl bromide in glacial acetic acid) was added. The tubes were capped and incubated in a water bath for 2 h at 50°C then allowed to cool for 5 min. To prepare standards 10 mg lignin (Sigma 471003) was added to 4.5 mL dioxane and 1.5 mL water and incubated at 50°C for 30 min (along with a dioxane/water blank) then allowed to cool for 5 min. Aliquots of the incubated standard solution (0.2, 0.3, 0.4, 0.5 and 0.6 mL) and the blank (0.6 mL) were added to separate glass test tubes and 0.5 mL of the acetyl bromide reagent was added to each. Samples, standards and the blank were made up to 16 mL with glacial acetic acid and 0.5 mL of this solution was transferred to a glass test-tube. To each test-tube, 2.5 mL glacial acetic acid, 1.5 mL sodium hydroxide (0.3 M) and 0.5 ml hydroxylamine hydrochloride (0.5 M) were added. Sample volume was adjusted to 10 mL with glacial acetic acid and then

transferred to quartz cuvettes for analysis at 280 nm using a spectrophotometer. Lignin content was calculated using the standard curve detailed. Four analyses were performed for each sample.

2.2.8 Tannin

Sorghum grain tannin content was determined using the Vanillin-HCl method (Price, Vanscoyoc, & Butler, 1978). Milled Sample (200 mg) was weighed into a polypropylene tube, to this, 10 mL of 1 % (v/v) HCl was added; samples were agitated on a roller bed for 20 min. Samples were centrifuged at 3000 x g to clear the supernatant of particulate matter. A 1 mL aliquot of sample was added to a glass test tube, to this 5 mL working vanillin reagent was added (using a blank of 1 mL sample and 5 mL 4 % (v/v) HCl). Samples were incubated at 30°C for 20 min and measured immediately at 500 nm. Five replicate samples were analysed.

2.3 Determination of α-amylase and β-amylase in sorghum flours

2.3.1 Enzyme extraction

Enzyme extracts for both assays were produced using a Megazyme Betamyl-3 kit (K-BETA3; Megazyme, Co. Wicklow, Ireland). Grain sample was milled using a DLFU laboratory disc mill using the EBC fine setting. Milled grain (0.5 g) was weighed into a 15 mL polypropylene tube, to this, 5 mL extraction buffer (1 M Tris/HCL, 20 mM disodium EDTA solution) was added. Extractions proceeded on a Stuart SRT60 roller bed (Bibby Scientific) for 1 h and were then centrifuged for 10 min at 2000 x g. Kit efficacy was monitored using wheat flour controls of known α-amylase and β-amylase activity.

2.3.2 Determination of α-amylase and β-amylase

Amylase activities in sorghum flour extracts were assayed using Megazyme test kits (Megazyme, Bray, Ireland) and standard methodologies. α-amylase was determined

using the Ceralpha kit (K-CERA) whilst β-amylase was determined using the Betamyl-3 kit. Five replicate samples were analysed in each case.

2.4 Measurement of sorghum starch amylose content

Amylose content was determined using a Megazyme Amylose/Amylopectin kit (K-AMYL). Starch was precipitated from milled sample (25 mg) using 95 % (v/v) ethanol. Starch samples were dissolved in ConA solvent and filtered through Fisherbrand QL100 filter papers (Fisher Scientific, Loughborough, Leicestershire, UK). Upon addition of ConA solution, amylopectin was precipitated from solution and removed by centrifugation (14000 x g for 10 min). Amylose supernatant and total starch samples were hydrolysed to glucose with a mixture of amyloglucosidase and fungal α-amylase. Liberated glucose was treated with GOPOD reagent (glucose oxidase, peroxidase and 4-aminoantipyrine) and GOPOD buffer (*p*-hydroxbenzoic acid).

Absorbance was monitored at 510 nm for amylose and total starch samples allowing percentage amylose to be calculated. Amylopectin was calculated by subtraction of amylose from total starch. Samples were analysed in triplicate.

2.5 Estimation of grain hardness and 100 grain weight

Grain hardness was indirectly determined according to the sodium nitrate method of grain floatation (Hallgren & Murty, 1983). Sodium nitrate was dissolved in RO water to yield a solution of SG 1.300. A sample of 100 sorghum grains were weighed to give 100 grain weight, the same samples were then used for grain floatation. Grain samples were placed into the sodium nitrate solution and stirred for 30 seconds; floating kernels were removed from the solution and counted. Five replicate readings were taken for each sorghum sample.

2.6 Imaging of sorghum grain ultrastructure using Scanning Electron microscopy (SEM)

Sorghum samples were deposited onto a conductive carbon pad and then mounted on a standard 12 mm SEM stub and transferred directly to the SEM. All samples were imaged at an accelerating voltage of 5-10 kV. The microscope used was an FEI Quanta 3D 200 (FEI, Hillsboro, Oregon, USA).

2.7 Starch extraction and purification from sorghum grain samples

Sorghum starch was extracted according to the method of Beta, Corke, Rooney, & Taylor (2001). Whole sorghum grain was steeped in 0.25 % (w/v) sodium hydroxide (200 mL) for 24 h at 5°C. Steeped grains were drained and washed with 200 mL RO water, then milled in a Waring blender. Sorghum slurry was passed through a 75 µm pore size sieve; materials left on the sieve were milled again until they could pass through the sieve. The filtrate was collected in polypropylene tubes and allowed to settle for 1 h. Tubes were centrifuged at 760 x g for 10 min. The supernatant was discarded and protein (grey material) was scraped from the top of the pellet using a metal spatula; samples were washed with excess water until the pellet was white. Recovered starch was dried at 40°C for 24 h.

2.8 Starch pasting properties: Rapid Visco Analyser measurements

Pasting profiles were established for sorghum grains and extracted sorghum starches with the use of an RVA super 4 (Newport Scientific, Jessup, Maryland) using Thermocline for Windows software. Milled sorghum grain (3 g) was weighed into an aluminium beaker; to this either 25 mL RO water or 25 mL 10 mM silver nitrate (to inhibit native amylases) was added (Batey, Hayden, Cai, Sharp, Cornish, Morell, et al., 2001). Samples were stirred at 960 rpm for the first 10 seconds and 160 rpm for the remainder of the test. Samples were heated with the following temperature

253 profile: hold at 50°C for 2 min, heat to 95°C at 7.15°C/min, hold at 95°C for 12 min,
254 cool to 50°C at 9°C/min, hold at 50°C for 4 min. Samples were analysed in triplicate.
255 *2.9 Differential scanning calorimetry*
256 Samples (approximately 5 mg) were weighed into aluminium pans and dispersed in
257 15 mg RO water. To ensure homogenous sample dispersion the aluminium pans were
258 mixed overnight on a roller bed. Mixed samples were analysed using a DSC823e
259 differential scanning calorimeter (Mettler-Toldeo, Greifensee, Switzerland). Samples
260 were measured between 10°C and 95°C (temperature ramp of 10°C/min).
261 *2.10 Mashing schedules*
262 Brewing liquor (reverse osmosis water; RO) supplemented with potassium
263 metabisulphite (1 g/kg) and calcium chloride dihydrate (2 g/kg) was heated to 50°C
264 using a water bath. Sorghum grain was milled to EBC fine grade (0.2 mm gap
265 setting) using a DFLU laboratory disc mill (Bühler Group, Uzwil, Switzerland). Grist
266 (100 g) was weighed into a metal mashing beaker and mixed with the atemperated
267 brewing liquor (300 mL). Mash pH was adjusted to pH 5.5 by addition of 10 % (w/v)
268 aqueous lactic acid. Enzymes were added as per either the low or high temperature
269 mashing regimes (Figure 1) and the mashing beakers were added to a bench top mash
270 bath (1-cube R12, Havlickuv Brod, Czech Replublic). The 1-cube mash bath was
271 preheated to 50°C prior to mashing; upon sample addition a temperature profile was
272 selected according to the enzyme system being used (Figure 1). Mash was stirred at
273 the Hartong speed setting as the Congress setting was insufficient to stir the mash.
274 Beakers were covered with aluminium foil for the duration of the mash to minimise
275 evaporation.

