

Pilot Scale Production of a Lager Beer from a Grist Containing 50% Unmalted Sorghum

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ABSTRACT

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Pilot scale (1000 L) brews were carried out with a grist comprising of unmalted sorghum (50% of total wet weight of grain) (South African variety) and malted barley (50% of total wet weight of grain) grist using a mashing program with rests at 50°C, 95°C and 60°C. Mashers were supplemented with a high heat stable bacterial α -amylase, a bacterial neutral protease and a fungal α -amylase. A control brew containing 100% malted barley was also carried out. Saccharification difficulties were encountered during mashing, and extraction of the grist was lower for the sorghum mashers. The sorghum mashers showed comparable lautering behaviour to that of the control mash. At mashing off the sorghum worts were starch positive. Apparent degree of fermentation of the sorghum gyles were less than the control gyles. Green beer filtration proved unproblematic. The sorghum beers compared quite closely with the control beer with regard to colour, pH and colloidal stability. Foam stability deficiencies were apparent with the sorghum beer. However, the fermentability of the sorghum worts were lower. Hence the sorghum beers were lower in total alcohol. Sensory analysis indicated that no significant differences existed between the sorghum beer and both the control beer and a commercial malted barley beer with regard to aroma, mouth-feel, after-taste and clarity. However, the sorghum beer was found to be significantly different to both of the other beers with regard to colour, initial taste and foam stability.

Key words: Enzymes, heat stable α -amylase, lauter tun, malt, sorghum, unmalted sorghum

INTRODUCTION

The production of clear lager beers from sorghum has been reported in many parts of the world. Canales and Sierra¹² reported the brewing of a lager type beer from sorghum in Mexico. Skinner⁴¹ reported the brewing of lager beer from sorghum in Botswana. A lager beer produced exclusively from sorghum was reported from Cameroon¹³, while Jayatissa *et al.*²⁴, examined varieties of sorghum from Sri Lanka, with the aim of selecting the most suitable variety for the production of a lager type beer. Etokakpan¹⁸ reported on the brewing of lager beer using

sorghum as adjunct in the United States. Perhaps the most successful developments in the brewing of lager type beers from sorghum have been made in Nigeria. In 1988 due to a government ban on the importation of malted barley, local breweries were forced to use alternative indigenous cereals such as maize and sorghum as replacements for malted barley²³.

Reviews of brewing with sorghum highlighting the technological difficulties incurred in malting and brewing of sorghum were compiled by Ogbonna³⁶ and Adejemilua¹. More recent comprehensive reviews on brewing lager beer from sorghum, particularly, malted sorghum have been compiled by Agu and Palmer² and Owuama³⁸. Due to problems associated with malted sorghum such as the development of insufficient diastatic power, limited protein modification, high malting costs, high malting losses, together with the need to supplement mashers with exogenous enzymes, it could seem more feasible to mash with unmalted grains and commercial enzymes^{3,4,8,14,21,22,30,31}.

Stewart and Hahn⁴³ used a grist of unmalted sorghum as adjunct to malted barley at 40% and discovered that it compared well with that of maize and rice. Dhamija and Singh¹⁵ suggested the use of sorghum as an adjunct to barley malt at a level of 50%. Satyanarayar and Narasimham⁴⁰ advocated the use of 40% sorghum as an adjunct in brewing and concluded that it compared very favourably with beer made from 100% malted barley. Likewise, Owuama and Okafor³⁹ advocated the use of an unmalted sorghum variety for brewing beer. Ugboaja *et al.*⁴⁴, produced a lager beer with 25% sorghum adjunct and concluded that a beer of good quality could be produced when barley malt was replaced with up to 25% unmalted sorghum.

Various mashing programs have been proposed for use with unmalted sorghum. When brewing with low levels of unmalted sorghum as adjunct to barley malt, the endogenous enzymes of the malted grain can be sufficient for complete saccharification. When brewing with high-unmalted sorghum levels, exogenous enzymes are required to ensure adequate extraction and liquefaction of the mash. This is achieved by the addition of a heat stable α -amylase²³. When mashing with unmalted sorghum and malted barley, double mash systems have been proposed³¹. When mashing with 100% unmalted sorghum, a single infusion mash, which is currently being used in the industrial production of sorghum lager beer is suggested^{21–23,28,30,31,34}. This has temperature stands at 50°C, 80–90°C, and 60°C. Various enzyme regimes have been suggested. However, Little³⁰ suggested that a heat stable α -amylase, a protease, and a fungal α -amylase are all that are needed to produce

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a beer from sorghum successfully. Goode *et al.*²², found that, when using optimised mashing conditions acceptable worts could be produced from a grist of 100% unmalted sorghum when optimised levels of a heat stable α -amylase, fungal α -amylase and a bacterial protease were incorporated into the system. Goode *et al.*²¹, also found the mashing program suitable when brewing with Nigerian white unmalted sorghum (Fara Fara variety) as adjunct to various proportions of malted barley.

Unlike malted barley, sorghum does not have a husk, and therefore, there can be filtration problems when a traditional lauter tun is used in mash separation³⁶. Okafor³⁷, Arri⁷ and Obilana³⁵ advocated that a mash having a relatively low malted barley content (<50%) may be separated in a mash filter, while Okafor³⁷ suggested the use of the lauter tun with the aid of artificial husks manufactured from nylon materials or plant fibres.

The 1988 ban on the importation of sorghum into Nigeria has since been lifted. This could lead to a proportion of malted barley been used in the production of sorghum lager beer. Also, in countries such as South Africa where sorghum (malted and unmalted), is being used in the production of traditional opaque beer and malted barley is being used in the production of lager beers, there is current interest in the production of lager beers from the locally grown sorghum crops.

The present investigation was aimed at assessing the difficulties and differences incurred in brewing a lager beer from a grist consisting of 50% unmalted sorghum (South African red variety) and 50% malted barley compared to a control beer produced from a grist of 100% malted barley beer, using a 1000 L pilot scale lauter tun as the method of mash separation. Also the resulting beers were compared using sensory taste panels.

MATERIALS AND METHODS

Materials

Unmalted sorghum (red in appearance) originating from South Africa was supplied by Quest International Ltd (Kilnagleary, Carrigaline, Co. Cork, Ireland). Malted barley (var. Cooper) (Table I) was obtained from The Malting Company of Ireland (Togher, Cork, Ireland). Mains water, treated to remove chlorine, iron and manganese, was used. The commercial enzyme preparations Hitempase 2XL (high heat stable bacterial α -amylase, IUB/EINECS/CAS no: 3.2.1.1/232-565-6/9000-90-2), Bioprotease N100-L (bacterial neutral protease, IUB/EINECS/CAS no: 3.4.24.4/232-991-1/9080-56-2) and Bioferm L (fungal α -amylase, IUB/EINECS/CAS no: 3.2.1.1/232-588-1/9001-19-8) were kindly provided by Quest International Ltd (Cork, Ireland). Details of the enzymes have

Table I. Specification of malted barley sample used in study (EBC Congress mash).

Variety	Cooper	Total soluble N	0.73% dm
Moisture	3.9%	Free amino N	161 mg/L
Extract	79% dm	Kolbach index	41.2
Colour	3.0 EBC	Friability	96.3%
Diastatic power	205 WK	Homogeneity	98.5%
Total nitrogen	1.65% dm	β -Glucan	0.25%
Protein	10.31% dm	Filtrate (30 min)	193 mL

been previously outlined by Goode *et al.*^{21,22}. Further details regarding the enzymatic side activities of the enzyme preparations are outlined in Table II. Hops were purchased from Lupex GMBH, Hallertau, Germany.

Enzyme units

Throughout this paper the enzyme amounts are expressed as units of individual enzyme activity per gram of grist. In respect to Table II the definitions of the individual enzyme activities of the various enzyme preparations are as follows:

NPU: One neutral protease unit is the quantity of enzyme required to produce the equivalent of 1 μ g of tyrosine per minute from casein in acetate buffer (pH 5.8–6.2). Casein is hydrolysed by the neutral protease at 30°C to produce smaller soluble peptide units. After 10 min the reaction is stopped and the excess casein is precipitated with trichloroacetic acid and removed by filtration. The soluble peptides are reacted with dilute Folin reagent. The optical density is determined at 660 nm. By using a standard curve, the values can be converted to μ g of tyrosine.

BG: One glucanase unit is defined as the amount of enzyme, which will produce 1 mg/mL maltose at pH 5.0 and at 50°C. β -D-glucans are hydrolysed by the β -glucanase to yield a variety of oligosaccharides. The reducing sugar groups released are reacted with 3,5 dinitro salicylic acid (DNS). The optical density is read at 540 nm and is converted into mg of reducing sugar (expressed as maltose) using a standard curve.

BAA: One bacterial α -amylase unit is the amount of enzyme, which breaks down 5.26 mg of starch per hour at pH 6.0 and 40°C (0.1 M sodium acetate buffer). The α -amylase breaks down the α -1,4 glycosidic linkages of buffered starch to yield maltose and smaller dextrins. The breakdown products are reacted with an iodine solution. This change in colour (blue to a red/brown) is followed spectrophotometrically and the end point (dextrinisation time) determined. For accuracy the dextrinisation time should take place between 10 and 20 min.

FAA: One fungal α -amylase unit is the amount of enzyme that breaks down 5.26 mg of starch per hour at pH 5.0 and 40°C. The method of determination is the same as for BAA, except the starch solution is buffered to pH 5.0

XYL: One xylanase unit is the amount of enzyme which will produce 1 μ mole of reducing sugar (xylose) per min. Xylan is reacted with xylanase to produce a reducing sugar. The reducing sugar is then reacted with 3,5 dinitrosalicylic acid. The optical density is read at 540 nm and is converted into μ moles of xylose produced, by using a standard curve.

HUT: One unit is the amount of enzyme that produces in 1 min under specified conditions a hydrolysate whose absorbance at 275 nm is the same as that of a solution containing 1.1 μ g/mL of tyrosine in 0.006 N HCl. The results are expressed as 'haemoglobin units on a tyrosine basis (HUT)'. A buffered enzyme solution is added to a buffered solution of haemoglobin at pH 4.7. The mixture is then digested at 40°C for 30 min. The excess haemoglobin is precipitated with trichloroacetic acid and removed by filtration. The quantity of solubilised haemoglobin in the filtrate is determined spectrophotometrically at 275 nm.

Table II. Enzymatic activities of the commercial enzyme preparations.

	Principal activity	Side activities
Hitempase 2 XL	α -Amylase: 120,000 BAA U/mL	β -Glucanase: trace
Bioprotease N-100L	Protease: 120,000 NPU/mL	β -Glucanase: 100 BG U/mL α -Amylase: 800 BAA U/mL Xylanase: not detected
Bioferm L	α -Amylase: 600,000 FAA U/mL	β -Glucanase: >1 BG U/mL Xylanase: 10 XLY U/mL Protease: HUT 70 U/mL Amyloglucosidase: 55 AMG U/mL

AMG: One amyloglucosidase unit is defined as the quantity of enzyme which consumes 1 μ mole of maltose per min at 40°C and at pH 4.3. Amyloglucosidase hydrolyses maltose with the formation of D-glucose. The enzyme reaction is stopped by shifting the pH with TRIS buffer to pH 7.6. The glucose formed is reacted with glucose oxidase to produce gluconate and hydrogen peroxide. The hydrogen peroxide is reacted with 4-aminophenazone together with phenol peroxidase to produce a colour compound 4-(p-benzo-quinone-mono-imino) phenazone. Colour development is measured at 500 nm.