After mashing, samples were placed immediately into a 20°C water bath and allowed to cool for 20 min. Cooled samples were made up to a standard weight of 700 g with RO water.

2.11 Standard wort (and fermented wort) analyses

2.11.1 Wort run-off volume after 10 minutes

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

2.11.2 Analysis of wort turbidity

Wort haze was measured using a Vos Rota turbidity meter (Haffmans, Venlo, Netherlands). The Vos Rota chamber was rinsed and filled with RO water. Glass cuvettes (60 mm diameter) were filled with filtered wort sample, capped and placed into the Vos Rota chamber. 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 appropriately to fit within the calibrated range of the device.

2.11.3 Wort Colour

Wort colour was determined according to Analytica-EBC method 4.7.1. (http://www.analytica-ebc.com/).

2.11.4 Specific gravity and percentage alcohol of samples

Density, specific gravity (SG) and alcohol content of wort and fermented samples were determined using an Anton Paar DMA 4500 and Alcolyzer Plus (Anton Paar,

Graz, Austria). Sample was passed through a Minisart cellulose acetate 0.45 µm syringe filter (Sartorius, Göttingen, Germany) into a 50 mL polypropylene tube. Sample (30 mL) was passed through both the DMA 4500 and Alcolyzer Plus and was equilibrated to 20.00°C before measurement..

2.11.5 Free amino nitrogen determination

The free amino nitrogen (FAN) content of samples was determined according to Analytica-EBC method 8.10 (ninhydrin method; http://www.analytica-ebc.com/). Samples absorbance values (570 nm) were compared against a glycine standard solution (2 mg/L). Samples were analysed in triplicate.

2.12 Small scale fermentation of wort

Small scale fermentations (100 mL) were conducted on worts produced using both the high and low temperature mashing regimes.

2.12.1 Yeast propagation

A metal loop was used to transfer *Saccharomyces cerevisiae* strain Bry 96 ale yeast (Siebel Institute, Chicago, Illinois, USA) from an agar slope into 10 mL autoclaved YPD media (1 % (w/w) yeast extract, 2 % peptone, 2 % glucose in RO water). The culture was incubated in a Ceromat BS-1 incubator (Sartorius) heated to 25°C and shaking at 120 rpm. After 4 days the culture was transferred to a 250 mL Erlenmeyer flask containing 90 mL YPD media. After a further 3 days the culture was transferred to a 2 L flask containing 900 mL YPD. Finally, after 4 more days the cells were harvested. Yeast slurry was centrifuged at 1,370 g in a J2-21 centrifuge (Beckman Coulter Inc, Brea, California); the supernatant was discarded and the pellet resuspended in RO water. A total yeast cell count was performed.

2.12.2 Simulated wort boiling and wort aeration

Wort was placed uncovered onto a Stuart SB162 stirring hot plate (Bibby Scientific; preheated to 300°C) and allowed to heat for 55 min, samples were then capped and heated for an additional 5 min before being removed from the heat. Samples were immediately plunged into iced water for 30 min to cool. Cooled wort (100 mL) was transferred aseptically into autoclaved 125 mL Wheaton serum bottles (containing a 12 x 4.5 mm stirrer bar) that were then sealed with a foam bung. Vessels were placed onto magnetic stirrer plates inside a cooled incubator (LMS Ltd, Sevenoaks, United Kingdom) set to 4°C and left to aerate overnight. Incubator temperature was increased to 18°C two hours before pitching.

2.12.3 Fermentation conditions

Yeast cells were pitched into wort at a rate of $1x10^6$ cells/mL/°Plato (Casey & Bamforth, 2010; Fix, 1999) before vessels were sealed with butyl rubber bungs and crimp caps. The butyl rubber bungs were then pierced with a Bunsen valve to allow CO_2 formed during fermentation to exit the vessel whilst preventing the entrance of potential contaminants. Finally, fermentation vessels were placed onto stirrer plates (300 rpm) and incubated at 18°C for 236 h. Fermentation progress was monitored regularly by measuring the weight of the vessel.

3. Results & Discussion

3.1 Characterisation and analysis of sorghum samples

Measurement of 100 grain weight for each sample (Table 1) confirmed the visual observation that the two brewing sorghum cultivars (the yellow sorghums from Nigeria and Cameroon) were larger in size than the agricultural cultivars. Looking at the grain compositional analysis (Table 1), the brewing cultivars were notably lower in protein and higher in starch than the forage sorghums, confirming their value as brewing raw materials. The starch contents reported are within the broad range expected for sorghum grain (55.6-75.2 % db; Jambunathan & Subramanian, 1988), whilst the range of protein contents reported (8.5-10.6 % db) falls in a tight band relative to the overall range for sorghum cultivars (4.4-21.1 % db) suggested by Jambunathan & Subramanian (1988). The Ghanaian white sorghum had the lowest starch content of the varieties tested and a surprisngly high cellulose content (22.4% db, versus 3.6-15.2% db for the remaining samples).

Tannins are usually associated with the pigmented seed coat of the sorghum grain (Dlamini, Taylor, & Rooney, 2007). Thus, it was not surprising that the highly pigmented, red sorghum had the highest concentration of condensed tannins (measured in catechin equivalents, Table 1). However, it is interesting to note that, apart from the yellow (Nigeria) sample, all of the sorghum cultivars contained significant amounts of tannin. The tannin contents reported here are within the ranges typically quoted for sorghum cultivars (Earp, Akingbala, Ring, & Rooney, 1981). Increased tannin content in sorghum has been linked to a number of issues during brewing, mostly attributed to the ability of tannins to bind proteinaceous material. Tannins have been found to negatively impact the diastatic power of sorghum malts through amylase binding (Beta, Rooney, Marovatsanga, & Taylor, 2000).

Furthermore, tannins have been implicated in inhibition of protease activity (Elmaki, Babiker, & El Tinay, 1999); this is usually associated with poor digestibility in human or livestock diet, but could likewise result in reduced proteolysis during brewery mashing.

Based on the amount of amylose (Table 1) in the sorghum starches, all of the cultivars investigated here fell into the heterowaxy classification (Sang, Bean, Seib, Pedersen, & Shi, 2008). Waxy sorghum starch contains very little amylose (<3.5 %) compared to normal sorghum starch (>23.6 %), heterowaxy starch amylose content is intermediary between these two categories. The yellow (Nigeria) sorghum was highest in amylose content (21.4%) whilst the Mexican red sorghum had the lowest amylose content (13.0%).

α-amylase activity was only detectable at low levels in the white sorghum from Nigeria (Table 1). This is not surprising as α-amylase is mainly produced 24-36 h after the onset of germination and is not thought to be present in the grain before this (Aisien & Palmer, 1983). The activity of β-amylase was either not detectable, or present at very low level (Table 1). This finding is in agreement with the current literature which suggests β-amylase in sorghum grain is either not present or is present with limiting quantities (Taylor, Dlamini, & Kruger, 2013). In spite of the low diastatic activities identified, it was important to complete this analysis by way of context for the RVA and brewing experiments.