Methods

Two 1000 L brew types were carried out in this study. The first brew type (sorghum brew) was made from a grist comprising of unmalted sorghum (50% of total wet weight of grain) and malted barley (50% of total wet weight of grain). The second brew type (control brew) was made from a grist comprising of 100% malted barley. The brews were carried out at the 1000 L Pilot Research Brewery at the Faculty of Food Science and Technology, University College Cork, Ireland. Each brew was carried out in duplicate. The sorghum brew was carried out with commercial enzymes Hitempase, Bioprotease and Bioferm all added at dosage rates of 0.1% (v/w) of grist. This corresponds to a specific enzyme activity of 120 bacterial amylase u/g of grain, 100 neutral protease u/g of grain and 600 fungal amylase u/g of grain for the enzymes Hitempase 2XL, Bioprotease N100L and Bioferm L respectively. The control brew was carried out with no commercial enzymes added.

Milling regime. 150 kg of grist (75 kg unmalted sorghum + 75 kg of malted barley) were milled in the dry 2-roller mill (Winfried Sauer, Frensdorf, Germany). The sorghum grain was milled at a mill setting of 0.125 mm. The malted barley was milled at a setting of 0.7 mm. The 2-roller milling of the sorghum grist was subjected to a plansifiteu analysis³² (Bühler GmbH, Braunschweig, Germany). These plansifiteu results were then compared with ideal milled grist size results for lauter tuns and mash filters³².

Mashing regime. The milled grist was mixed with mash-in liquor to give an initial liquor to grist ratio of 2.5:1. The mash pH was not adjusted. Mashing was carried out in a 3-vessel 1000 L brew house (Beraplan Harter GmbH; Tuchenhausen Ireland Ltd, Dublin). The bacterial heat stable α -amylase and the bacterial neutral protease enzymes were added at mashing-in (50°C) followed by a temperature rest at 50°C for 60 min. The temperature was then raised to 95°C at a rate of 1.5°C/min. The temperature was then held at 95°C for 40 min. The temperature

was then reduced to 60°C in 30 min by the addition of cold water (<5°C) to give the liquor to grist ratio of 4.2:1. The fungal α -amylase was added at this point. After a 30 min temperature stand at 60°C, the temperature was then increased to 78°C at a rate of 1.5°C/min and held for 10 min. Mashing off was performed at 78°C giving a total mashing time of 212 min.

Control mash. In order to compare results with a standard 100% malted barley brew; a 100% malted barley standard mash was performed using the following mashing programme: 50°C for 30 min, 63°C for 60 min, 75°C for 10 min and 78°C for 5 min. No commercial enzymes were added.

Analytical methods

Monitoring mash performance. The mashing vessel used was equipped with a pre-masher, mash agitator and was bottom steam jacketed. The temperature in the mash vessel was controlled by a Siemens C7 programmable logic controller (Nürnberg, Germany). Mashing performance was monitored using the following standard methods: pH, iodine saccharification test and extract of the sample¹⁹. The pH was measured using a pH meter calibrated to the temperature of the mash sample to be measured. Saccharification was measured by transferring at regular intervals a drop of the mash to a porcelain plate, cooling the sample to 20°C, and adding a drop of iodine solution. The specific gravity and °Plato (% extract w/w) of the samples were measured using a Servo Chem Automatic Beer Analyser (SCABA) (Foss Tecator, Dublin). Extract was calculated as described earlier^{21,22}.

Monitoring lautering performance. Lautering performance (8 knifed lauter tun rake) was monitored by recording, the pressure differences across the lautering plates, the lautering times and the lautering difficulties that occurred.

Wort boiling, hot trub separation, wort cooling, yeast pitching. The wort was boiled for 60 min, in a wort kettle (bottom steam jacketed and internal calandria). Hops were added in two stages. At the start of boiling 0.57 g/L Hallertau Brewers Gold 98 (9% α -acid) and 0.205 g/L Hallertau Perle 98 P45 (10% α -acid) were added to the boil. With 5 min remaining to boil 0.103 g/L Hallertau Hallertauer 98 P45 and 0.103 g/L Hallertau Perle 98 P45 were added. The boiled wort was then transferred to the whirlpool vessel (2000 L) and rested for 15 min. The wort was then cooled to a pitching temperature of 11°C using a 2-stage plate heat exchanger. Aeration of the wort was achieved. Saflager dried yeast S-189 (*Saccharomyces carlsbergensis*) (DCL Yeast Ltd, Surrey, UK), a bottom

fermenting yeast used in the production of lager and pilsen beers, was used for fermentation. Yeast (1000 g) was rehydrated in 10 L of the brew wort at a temperature of 23°C. Following a period of hydration (30 min), this was pitched into wort at a temperature of 11°C.

Fermentation. The temperature during fermentation was allowed to increase from the pitching temperature of 11°C to 13°C. Following primary fermentation and a period of warm maturation (13°C) the temperature was lowered at 1.5°C/day to -1°C. The total time of fermentation/maturation period from pitching the yeast to filtering the green beer was 28 days. Fermentation was monitored on a daily basis by taking samples and analysing them for pH, specific gravity, yeast cell count, free amino nitrogen and amino acids¹⁹.

Green beer filtration. Before filtration, yeast was removed from the cone of the cylindrical fermentation vessel (60° angle). The green beers were filtered using a diatomaceous earth (kieselguhr) filter consisting of 8 horizontal plates giving a total surface area of 0.75 m² (Della Toffola S.p.A., Treviso, Italy). A first pre-coat (0.3 kg Speedplus + 0.2 kg Arbocel) and a second pre-coat (0.6 kg Speedflow) were used. The body feed used was speedflow (Lafarge Redlands Minerals, Worksop, Nottinghamshire). Green beer kieselguhr filtration was monitored by recording the amounts of pre-coats and body feed used, the pressure differences across the filter plate, the speed of the feed pump and the amount of filtrate recovered after a set time. Following kieselguhr filtration, the beer was passed through a trap filter (10 µm) and a pre-filter (1.5 µm) (Pall Corporation, Portsmouth, United Kingdom) and then into the bright beer tank.

Packaging of the beer. The beers were transferred from the bright beer tank to the keg filler via a sterile filter (0.45 µm) (Pall Corporation, Portsmouth, United Kingdom). The filtered beers were packaged into both 30 L and 50 L kegs using a Keg-Boy C2 keg filling and cleaning/sterilising machine (GEA Till GmbH and Co., Kriftel, Germany). Later beer was bottled and crowned from the kegs into 330 mL amber coloured, long neck beer bottles. This was done using an Esau and Hueber siphon manual bottle filler and manual bottle crowner (Klaus Esau Maschinen Anlagen and Hans Hueber GmbH, Schrobenhause, Germany). The bottled beers were then used for further beer characterisation.

Beer analysis. The beers were characterised using the following analyses: alcohol, apparent degree of fermentation, starch test, colour, viscosity, free amino nitrogen, foam stability (NIBEM method) and dissolved CO₂ in bottled beers¹⁹. The haze forming potential of the beers was determined by measuring the turbidities of the beers at 0°C. The beers were then placed in a water bath at 60°C for 48 h. The samples were then cooled and kept at 0°C overnight. The turbidities of the beers were measured using a beer photometer (Dr. Bruno Lange GmbH Berlin, Dusseldorf). ΔEBC is the difference between the initial haze reading at 0°C and the final haze reading at 0°C.

Amino acid profiles of the resultant beers. This was carried out as described earlier^{21,22} using a standard method³² based on the principle of reversed phase chromatography with precolumn derivatisation and fluorescence detection.

Total soluble nitrogen. Total soluble nitrogen levels of the samples were determined using the Kjeldahl method for nitrogen determination¹⁹. A Kjelttec distillation unit was used in these determinations (Kjelttec Systems 1026, Tecator, Masons Technology Ltd, Dublin).

Maltodextrin analysis. A Waters HPLC system consisting of a 717 Autosampler, 600 Controller, 410 Differential Refractometer all controlled by Millennium software was used. The column used was a Phenomenex Resex-Oligo Column (Torrance, CA, USA). The mobile phase (eluent) was deionised water at a flow rate of 0.3 mL/min and the column was heated in a heating block at 85°C. Samples (worts, syrups etc) were diluted 1/10 and filtered through a 0.45 µm membrane filter prior to injection. The injection volume was 100 µL. Quantification of sugars was by comparison with known external standards (glucose, maltose, maltotriose etc).

Sensory analysis. Taste testing was carried out using the following standard sensory analysis methods: a paired comparison test¹⁹, in which twenty-three untrained assessors took part and a ranking test in which the sorghum beer, the control beer and a commercial beer were presented to a group of twenty-one untrained assessors. The assessors were instructed to avoid ties by making a “best guess” about adjacent samples, even if they appeared to be the same. Seven preference tests¹⁹ were carried out in order to determine the assessors’ preference among the three beers with respect to the following criteria: aroma, colour, mouth-feel, initial taste, aftertaste and overall acceptability of the product. Intensity ranking tests¹⁹ were also carried out in order to determine the tasters’ perception of the intensity of the following product attributes: foam stability and beer clarity. The commercial beer brand used in the sensory tests was a well-known, internationally available product.

Experimental procedure. Each brew was carried out in duplicate while each analysis was repeated three times. The results quoted are the mean of the repeated brews together with standard deviations.

RESULTS AND DISCUSSION

Milling

When using a lauter tun for mash filtration, a suitable grist size fraction distribution is needed for optimum sugar extraction and efficient separation of wort from grains. If the grist particle size is too coarse, sugar extraction difficulties can occur. If the grist particle size is too fine, high lauter pressure differences can occur leading to slow problematic filtration. Optimised roller milling provides a con-

Table III. Plansifiteu analysis results of unmalted sorghum milled at various grind levels.

	2-Roller mill (0.125 mm roller gap)	Lauter tun ³²	Mash filter ³²
>1.250 mm	22.98 ± 0.65	18–25	11
>1.000 mm	16.52 ± 0.59	<10	4
>0.500 mm	33.56 ± 0.46	35	16
>0.250 mm	13.75 ± 0.07	21	43
>0.125 mm	5.78 ± 0.01	7	10
<0.125 mm	7.40 ± 0.29	<12	16

trolled way of achieving an optimal grist particle size distribution²⁷. In this study a plansifiteu analysis of sorghum grists milled at different 2-roller mill grind settings was carried out, in order to determine the most suitable 2-roller distance setting when lautering with grists containing high levels of unmalted sorghum. For the purpose of milling the unmalted sorghum grain, it was concluded that the 2-roller mill grind setting of 0.125 mm was the most suitable grind. This mill grind setting was chosen because of its particle size distribution (Table III) when compared to reported³² optimal particle size distributions for malted barley (Table III).

Mashing

The mashing programme used was similar to that being used in commercial sorghum brewing as reported by MacFadden and Clayton³¹, Little³⁰, Hallgren²³, Lamidi and Burke²⁸, O'Rourke³⁴ and Goode *et al.*^{21,22}. The sorghum-mashing programme has been used commercially for the production of wort made from a grist consisting of 50% unmalted sorghum and 50% unmalted maize together with commercial enzymes. The mashing programme takes into account the fact that unmalted cereals have virtually no enzyme complement. It is designed to cater for the high gelatinisation temperatures of both sorghum and maize starches while optimising the mashing temperatures for the added microbial enzymes. The programme does not fully utilise the endogenous enzymes of malted barley. Nevertheless, the 60 min stand at 50°C does utilise the endogenous proteolytic enzymes of the malted cereal. The programme was previously deemed advantageous for mashing with increasing percentages of unmalted sorghum mixed with malted barley when exogenous enzymes are incorporated²¹.