3.2 SEM imaging of sorghum grain samples

Scanning electron microscopy (SEM) allowed for high resolution imaging of the interior of each grain sample (e.g. Figures 2A & B). Cursory investigation of the samples by SEM showed the grains to be relatively similar (excluding overall size and shape), with all samples displaying the characteristic sorghum grain features of an

embryo, an endosperm and a pericarp-testa (the outer-coat of the grain). However, use of higher magnification SEM enabled a closer look at the detailed structures of the different cultivars. The endosperm tissue of the grains all displayed areas of tightly packed and loosely packed starch granules, defined as corneous and floury endosperm tissue respectively (Hoseney, Davis, & Harbers, 1974). However, within these structures there was noticeable variation between the grains. The two brewing cultivars possessed a clear delineation between the corneous and floury endosperm (e.g. Figure 2C), this was not evident in the other varieties. The border between corneous and floury endosperm was not clear in the red variety, with tightly packed granules transitioning gradually to a looser structure toward the centre of the caryopsis. In addition, the floury region of the red cultivar was not as loosely packed as the brewing varieties. A feature unique to the white variety from Nigeria was the presence of extensive regions of loosely packed starch granules at the periphery of the endosperm. This was interesting as floury (loosely packed) endosperm tissue is usually associated with the centre of the sorghum caryopsis (Rooney & Miller, 1981). The central region of this cultivar possessed very little observable floury endosperm tissue. The other white cultivar, from Ghana possessed little observable floury endosperm with corneous endosperm extending throughout the grain (Figure 2B).

Spherical structures were observed between the starch granules of sorghum samples (e.g. Figures 2D & E). Confocal laser scanning microscopy and fluorescent staining with Rhodamine B was used to confirm the identity of these structures as protein (data not shown). These are probably prolamins, the storage protein that accounts for 60-70 % of sorghum protein (Duodu, Taylor, Belton, & Hamaker, 2003).

In agreement with prior literature (Seckinger & Wolf, 1973), protein bodies were abundant towards the endosperm periphery, becoming less so in the corneous

endosperm and floury endosperm. In the corneous endosperm, spherical protein bodies were concentrated between starch granules (e.g. Figure 2E). Starch granules in corneous endosperm were less spherical and irregularly shaped (Figure 2E). Polygonal starch granules are thought to be formed by constriction by storage proteins caused by water loss during maturation of the caryopsis (Hoseney, Davis, & Harbers, 1974). As the starch granules become packed together, protein bodies are compacted and concentrated between starch granules.

Imaging of crudely purified sorghum starch further illustrated the close interaction between protein matrix and starch granule (Figure 2F). Many starch granules had clear indentations, with some containing protein that survived purification. The white sorghum from Ghana displayed the greatest degree of protein surviving crude starch isolation, indicating a particularly strong protein-starch interaction in this cultivar. Such interactions have the potential to hinder starch swelling and hydration during brewery mashing (Almeida-Dominguez, Suhendro, & Rooney, 1997).

3.3 Thermophysical properties of sorghum flours and extracted/purified starches

Pasting profiles of sorghum flours in water revealed key differences between the sorghum varieties investigated (Figure 3A). The pasting profile of the yellow cultivar from Nigeria closely resembled that of a barley control (not shown) and displayed the highest peak viscosity and final viscosity. Both yellow (Cameroon) and white (Nigeria) displayed low peak and final viscosities (Table 2), this was hypothesised to be due to enzyme activity within the sorghum flours, although only the White (Nigeria) sorghum contained detectable α-amylase activity (Table 1). Use of silver nitrate (10 mM) to inhibit enzymes during Rapid Visco Analyser (RVA) testing revealed a pasting profile markedly different to that obtained with water (Figure 3B & Table 2). During enzyme-inhibited RVA all sorghum flours displayed an increase in

viscosity as compared to RVA using water. This suggested the presence of enzyme activity within the sorghum flours. For silver nitrate RVA, white (Nigeria) and yellow (Cameroon) displayed pasting profiles similar to the other sorghums with the exception of the white sorghum cultivar from Ghana, which displayed a unique pasting profile (Figures 3A and 3B), with neither a clear viscosity peak nor viscosity trough being observed. The characteristic lack of a viscosity peak was observed with or without silver nitrate addition, suggesting that enzyme activity was not the cause of this feature. Lack of a clear viscosity peak in maize has been linked to poor starch granule hydration and swelling as a result of protein-starch interactions (Almeida-Dominguez, Suhendro, & Rooney, 1997). The hypothesis that protein starch interactions inhibited starch granule swelling in the white sorghum from Ghana is supported by the SEM imaging results (Figure 2F, Section 3.2). The impact of protein-starch interaction on starch granule swelling is thought to be exacerbated in material originating from the corneous endosperm due to the tightly packed condition of the starch (Almeida-Dominguez, Suhendro, & Rooney, 1997). In agreement with findings from SEM imaging, a simple floaters test for grain hardness (Table 1) suggested the white sorghum from Ghana contained the highest proportion of corneous endosperm as compared to the other sorghum samples (since increased endosperm density, reflecting a higher proportion of corneous material, will cause the grains to sink rather than float)Furthermore, RVA analysis of starch isolated from the white sorghum (Ghana) revealed a pasting profile similar to the other sorghums analysed (Figure 3C and Table 2). This suggests that poor swelling was not an indigenous characteristic of the starch in that cultivar and was instead mediated by a component removed during purification.

One of the primary issues associated with sorghum brewing is a high starch gelatinisation temperature. Use of differential scanning calorimetry revealed that all of the sorghum cultivars studied here had a gelatinsation temperature (Table 2) in excess of that expected for barley malt (62-63°C; Palmer, Etokakpan, & Igyor, 1989). The red sorghum sourced from Mexico had the lowest gelatinisation peak tempeature (68.9°C) whilst the other sorghums gelatinised at higher temperatures (peak temperature 72.9-74.5°C) Interestingly, an association was observed between starch amylose content and peak gelatinisation temperature (Tables 1 and 2). This is in agreement with the findings for rice and maize previously determined by other researchers (Knutson, 1990; Varavinit, Shobsngob, Varanyanond, Chinachoti, & Naivikul, 2003). The complex nature of starch gelatinisation is highlighted by comparison of DSC analysis of sorghum flour and sorghum starch in Table 2. Gelatinisation of isolated sorghum starches was achieved at a lower value than their counterpart sorghum flours. Swelling of starch granules is required for efficient gelatinisation, this process has been found to be restricted by interactions of starch with lipids and proteins (Debet & Gidley, 2006). The lower gelatinisation temperatures observed in isolated sorghum starches can probably be accounted for by the removal of lipids and proteins that could inhibit granule swelling.

3.4 Laboratory mashing of unmalted sorghum samples

Each of the five sorghum samples were mashed using both the high and low temperature mash schedules depicted in Figure 1. Analytical data for the resulting wort samples is presented in Table 3, alongside post-fermentation data indicating ethanol yield and fermentability when each wort was fermented at laboratory scale. Together these data enable the brewing value of the worts to be appraised, with

reference both to the efficacy of the novel low temperature mashing schedule and also to the impacts of sorghum grain composition and structure on the mashing process.

Hot water extract (HWE) is a key indicator of brewing efficiency. It represents the proportion of grist material solubilised during mashing and is calculated based on the extract content of wort (expressed in °Plato) and the amount of dry matter in the grist. The yellow (Nigerian) brewing sorghum had the highest HWE (82.6%; Table 3) using the high temperature (conventional) mash schedule. Surprisingly the other brewing cultivar from Cameroon had a lower HWE (78.6%) than two of the forage cultivars using this mash schedule. Most interestingly, the low temperature mashing schedule evened out the differences between cultivars, yielding HWE values ranging between 81.1-82.7% for all samples bar the Ghanaian white sorghum (72.9%). This probably reflects the activity of the Promalt S-LTP enzyme blend which was apparently able to convert starch to sugars at low temperature consistently and irrespective of grain protein content. The white sorghum from Ghana performed worst in terms of HWE with either mashing schedule and has previously (Section 3.2) been noted to exhibit a high proportion of corneous endosperm and strong starch-protein interactions. This presumably caused problems with starch swelling and conversion using either brewing schedule. Increased corneous endosperm has been associated with reduced saccharification during mashing as a result of strong starch-protein interactions causing inferior amylase access (Espinosa-Ramirez, Perez-Carrillo, & Serna-Saldivar, 2014). This hypothesis is corroborated by the RVA results (Table 2). Furthermore the Ghanaian sorghum had the lowest starch content of all of the samples (49.3% db; Table 1).