Of particular importance is the requirement for mash cooling after the gelatinisation stand at 95°C and before the addition of the fungal α -amylase. This was achieved by the addition of cold water. Therefore, at mashing-in (50°C), a thick mash at a liquor to grist ratio of 2.5:1 was employed. With the later addition of cold water, this ratio was brought to 4.2:1. If a high gravity brew were desired then an external mash cooler would be required.

Mash pH

The pH was monitored throughout the mashing regimes. The pH for the sorghum mashes remained steady throughout the mashing regimes at 5.82. With the addition of cold water to cool the mash from 95°C to 60°C, the pH of the mashes decreased to 5.75. The pH of the control mash rose from pH 5.53 to pH 5.57 during proteolysis and then drops to pH 5.4 at the 63°C stand.

Saccharification

The saccharification test was monitored at regular intervals throughout all mashes. For the sorghum mash at the beginning of the 95°C stand, the mash was found to be starch negative. However, after 15 min at 95°C, the cooled mash (20°C) was starch positive. At the end of mashing, the sorghum mash was starch positive. The control mash was starch negative at the end of mashing. These results confirmed previous reports by Nout and Davies³³, Stark *et al.*⁴², Aisien and Muts⁵ and Aniche and Palmer⁶, whereby problems were encountered during sorghum mashing due to slow and incomplete dextrinisation and saccharification, leading to starch positive worts and hence starch positive beers. Incomplete saccharification is mainly due to either incomplete gelatinisation of the starch or insufficient amylolytic activity during subsequent liquefaction and saccharification^{11,42}. Viscoamylograph analysis of sorghum malt starches (in the absence of commercial enzymes) by Dufour *et al.*¹⁷ showed that gelatinisation of sorghum starches started around 75°C and completed at temperatures of 90–97°C (depending on the sorghum cultivar). Prior to the hold at 95°C, the mashes were starch negative, which suggested that all of the gelatinised starch was fully liquefied and saccharified. During the hold at 95°C, at this high temperature more starch was released into solution. It is possible that at this point the heat stable α -amylase had lost its activity. To achieve complete liquefaction, either a higher dose of heat stable α -amylase was required, or a portion of heat stable α -amylase could have been added during the stand at 95°C as opposed to adding all the enzyme at mash-in. In these trials calcium was not added to the mash and pH adjustment was not made. In a previous publication by Goode *et al.*²² it was shown when

Table IV. The amino acid profiles (nmol/mL) of the sorghum brew fermentation (days 0–5) and of the final filtered beer.

Sorghum brew	Typical* wort	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Final beer	Typical* beer
Aspartic acid (A,1)	301–751	845	423	400	395	177	170	192	150–375
Glutamic acid (A,1)	238–884	583	370	325	245	174	126	171	204–340
Asparagine (A)	303–1362	954	371	157	98	0	0	0	<76
Serine (A,1)	381–1332	1479	597	301	211	0	0	0	95–285
Glutamine (A,1)	68–753	224	100	90	70	0	0	0	<68
Glycine (C,2)	266–799	851	739	711	608	538	662	490	266–666
Threonine (A,1)	336–923	678	475	436	307	157	0	0	42–126
Alanine (C,2)	673–2245	2735	1434	1386	1383	1380	1361	1280	898–1347
Arginine (A,3)/amino butyric	344–2409	3287	1265	1260	1257	1175	1188	1153	657–1544
Tyrosine (C,2)	331–1104	1091	561	510	443	466	446	371	221–552
Valine (B,2)/methionine (B)	817–2262	1110	598	581	532	464	394	323	494–921
Tryptophan (C)	0–196	318	92	100	92	86	83	81	5–98
Isoleucine (B)/phenylalanine (C,2)	744–2476	1222	609	581	511	450	361	274	137–865
Leucine (B,3)	762–2287	2678	1146	1053	847	671	465	293	76–762
Lysine (A)	410–1368	1075	440	370	301	254	0	0	68–342

Results represent the absolute mean value of one wort sample, A = Group A amino acids, B = Group B amino acids, C = Group C amino acids, 1 = Class 1, 2 = Class 2, 3 = Class 3, * = typical value ranges (nmol/mL) for wort/beer produced from 100% malted barley (modified from MEBAK³²).

mashing with 100% unmalted sorghum that both the inclusion of calcium in the mash-in liquor (200 ppm) and the adjustment of the mash-in pH to 6.5 was beneficial for starch conversion. Other factors which might retard starch conversion include the mash thickness and the high tannin content of the sorghum variety. Some sorghum varieties are rich in tannin, which may interact with and inactivate enzymes¹⁰.

Extract recovery

A higher percentage extract of 78.01% (dry wt) was achieved in the control mash compared to sorghum mash at 72.91% (dry wt). It was observed that during the stand at 95°C, the mash became cloudier and lost its intense colour. This may have been due to precipitation of proteins and tannins. Hot trub formation (protein-polyphenol complexing) was happening at this stage of the brew house process as opposed to malted barley brewing where coagulable protein comes out of solution during wort boiling²⁷.

Assimilable nitrogen content of worts

It has been known for many years that the amino acid content of wort FAN is an important nutritional requirement of yeasts. The amino acids can be classified into four groupings (A, B, C and D) based on their rate and time of uptake during fermentation²⁵. Another classification system is based on their level of importance to the metabolism of the yeast (Class 1 (least important), Class 2 and Class 3 (most important))²⁶. The sorghum wort (Table IV) was found to be sufficient in all amino acids but particularly high in Group A amino acids (aspartic acid, glutamic acid, asparagine, serine and arginine/amino butyric) and Group B amino acids (leucine and isoleucine) and Group C amino acids (tyrosine, alanine, phenylalanine and tryptophan). When comparing them to the amino acid profile of the control wort, the sorghum wort is quite similar. Overall, when making comparison to the control wort (Table V), the amino acid content of the sorghum wort could be regarded as being adequate for fermentation.

Lautering

Lautering has been reported to be difficult for a number of reasons, when high levels of unmalted sorghum are used

in the mash. Some of the reasons contributing to this are the milling difficulties experienced when processing unmalted whole grain sorghum, lack of husk filtering material and also starch conversion difficulties. For the control mash, as expected, no lautering problems were experienced so the rake was not used, and high pressure differences were not observed. However for the sorghum mash, regular raking of the mash bed was needed to speed up filtration. The time taken from start of lautering to beginning of boiling for the control brew was 120 min (1100 L collected), whereas for the sorghum mash, the time taken was 130 min (880 L collected). Normal lautering times reported from industry are 120 min total. Considering that this was filtration of a mash containing 50% unmalted sorghum it might be acceptable in a commercial environment.

Fermentation performance

During fermentation, temperature, pH, specific gravity, alcohol development, yeast counts, free amino nitrogen and amino acid utilisation were monitored (Table VI) to provide comparison between the fermentation performance of the sorghum wort against the control wort.

Table VI. Fermentation characteristics of gyles.

	Sorghum brew	Control brew
Apparent degree of fermentation (%)	75.43	86.17
Highest yeast cell count (cells/mL)	59.4 * 10 ⁶ ± 3.9 * 10 ⁶	64.5 * 10 ⁶
Day 4		Day 2
Original extract (°Plato)	10.98 ± 0.1	11.22 ± 0.08
Extract (°Plato)		
Day 5	3.53 ± 0.08	2.31 ± 0.04
pH		
Day 0	5.7 ± 0.05	5.4 ± 0.03
Day 5	4.29 ± 0.02	4.30 ± 0.02
Day 10	4.3 ± 0.02	4.3 ± 0.01
Free amino nitrogen (mg/L)		
Day 0	195 ± 3	252 ± 5
Day 1	119 ± 5	185 ± 5
End of FAN utilisation (mg/L)	69 ± 4	77 ± 5
Final alcohol (% v/v)	4.3 ± 0.1	4.89 ± 0.15
Days taken to reach 95% of total alcohol	Day 6	Day 5

Table V. The amino acid profiles (nmol/mL) of the malted barley control brew fermentation (days 0–5) and of the final filtered beer.

Control brew	Typical* wort	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Final beer	Typical* beer
Aspartic acid (A,1)	301–751	1023	731	714	651	525	442	357	150–375
Glutamic acid (A,1)	238–884	510	394	375	367	309	303	238	204–340
Asparagine (A)	303–1362	879	526	417	263	159	91	96	<76
Serine (A,1)	381–1332	1321	1021	821	498	268	150	131	95–285
Glutamine (A,1)	68–753	257	189	158	123	100	97	90	<68
Glycine (C,2)	266–799	1020	689	658	632	611	725	725	266–666
Threonine (A,1)	336–923	988	741	633	398	215	167	93	42–126
Alanine (C,2)	673–2245	1821	1816	1846	1721	1657	1997	1783	898–1347
Arginine (A,3)/amino butyric	344–2409	2101	2059	2076	1874	1745	2180	1946	657–1544
Tyrosine (C,2)	331–1104	1166	856	724	679	565	615	524	221–552
Valine (B,2)/methionine (B)	817–2262	1308	851	815	696	596	572	489	494–921
Tryptophan (C)	0–196	201	164	156	145	121	147	132	5–98
Isoleucine (B)/phenylalanine (C,2)	744–2476	1165	910	853	689	555	499	423	137–865
Leucine (B,3)	762–2287	2354	1602	1389	1015	727	574	510	76–762
Lysine (A)	410–1368	915	716	591	403	307	282	266	68–342

Results represent the absolute mean value of one wort sample, A = Group A amino acids, B = Group B amino acids, C = Group C amino acids, 1 = Class 1, 2 = Class 2, 3 = Class 3, * = typical value ranges (nmol/mL) for wort/beer produced from 100% malted barley (modified from MEBAK³²).

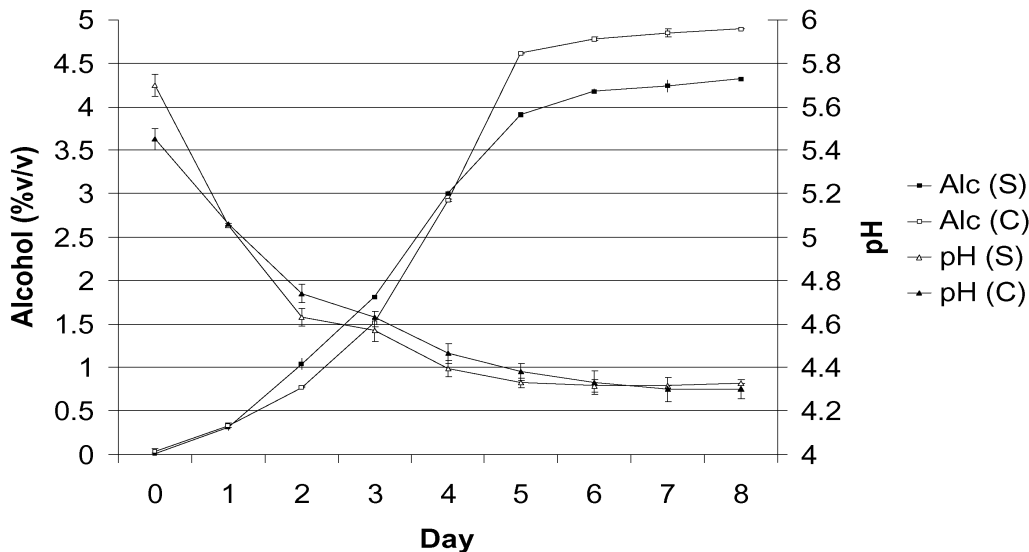


Fig. 1. Alcohol and pH profiles of the sorghum fermentation (S) and control fermentation (C) over the course of fermentation days 0–8.