Whilst extract is an important economic consideration, the brewer also needs to understand the value of that extract for alcohol production through fermentation. This

515 is appraised here in terms of the individual and total amounts of fermentable sugars
516 generated in wort. Whilst some of the forage sorghums performed reasonably well in
517 terms of extract potential, the known brewing cultivars resulted in significantly higher
518 total fermentable sugars using either mashing schedule (Table 3). Interestingly, the
519 yellow Nigerian brewing cultivar gave the highest fermentable sugars yield using the
520 high temperature mash schedule, but was exceeded in this regard by the other
521 (Cameroonian) brewing variety when mashed using the low temperature regime.
522 Furthermore, all cultivars yielded higher amounts of fermentable sugars using the low
523 temperature mash schedule relative to equivalent data for the high temperature mash.
524 The profile of fermentable sugars in wort is principally determined by the enzymes
525 present and their interaction with the mash time-temperature schedule. Thus, radically
526 different profiles were obtained when comparing the two mash schedules, but
527 comparing within each schedule, there was minimal impact of cultivar on fermentable
528 sugar spectrum (Table 3). The main feature of this data set is thus the very high
529 glucose concentrations (36.8-45.5 g/L) in low temperature mashed worts, due to the
530 inclusion of an amyloglucosidase enzyme in the formulation (Amylo 300). In
531 comparison, for the high temperature mashed worts, glucose concentrations ranged
532 from 9-12.5 g/L and maltose was the major wort fermentable sugar (30.9-47.3 g/L).
533 It has been suggested that tannins can be involved in amylase binding and inactivation
534 (Okolo & Ezeogu, 1996). Review of the present data set fails to support this
535 hypothesis, with analysed tannin levels (Table 1) showing no obvious association with
536 fermentable sugars yield (Table 3). We conclude that other factors were more
537 significant in determining the yield of sugars and that tannins were not limitng on
538 amylase activity at the concentrations noted (35-74 mg/g db catechin equivalents) and
539 with the concentrations of exogenous enzymes used.

Mashing with the white variety from Nigeria produced wort comparable to the brewing cultivars in both high- and low-temperature mashing systems in terms of extract. Despite this, worts of the white sorghum from Nigeria were lower in glucose, maltose and maltotriose content. This probably resulted from incomplete hydrolysis of soluble, yet unfermentable dextrins in the wort.

Based on the current results, the high-temperature system performed optimally with the yellow cultivar from Nigeria but with reduced efficiency when acting upon the other varieties. The low-temperature enzyme system is assumed to act on ungelatinised starch, without the need for efficient starch dissolution, and it is likely that starch characteristics had a lesser impact on mashing efficiency in this case.

The Free amino nitrogen (FAN) content of worts produced (44-94 mg/L; Table 3) were comparable to published data for worts produced from 100 % unmalted sorghum grain (e.g. 51 mg/L; (Bajomo & Young, 1993)). For all cultivars the low temperature mash schedule gave marginally higher FAN contents relative to those from the high temperature mashes. However, all of these worts would likely require supplementation with additional nitrogen sources prior to fermentation as they would not provide the minimum of 100-230 mg/L FAN (dependent on wort gravity) thought to be required for efficient yeast cell fermentation (Pierce, 1987). Worts produced from the Mexican red sorghum and the white variety from Nigeria gave higher FAN worts than did the brewing cultivars. However, they would sill be considered FAN deficient relative to a barley malt wort (e.g. 158 mg/L; Bajomo & Young, 1993). Worts produced using the Ghanaian sorghum had significantly lower FAN contents as compared to other worts when using either enzyme system. Since this variety had a similar protein content to the other agricultural varieties (Table 1) a reduced wort

564 FAN content implies issues with proteolysis during mashing, which might again
565 reflect the impacts of strong starch granule-protein interactions.
566 Another characteristic of note during mashing was turbidity in worts of the
567 Cameroonian and Mexican cultivars. During high-temperature mashing of both
568 cultivars high turbidity wort was produced (Table 3); this was not observed with use
569 of the low-temperature mashing system. Wort haze can be attributed to a number of
570 causative factors, including lipid content, polyphenol-protein interactions and the
571 survival of β-glucan in the wort (Steiner, Becker, & Gastl, 2010). Interestingly, these
572 two varieties were both of characteristically low amylose content (Table 1); perhaps
573 poor amylopectin hydrolysis could have contributed to haze formation. Wort samples
574 in this research were only run through a filter paper, it is possible that turbidity may
575 not be an issue in at industrial scale using a mash filter.
576 *3.5 Laboratory scale fermentation trials*
577 Worts produced from five different sorghum cultivars were fermented at small scale
578 (100 mL). The fermentations of the low-temperature mashed worts displayed higher
579 final alcohol contents (% ABV) as compared to those of the high-temperature system
580 (Table 3) although they took significantly longer to reach attenuation (final gravity).
581 In addition, fermentations of low-temperature mashing were lower in residual extract
582 and FAN content, suggesting a proportionately greater utilisation of wort components.
583 Despite the fact that worts produced using the low-temperature system contained
584 higher amounts of fermentable sugars and FAN as compared to high-temperature
585 mashed worts, fermentation profiles showed that they fermented relatively slowly by
586 comparison (data not shown). Fermentation of worts produced from the high-
587 temperature system were mostly complete within 120 h. For low temperature mashed
588 worts fermentation was not fully attenuated even after 236 h. This was most likely

589 due to the sugar profiles of the worts. Worts produced by low-temperature mashing
590 were rich in glucose (due to the amyloglucosidase enzyme addition), which has
591 previously been linked to inhibited glucose uptake, yeast growth and slow
592 fermentation (MacGregor, Bazin, Macri, & Babb, 1999; Phaweni, O'Connor-Cox,
593 Pickerell, & Axcell, 1993). The results illustrate that simply providing a greater
594 content of fermentable sugar and FAN does not guarantee an efficient fermentation.
595 The worts of the Mexican sorghum and agricultural white sorghum (Nigeria) from
596 low-temperature mashing were of comparable fermentability and final alcohol yield to
597 those produced using brewing cultivars. This was despite them having a lower starch
598 content in the original grist (Table 1).
599 The results obtained here suggest that worts produced using the low-temperature
600 mashing system can result in fermentation alcohol yields comparable to the high-
601 temperature mashing system. In addition, the low-temperature system appeared less
602 dependant on the raw materials used. However, fermentation of the low-temperature
603 mashed worts was relatively slow, indicating a deficiency in a component required for
604 efficient fermentation or the presence of a component at inhibitory concentrations.

4. Conclusions

A novel low-temperature mashing system was shown to produce worts of comparable brewing value to those resulting from a more traditional, energy intensive, high-temperature mash. The energy savings of operating with the low temperature system would be substantial at industrial scale because i) T_{max} for the schedule was reduced from 95°C to 78°C, ii) the energy requirements of heating a mash to 95°C and then cooling it back to 65°C to saccharify the mash are removed and iii) the overall mash schedule is shorter by approximately 2 hours. Furthermore, our results offer preliminary encouragement that the novel low-temperature mashing regime compensates for some raw material quality differences and narrowed the gap in brewing performance between the use of brewing and non-brewing sorghum cultivars. It thus has the potential to facilitate broader use of locally produced sorghum varieties in brewing, although full substantiation of this is beyond the scope of the present paper. The noted issue with long, sluggish fermentation times for the low temperature mashed worts is readily solvable in brewing practice. The excellent apparent fermentability results confirm that the worts had the required alcohol yield potential, albeit that the fermentations took a long time to attenuate. Fermentation vigour would most likely be improved by i) substituting different diastatic enzyme blends for the Amylo300 (amyloglucosidase) used here. This enzyme is not the component which confers the low temperature gelatinisation property and it generates high concentrations of glucose in worts which subsequently can slow yeast glucose uptake (Phaweni, O'Connor-Cox, Pickerell, & Axcell, 1993), or ii) the use of supplementary yeast nutrients (nitrogen source, Zn^{2+}, etc.).

With regard to the impacts of cultivar composition, starch properties and ultrastructure on brewing performance it was interesting to note that with either

mashing schedule the impacts of kernel structure, and in particular evidence of strong starch-protein interactions had a far greater influence than did starch gelatinisation temperature – although the latter is more frequently used to assess likely brewing performance. Thus the noted lower gelatinisation temperature range for the red sorghum from Mexico did not offer a significant advantage in terms of extract or fermentable sugars yield. Whilst the brewing varieties were of lower protein content, protein *per se* did not correlate with mashing performance. Thus, the red sorghum contained the highest amount of protein (and tannins) but yielded respectable brewing performance, particularly when mashed using the low temperature regime. Hence our work suggests that it is the way in which protein is structured and in particular the strength of protein-starch granule interactions which most influenced brewing performance. Thus the white (Ghana) sorghum performed poorly using either mash schedule. The RVA profile represented the easiest way of identifying this sorghum as potentially problematic for brewing use.

In the present work there was no support for the hypothesis that tannin levels negatively impact on brewing performance (with the levels of exogenous enzymes used here), although this was not the main focus of the study and no sensory tests were performed on beers to evaluate the levels of astringency conferred.

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Conflict of Interest

The authors are not aware of any conflict of interest relating to publication of the enclosed material.

Bibliography

Aisien, A. O., & Palmer, G. H. (1983). The sorghum embryo in relation to the hydrolysis of the endosperm during germination and seedling growth. *Journal of the Science of Food and Agriculture, 34*(2), 113-121.

Almeida-Dominguez, H. D., Suhendro, E. L., & Rooney, L. W. (1997). Factors affecting rapid visco analyser curves for the determination of maize kernel hardness. *Journal of Cereal Science, 25*(1), 93-102.

Analytica-ebc. http://www.analytica-ebc.com/. Fachverlag Hans Carl. Accessed: 23.03.16.

Bajomo, M. F., & Young, T. W. (1993). The properties, composition and fermentabilities of worts made from 100-percent raw sorghum and commercial enzymes. *Journal of the Institute of Brewing, 99*(2), 153-158.

Batey, I. L., Hayden, M. J., Cai, S., Sharp, P. J., Cornish, G. B., Morell, M. K., & Appels, R. (2001). Genetic mapping of commercially significant starch characteristics in wheat crosses. *Australian Journal of Agricultural Research, 52*(11-12), 1287-1296.

Beta, T., Corke, H., Rooney, L. W., & Taylor, J. R. N. (2001). Starch properties as affected by sorghum grain chemistry. *Journal of the Science of Food and Agriculture, 81*(2), 245-251.

Beta, T., Rooney, L. W., Marovatsanga, L. T., & Taylor, J. R. N. (2000). Effect of chemical treatments on polyphenols and malt quality in sorghum. *Journal of Cereal Science, 31*(3), 295-302.

Casey, T. R., & Bamforth, C. W. (2010). Silicon in beer and brewing. *Journal of the Science of Food and Agriculture, 90*(5), 784-788.

Cequier-Sanchez, E., Rodriguez, C., Ravelo, A. G., & Zarate, R. (2008). Dichloromethane as a solvent for lipid extraction and assessment of lipid classes and fatty acids from samples of different natures. *Journal of Agricultural and Food Chemistry, 56*(12), 4297-4303.

Daiber, K. H. (1975). Enzyme inhibition by polyphenols of sorghum grain and malt. *Journal of the Science of Food and Agriculture, 26*(9), 1399-1411.

Debet, M. R., & Gidley, M. J. (2006). Three classes of starch granule swelling: Influence of surface proteins and lipids. *Carbohydrate Polymers, 64*(3), 452-465.

Dlamini, N. R., Taylor, J. R. N., & Rooney, L. W. (2007). The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. *Food Chemistry, 105*(4), 1412-1419.

Dufour, J. P., Melotte, L., & Srebrnik, S. (1992). Sorghum malts for the production of a lager beer. *Journal of the American Society of Brewing Chemists, 50*(3), 110-119.

Duodu, K. G., Taylor, J. R. N., Belton, P. S., & Hamaker, B. R. (2003). Factors affecting sorghum protein digestibility. *Journal of Cereal Science, 38*(2), 117-131.

Earp, C. F., Akingbala, J. O., Ring, S. H., & Rooney, L. W. (1981). Evaluation of several methods to determine tannins in sorghums with varying kernel characteristics. *Cereal Chemistry, 58*(3), 234-238.

Elmaki, H. B., Babiker, E. E., & El Tinay, A. H. (1999). Changes in chemical composition, grain malting, starch and tannin contents and protein digestibility during germination of sorghum cultivars. *Food Chemistry, 64*(3), 331-336.

Espinosa-Ramirez, J., Perez-Carrillo, E., & Serna-Saldivar, S. O. (2014). Maltose and glucose utilization during fermentation of barley and sorghum lager beers as affected by beta-amylase or amyloglucosidase addition. *Journal of Cereal Science, 60*(3), 602-609.

Fix, G. J. (1999). *Principles of brewing science: A study of serious brewing issues.* Brewers Publications.

Hallgren, L., & Murty, D. S. (1983). A screening-test for grain hardness in sorghum employing density grading in sodium-nitrate solution. *Journal of Cereal Science, 1*(4), 265-274.

Hoseney, R. C., Davis, A. B., & Harbers, L. H. (1974). Pericarp and endosperm structure of sorghum grain shown by scanning electron-microscopy. *Cereal Chemistry, 51*(5), 552-558.

Iiyama, K., & Wallis, A. F. A. (1990). Determination of lignin in herbaceous plants by an improved acetyl bromide procedure. *Journal of the Science of Food and Agriculture, 51*(2), 145-161.

Jambunathan, R., & Subramanian, V. (1988). Grain quality and utilisation of sorghum and pearl millet. In: Proceedings of the International Biotechnology Workshop. International Crops Research Institute for the Semi-Arid Tropics.

Knutson, C. A. (1990). Annealing of maize starches at elevated-temperatures. *Cereal Chemistry, 67*(4), 376-384.

Kobue-Lekalake, R. I., Taylor, J. R. N., & de Kock, H. L. (2007). Effects of phenolics in sorghum grain its bitterness, astringency and other sensory properties. *Journal of the Science of Food and Agriculture, 87*(10), 1940-1948.

Lyumugabe, F., Gros, J., Nzungize, J., Bajyana, E., & Thonart, P. (2012). Characteristics of African traditional beers brewed with sorghum malt: A review. *Biotechnologie Agronomie Societe et Environnement, 16*(4), 509-530.

MacGregor, A. W., Bazin, S. L., Macri, L. J., & Babb, J. V. (1999). Modelling the contribution of alpha-amylase, beta-amylase and limit dextrinase to starch degradation during mashing. *Journal of Cereal Science, 29*(2), 161-169.

Morrison, I. M., Asiedu, E. A., Stuchbury, T., & Powell, A. A. (1995). Determination of lignin and tannin contents of cowpea seed coats. *Annals of Botany, 76*(3), 287-290.

Novellie, L. (1981). Fermented beverages. In: Proceedings of the International Symposium on Sorghum Grain Quality. International Crops Research Institute for the Semi-Arid Tropics.

Okolo, B. N., & Ezeogu, L. I. (1996). Enhancement of amylolytic potential of sorghum malts by alkaline steep treatment. *Journal of the Institute of Brewing, 102*(2), 79-85.