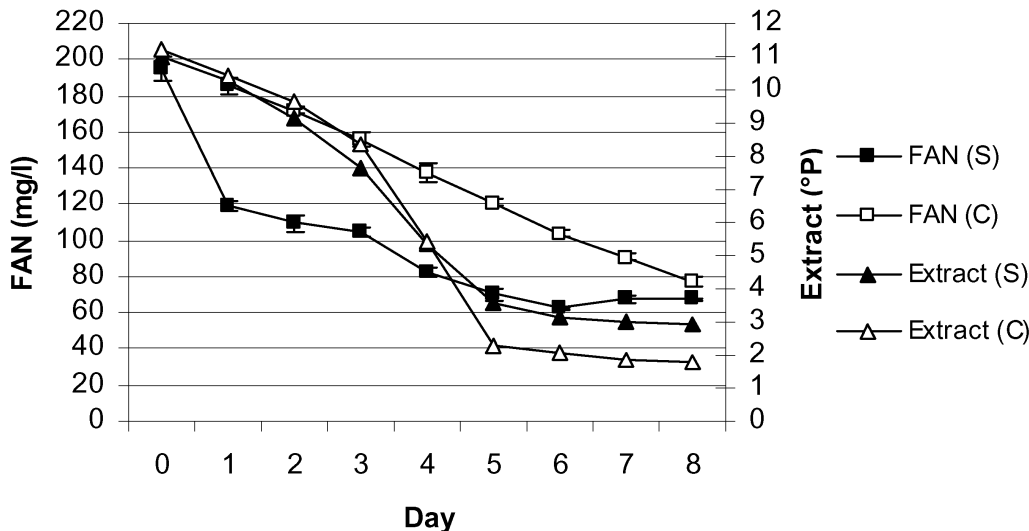


Fig. 2. Free amino nitrogen (FAN) and % extract profiles of the sorghum fermentation (S) and control fermentation (C) over the course of fermentation days 0–8.

pH

Beer fermentation is a natural acidification process²⁰. The greatest drop in pH should occur over the first 48 hr of fermentation. A rapid drop in pH can be correlated to the yeast viability and yeast growth. Wort amino nitrogen stimulates yeast growth, which in turn promotes pH decline. A low pH is desirable to inhibit gram-negative wort spoilage bacteria and also for the final flavour of the beer²⁰. With regard to pH changes during fermentation, in both the sorghum gyle and in the control gyle the pH dropped (Fig. 1). No great differences in pH were seen between the fermentations. By the 5th both gyles had reached a pH of 4.3.

Specific gravity/alcohol development

As in all healthy fermentations the % extract (°Plato) of the gyles decreased with time (Fig. 2), and the percent-

age alcohol (% v/v) increased (Fig. 1). The sorghum gyle fermentation proceeded at the same rate as the control gyle over the first 4 days with 2.94% (v/v) alcohol (specific gravity 1.021) being produced. However by the 5th day the control gyle achieved a higher alcohol content (4.61% (v/v)) against the sorghum gyle (3.91% (v/v)). Both gyles were fully attenuated by days 7–8.

Yeast cell count (cells/mL)

As previously mentioned the composition of wort is critical for a healthy yeast fermentation. Deficiencies in any of the critical components can be responsible for a sluggish fermentation. For the control gyle, the yeast cell count peaked on days 1–2 and decreased thereafter (Table VI). After day 4, the count dropped quite considerably. For sorghum fermentation, the cell count reached its highest on day 4 (59.4×10^6 cells/mL) and decreased thereafter.

Free amino nitrogen

The method used for determining free amino nitrogen gives an estimate of the amount of amino acids, ammonia, and, in addition, the terminal α -amino nitrogen groups of peptides and proteins. Proline is also partially estimated¹⁹. As the fermentation proceeded, the levels of free amino nitrogen (FAN) decreased linearly (Fig. 2). In all fermentations the FAN decreased to a minimum of 69–76 mg/L. At this point, the yeast no longer metabolised the free amino nitrogen. The control fermentation proceeded at a constant rate, using up 30–40 mg/L of FAN each day. It took the fermentation 9 days to reach a FAN level of 69–76 mg/L. The sorghum fermentation took 7 days to reach the same FAN level of 69–76 mg/L. Where all malt worts are employed, it is often found that the amount of free amino nitrogen supplied is considered greater than what the yeast will metabolise, so that substantial quantities are carried into the beer. These results show that the FAN content of the sorghum wort was nutritionally rich and contained ample FAN to satisfy the requirements of the yeast.

Amino acid utilisation during fermentation

The amino acid composition of the wort FAN is important because it affects its yeast feeding value which can have both direct and indirect implications on the rate of fermentation and the flavour profile of the resultant beer. Table IV and Table V show the amino acid compositions of the worts in nmol/mL. In the sorghum fermentation by day 5, the amino acids asparagine, serine, glutamine, histidine, threonine and lysine were fully utilised. In comparison the control fermentation (Table V), never fully utilised these amino acids and they remained in the final beer. With regard to all other amino acids, in the period after day 5 to the end of fermentation/maturation very small quantities of these amino acids were utilised.

The fermentation profiles of the sorghum gyle were quite similar to the profiles of the malted barley control gyle. The lesser degree of fermentation achieved in the sorghum brew might be improved by complete saccharification of the sorghum mash. Also, the controlled addition of an amyloglucosidase could have beneficial effects as demonstrated by Bajomo and Young⁹.

Green beer filtration

There are conflicting reports regarding the filterability of sorghum beer. Aisien and Muts⁵ reported major problems when filtering beer made from 100% malted sorghum. Dale *et al.*¹⁴ however filtered sorghum beer without difficulty. In these trials, problems were not experienced when filtering the sorghum brews.