Palmer, G. H., Etokakpan, O. U., & Igyor, M. A. (1989). Sorghum as brewing material. *Mircen-Journal of Applied Microbiology and Biotechnology, 5*(3), 265-275.

Phaweni, M., O'Connor-Cox, E. S. C., Pickerell, A. T. W., & Axcell, B. C. (1993). Influence of adjunct carbohydrate spectrum on the fermentative activity of a brewing strain of *saccharomyces cerevisiae*. *Journal of the American Society of Brewing Chemists, 51*(1), 10-15.

Pierce, J. S. (1987). Brown, Horace memorial lecture - the role of nitrogen in brewing. *Journal of the Institute of Brewing, 93*(5), 378-381.

Price, M. L., Vanscoyoc, S., & Butler, L. G. (1978). Critical evaluation of vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry, 26*(5), 1214-1218.

Rooney, L. W., & Miller, F. (1981). Variation in the structure and kernel characteristics of sorghum. In: Proceedings of the International symposium on sorghum grain quality. International Crops Research Institute for the Semi-Arid Tropics.

Sang, Y. J., Bean, S., Seib, P. A., Pedersen, J., & Shi, Y. C. (2008). Structure and functional properties of sorghum starches differing in amylose content. *Journal of Agricultural and Food Chemistry, 56*(15), 6680-6685.

Santos, M., Jimemez, J. J., Bartolome, B., Gomez-Cordoves, C., & del Nozal, M. J. (2003). Variability of brewer's spent grain within a brewery. *Food Chemistry, 80*(1), 17-21.

Seckinger, H. L., & Wolf, M. J. (1973). Sorghum protein ultrastructure as it relates to composition. *Cereal Chemistry, 50*(4), 455-465.

Steiner, E., Becker, T., & Gastl, M. (2010). Turbidity and haze formation in beer-insights and overview. *Journal of the Institute of Brewing, 116*(4), 360-368.

Taylor, J. R. N., Dlamini, B. C., & Kruger, J. (2013). 125th anniversary review: The science of the tropical cereals sorghum, maize and rice in relation to lager beer brewing. *Journal of the Institute of Brewing, 119*(1-2), 1-14.

Varavinit, S., Shobsngob, S., Varanyanond, W., Chinachoti, P., & Naivikul, O. (2003). Effect of amylose content on gelatinization, retrogradation and pasting properties of flours from different cultivars of Thai rice. *Starch-Starke, 55*(9), 410-415.

Table 1: Analytical data for the five sorghum cultivars used in the trial.

	Yellow (Nigeria)	**Yellow (Cameroon)**	**Red (Mexico)**	**White (Nigeria)**	**White (Ghana)**
Ash (g/100g db)	**1.8** ±0.19	**1.8** ±0.16	**2.3** ±0.18	**2.2** ±0.35	**2.5** ±0.18
Cellulose (g/100g db)	**11.7** ±3.1	**3.6** ±2.39	**13.1** ±3.28	**15.2** ±4.22	**22.4** ±0.95
Hemicellulose (g/100g db)	**7.6** ±0.60	**6.6** ±2.11	**6.9** ±2.67	**5.6** ±1.79	**4.1** ±0.31
Lignin (g/100g db)	**5.3** ±1.09	**7.4** ±1.66	**6.7** ±1.8	**5.0** ±0.95	**4.9** ±1.03
Lipid (g/100g db)	**3.1** ±0.2	**3.2** ±0.16	**2.7** ±0.15	**3.2** ±0.23	**3.9** ±0.53
Protein (g/100g db)	**9.4** ±0.06	**8.5** ±0.59	**10.6** ±0.2	**9.8** ±0.36	**10.2** ±0.30
Starch (g/100g db)	**61.7** ±6.04	**64.4** ±2.33	**58.1** ±2.39	**55.8** ±1.75	**49.3** ±0.62
TOTAL	**100.49**	**95.53**	**100.32**	**96.7**	**97.19**
Moisture content (%)	**11.2** ±0.16	**10.4** ±0.04	**14.7** ±0.13	**11.5** ±0.05	**11.4** ±0.21
Amylose (%)	**21.4** ±2.60	**14.1** ±0.00	**13.0** ±1.20	**18.4** ±3.10	**18.9** ±1.80
Floaters (%)	**94.0** ±1.00	**99.7** ±0.60	**90.3** ±3.20	**95.3** ±0.60	**32.0** ±1.00
100 grain weight	**3.94** ±0.31	**5.24** ±0.43	**2.26** ±0.07	**3.22** ± 0.28	**2.55** ±0.28
α-amylase (DU/g)	nd	nd	nd	1.66±0.24	nd
β-amylase (betamyl-3-units)	nd	0.19±0.096	0.21±0.058	0.23±0.081	nd
		Catechin equivalents mg/ g (dry weight basis)			
Tannins (Vanillin-HCl method)	nd	**48** ±27	**74** ±6	**43** ±7	**35** ±6

Results are the mean of at least triplicate independent analyses ± standard deviation

Table 2: Thermophysical properties of sorghum flours and starches according to Differential Scanning Calorimetry (DSC) and Rapid Visco Analysis (RVA) in the presence of 10 mM silver nitrate.

	Yellow (Nigeria)	Yellow (Cameroon)	Red (Mexico)	White (Nigeria)	White (Ghana)
Sorghum flour samples (DSC)					
Gelatinisation Onset (°C)	72.49	71.07	64.54	72.54	71.87
Gelatinisation Peak (°C)	77.34	76.48	71.47	76.83	76.87
Gelatinisation Endset (°C)	84.73	83.97	78.76	83.65	85.60
Sorghum flour samples (RVA)					
Time of gelatinization (s)	371	347	314	355	360
Peak Viscosity (cP)	733	1010	782	765	-
Trough Viscosity (cP)	661	786	605	723	-
Breakdown (cP)	72	224	177	41	-
Final Viscosity (cP)	1388	1617	1305	1469	1419
Total Setback (cP)	726	831	700	746	-
Sorghum starches (DSC)					
Gelatinisation Onset (°C)	70.32	68.43	63.36	69.83	69.95
Gelatinisation Peak (°C)	74.47	72.90	68.92	73.50	74.16
Gelatinisation Endset (°C)	80.28	79.14	75.58	79.35	80.79
Sorghum starches (RVA)					
Time of gelatinization (s)	325	315	274	319	322
Peak Viscosity (cP)	3928	4159	4893	3986	4068
Trough Viscosity (cP)	993	1044	1027	1229	1206
Breakdown (cP)	2935	3115	3866	2757	2862
Final Viscosity (cP)	2605	2718	2451	3137	3317
Total Setback (cP)	1612	1674	1424	1908	2111

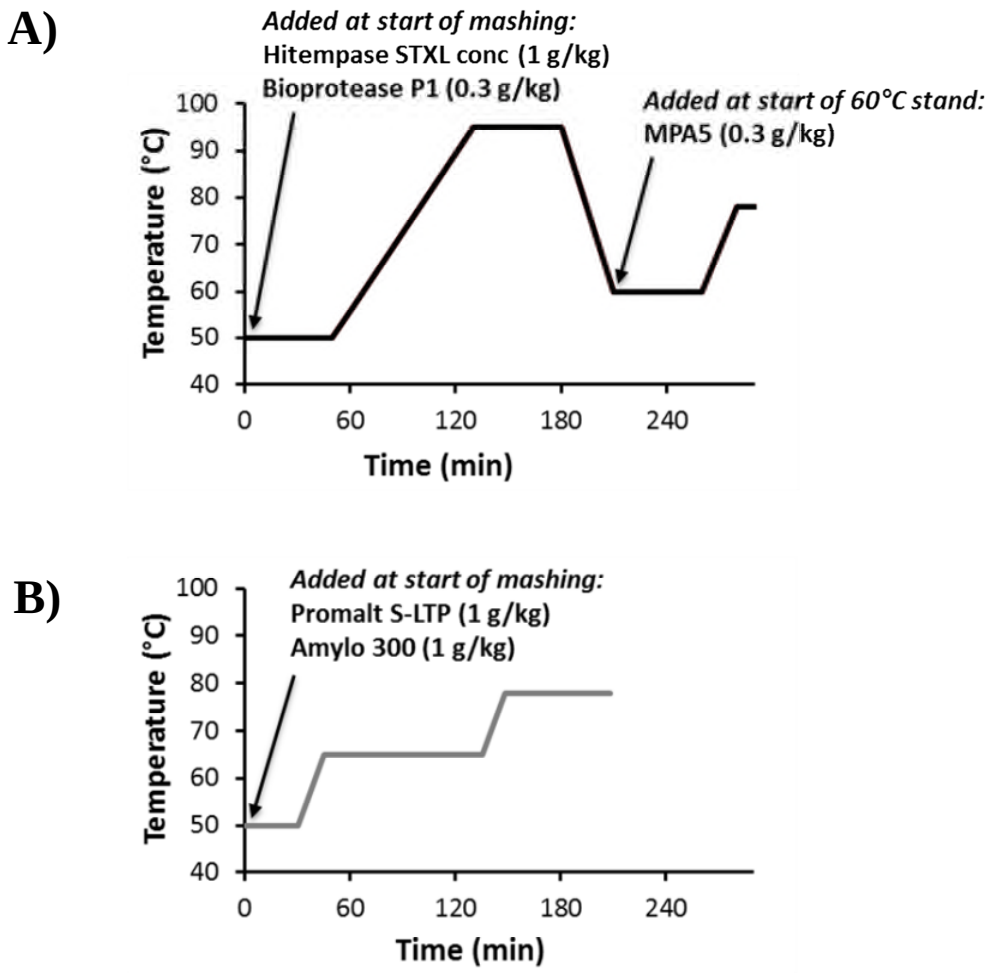
Results are the mean of triplicate analyses.

Table 3: Results to mashing and fermentation trials using five sorghum cultivars mashed using either the high temperature or low temperature mashing schedule. Standard conditions: mashing-in pH 5.5, KMS 1 g/kg, $CaCl_2 \cdot 2H_2O$ 2 g/kg, enzymes.

	HT mashing system					LT mashing system				
Wort analyses	**Yellow Nigeria**	**Yellow Cameroon**	**Red Mexico**	**White Nigeria**	**White Ghana**	**Yellow Nigeria**	**Yellow Cameroon**	**Red Mexico**	**White Nigeria**	**White Ghana**
Extract (°P)	**10.71** ±0.1	**10.34** ±0.06	**9.86** ±0.08	**10.42** ±0.34	**10.12** ±0.05	**10.62** ±0.06	**10.71** ±0.06	**10.12** ±0.08	**10.69** ±0.12	**9.55** ±0.25
Hot Water Extract (% db)	**82.6**	**78.6**	**78.8**	**80.4**	**77.7**	**81.8**	**81.7**	**81.1**	**82.7**	**72.9**
Wort colour (EBC)	**5** ±0	**6** ±1	**7** ±1	**3** ±0	**2** ±0	**5** ±0	**6** ±0	**5** ±0	**3** ±0	**2** ±0
Wort pH	**5.71** ±0.01	**5.74** ±0.01	**5.67** ±0.02	**5.80** ±0.01	**5.63** ±0.04	**5.70** ±0.01	**5.80** ±0.01	**5.71** ±0.02	**5.88** ±0.03	**5.72** ±0.04
FAN (mg/L)	**61** ±5	**70** ±1	**78** ±1	**86** ±5	**44** ±2	**63** ±1	**73** ±0	**82** ±4	**94** ±2	**49** ±1
fructose (g/L)	**0.7** ±0.1	**1.3** ±0	**0.3** ±0	**0.7** ±0.1	**0.5** ±0	**0.6** ±0	**1.1** ±0.1	nd[a]	**0.7** ±0	**0.3** ±0
glucose (g/L)	**9.9** ±0.4	**12.5** ±1.5	11.1 ±0.3	**11** ±0.7	**9** ±0.3	**45.3** ±3.2	**45.5** ±3.9	**44** ±1.3	**41.4** ±1.5	**36.8** ±1.6
maltose (g/L)	**47.3** ±2.8	**41.1** ±3.4	**37.5** ±1.4	**32.9** ±2.1	**30.9** ±1.9	**26.7** ±3	**27.6** ±1.8	**24.9** ±0.9	**26.6** ±0.9	**22.3** ±2.4
maltotriose (g/L)	**29.2** ±1	**26.7** ±2.9	**23.9** ±0.9	**21.8** ±1.7	**23.9** ±1.8	**15.3** ±2.1	**15.4** ±1.4	**11.3** ±0.2	**13** ±0.7	**12.1** ±0.3
[a]Fermentable sugars (TOTAL)	**87.1**	**81.6**	**72.8**	**66.4**	**64.3**	**87.9**	**89.6**	**80.3**	**81.7**	**71.5**
haze 25° (EBC)	**1.86** ±0.01	**8.78** ±0.19	**7.79** ±2.13	**1.9** ±0.47	**0.13** ±0.04	**1.7** ±0.2	**1.08** ±0.06	**0.63** ±0.24	**2.18** ±0.07	**0.22** ±0.07
haze 90° (EBC)	**1.12** ±0.03	**3.71** ±0.13	**2.98** ±0.72	**1.19** ±0.22	**0.25** ±0.05	**1.41** ±0.1	**2.04** ±0.22	**0.37** ±0.09	**1.09** ±0.02	**0.37** ±0.11
run-off volume (mL)	**327** ±28	**425** ±22	**319** ±19	**245** ±9	**300** ±6	**448** ±8	**443** ±23	**441** ±5	**366** ±15	**383** ±14
Post-fermentation analyses										
Alcohol content (% ABV)	**4.68** ±0.16	**4.37** ±0.59	**4.40** ±0.09	**4.17** ±0.41	**4.11** ±0.15	**4.69** ±0.25	**5.13** ±0.61	**4.71** ±0.25	**5.11** ±0.17	**4.36** ±0.12
Residual Extract (° Plato)	**2.69** ±0.06	**2.09** ±0.23	**2.04** ±0.03	**2.71** ±0.28	**2.99** ±0.1	**1.39** ±0.11	**1.55** ±0.06	**0.83** ±0.2	**1.28** ±0.04	**1.37** ±0.07
FAN (mg/L)	**27** ±0	**24** ±1	**31** ±2	**29** ±1	**16** ±4	**17** ±3	**21** ±1	**19** ±1	**19** ±8	**15** ±1
Apparent fermentability (%)	**76.8**	**81.2**	**81.0**	**75.4**	**72.4**	**87.2**	**86.1**	**92.0**	**88.7**	**86.5**

Results are the mean of triplicate independent mashes ± standard deviation. [a]sum total of fructose, glucose, maltose and maltotriose.

Figure 1: Details of A) traditional high temperature and B) novel low temperature mashing regimes used in the research, together with details of the respective exogenous enzymes added.



Enzyme preparation	Principal Activities	Enzyme source	Temperature optimum	pH optimum
Amylo 300	amyloglucosidase	*A. niger*	75	4.0
Bioprotease P1	protease	*Bacillus spp.*	70	6.0
Hitempase STXL	α-amylase	*B. lichenformis*	90	6.0
MPA 5	α-amylase	*A. oryzae*	60	6.0
Promalt S-LTP	Amylolytic and proteolytic	*GM and non-GM strains*	50-70	5.0-7.0

Figure 2 Scanning electron micrographs showing: Longitudinal cross section through an entire caryopsis of A) yellow sorghum from Nigeria and B) white sorghum from Ghana. C) the border between floury and corneous endosperm in the yellow (Nigeria) sample D) High magnification image of the floury endosperm of yellow Nigerian sorghum E) corneous endosperm of the white Ghanaian sorghum and F) a starch granule isolated from the white sorghum originating in Ghana, labelled with (i) protein body and (ii) indentation.