Characteristics of final beers

Table VII shows the characteristics of the final beers. With regard to colour and viscosity, the beers compared quite closely (Table VII). However, the apparent degree of fermentation percentage differed quite considerably between the sorghum brew and the control brew. This was also seen in the alcohol percentages of both beers. It must also be remembered that the sorghum brew was starch positive.

The foam stability readings of the beers differed quite considerably. The sorghum beer displayed poor foam re-

tention ability (NIBEM 118 s) compared to the control beer (NIBEM 266 s). According to MEBAK³² (NIBEM method) a reading of <220 s for a bottom fermented beer is poor, while a reading of >300 s is very good.

The haze forming potential readings were quite low for all the beers. The sorghum beer's potential to form a haze under the test conditions was slightly higher than the control beer. In contrast to the control beer, less residual nitrogen remained in the sorghum beers. This can be a desirable factor and could help to reduce the risk of growth of contaminating microbes. With regard to the amino acid content of the beers, the control beer had the highest quantity of total amino acids (Table V) in comparison to the sorghum beers (Table IV). The sorghum beer was devoid of amino acids asparagine, serine, glutamine, histidine, threonine, and lysine (Table IV). The sorghum beers also contained a lower quantity of the remaining amino acids in comparison to the all malt control beer.

The sugar profiles of the beers (Table VIII) showed that both beers contained no glucose. In comparison to the control beer, the sorghum beers had higher remaining levels of maltose and maltotriose. The sorghum beer was higher in the dextrin fractions DP4–DP10 and >DP10 (DP = degree of polymerisation). In conventional fermentation systems the yeast utilises the glucose fractions at the start of fermentation²⁷. The main fermentation sugar utilised is maltose, while maltotriose is referred to as the secondary fermentation sugar²⁷.

Table VII. Characteristics of final beers.

	Sorghum beer	Control beer
Present gravity	1.01098 2.81 ± 0.32 ^P	1.00654 1.67 ± 0.11 ^P
Apparent degree of fermentation (%)	75.43	86
Alcohol (% v/v)	4.30 ± 0.1	4.89 ± 0.15
Calories (100 mL)	43.46	40.2
pH	4.51 ± 0.08	4.46 ± 0.05
Colour (EBC units)	5.49 ± 0.20	5.65 ± 0.10
Viscosity (mPas)	1.67 ± 0.02	1.76 ± 0.01
Starch	Positive	Negative
Nibem (s)	118	266
Dissolved CO ₂ in bottles	4.1 ± 0.1 g/L 2.09 volumes of CO ₂	4.4 ± 0.1 g/L 2.27 volumes of CO ₂
Total soluble nitrogen (mg/L)	478 ± 32	716 ± 44
Haze forming potential (ΔEBC)	0.8 ± 0.05	0.215 ± 0.02

Table VIII. Sugar spectrum of final beers.

	Sorghum beer (g/100 mL)	Control beer (g/100 mL)
Glucose	0.000	0.000
Maltose	0.166	0.089
Maltotriose	0.331	0.210
DP4	0.808	0.249
DP5	0.205	0.051
DP6	0.226	0.089
DP7	0.268	0.157
DP8	0.193	0.141
DP9	0.181	0.132
DP10	0.134	0.092
>DP10	1.706	1.466

DP = Degree of polymerisation.

Sensory analysis of beers

The paired comparison test revealed that ten assessors preferred the sorghum beer, while thirteen preferred the control beer. Referring to EBC 13.6¹⁹, the control beer was not significantly preferred to sorghum beer.

A series of ranking tests (EBC 13.11)¹⁹ were carried out. From the ranking tests no significant differences were found between the sorghum beer, the control beer and the commercial beer samples with regard to aroma. The beers were judged to have significantly different colours ($p < 0.05$). The colour of sorghum beer was preferred over the colour of the control beer ($p < 0.01\%$). There were no significant differences observed between the three beers samples with respect to mouth-feel. With regard to the initial taste of the sorghum beer and the control beer samples, they were found to differ significantly ($p < 0.01$). However with regard to initial taste the sorghum beer and the commercial beer were not judged to differ significantly. No significant differences were observed between the samples with regard to aftertaste. With regard to overall acceptability, the 3 beers did not differ significantly ($p < 0.05$).

With regard to the visually perceived foam stability of the beers, the three samples were significantly different ($p < 0.01$). The commercial beer was judged to have superior foam stability to the sorghum beer and the control beer ($p < 0.01$), while the control beer was judged to have a superior foam stability to the sorghum brew ($p < 0.05\%$). No significant differences were observed between the 3 samples with regard to clarity. Overall, the sensory results were quite favourable towards the sorghum beer. With the exception of foam stability it was not found to differ significantly with the commercial beer or the control beer.

CONCLUSION

As previously stated the sorghum-mashing program is designed specifically to cater for the higher gelatinisation temperature of sorghum and the optimum temperatures of the commercial enzymes. From the extract amounts developed in the mashes containing 50% unmalted sorghum and 50% malted barley it can be concluded that this mashing procedure is effective in extracting soluble material from both the sorghum and the malted barley. Earlier results by Goode *et al.*²¹ show that the mash extraction/conversion can be improved further. This mashing regime also means that only one mash vessel is needed throughout mashing, in comparison to double mash systems where two mashing vessels are required.

In comparison to the control mash, the lautering rates of the sorghum mashes were reduced. However, the result confirmed the reports and opinions of others that at a level of 50% sorghum grain in the grist, the use of a lauter tun is possible. Okafor³⁷, Arri⁷ and Obliana³⁵ have all advocated the use of a mash filter when separating mashes consisting of >50% sorghum.

By combining the technical data of the pilot brews with the analytical results for the beers, together with the sensory results, it can be concluded that a lager beer of comparable quality to a 100% malted barley beer could be produced without difficulty from a grist containing 50% unmalted sorghum and 50% malted barley.

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