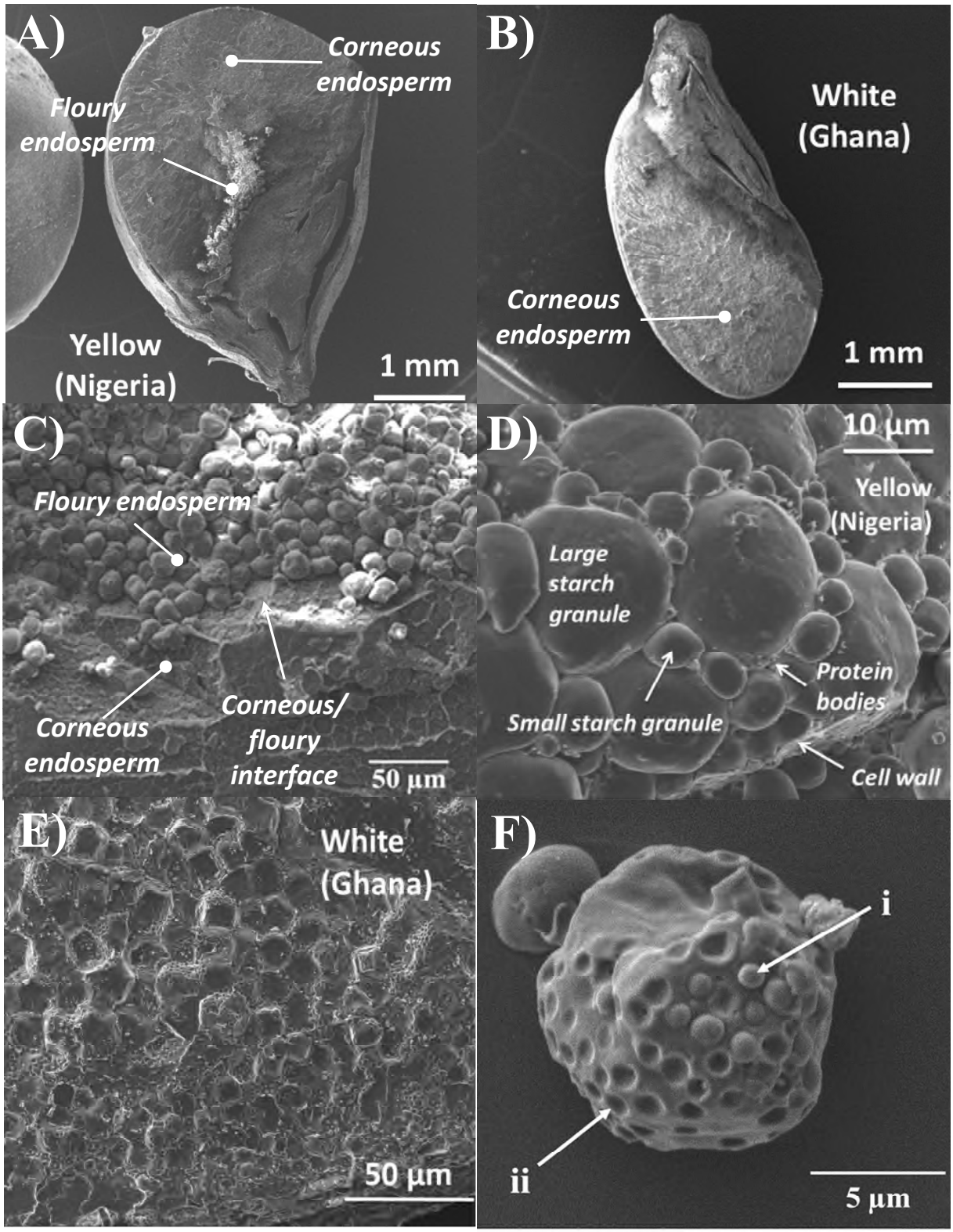
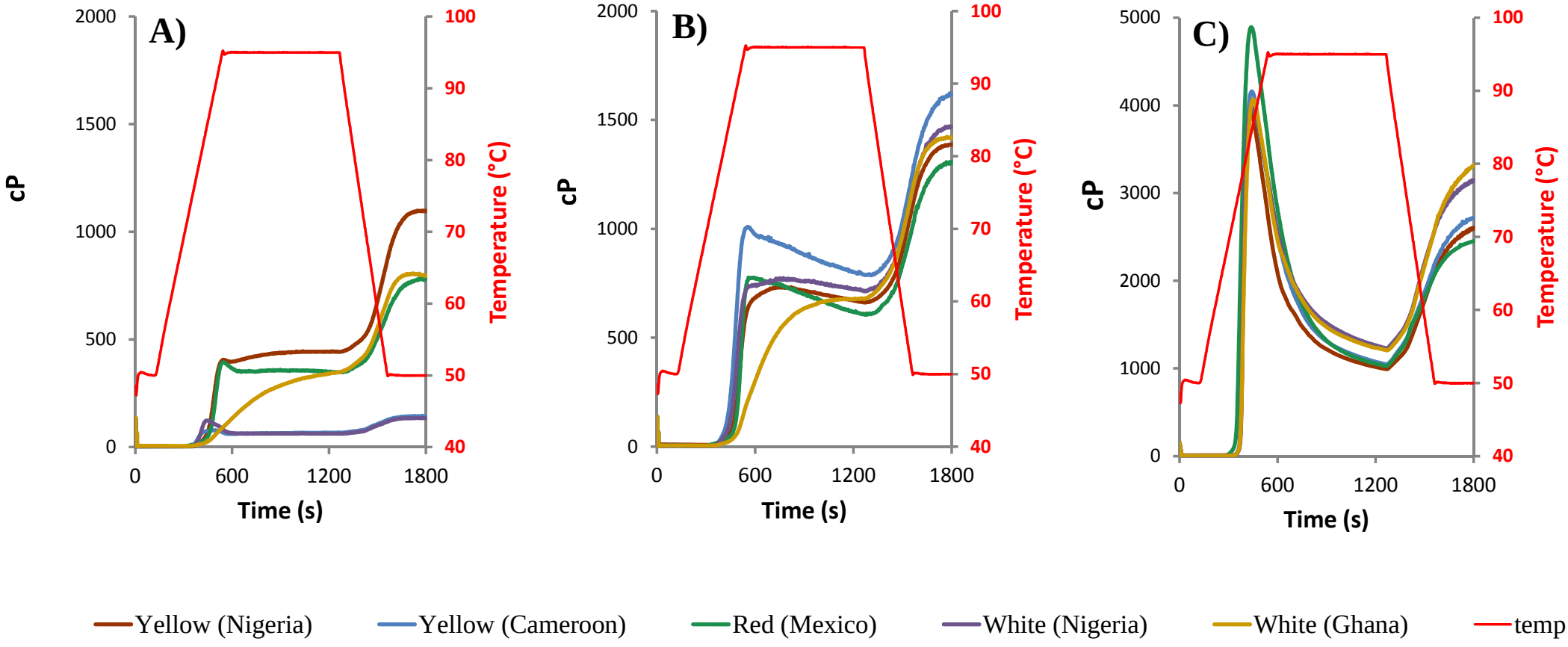


Figure 3: RVA pasting profiles of (A) sorghum flours tested in water (B) sorghum flours tested in 10 mM silver nitrate and (C) extracted and purified sorghum starches in 10 mM silver nitrate.



Results displayed are the mean of triplicate analyses.