

Mashing Studies with Unmalted Sorghum and Malted Barley

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ABSTRACT

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The effects on wort quality when mashing with unmalted sorghum (0–100%) and malted barley (100–0%) in combination with industrial enzymes were evaluated. A mashing program with temperature stands at 50°C, 95°C and 60°C was used. Different combinations of commercial enzymes were evaluated. A heat stable α -amylase was found to be essential for efficient saccharification. The inclusion of a fungal α -amylase in mashes with a high sorghum content improved filtration rates to that of 100% malted barley mashes. Addition of a bacterial protease increased the amount of nitrogen solubilisation and peptide degradation. An increase of the relative proportion of sorghum in the grist resulted in decreases in wort filtration, colour, viscosity, attenuation limit, free amino nitrogen, high molecular weight nitrogen, and a corresponding increase in pH ($p < 0.01$). Overall the addition of malted barley in small proportions to unmalted sorghum mashes together with commercial enzymes was found to improve the potential for brewing a high quality lager beer from unmalted sorghum.

Key words: Adjuncts, enzymes, heat stable α -amylase, malted barley, sorghum, unmalted sorghum.

INTRODUCTION

Unlike barley, which is the predominant cereal being used in the brewing industry, sorghum has the ability to grow in the semi-arid climatic regions of the world²². In Africa, sorghum grain is used to prepare bread, porridges, and beverages both alcoholic and non-alcoholic²⁸. Since ancient times, it has been used for the production of traditional African opaque beer¹¹. Due to a demand for western type clear lagers, much research was carried out in the 1970's and 1980's on the use of sorghum as a brewing material. The importance of this research was highlighted when in 1988 the Nigerian government due to economic reasons, put a ban on the importation of malted barley into Nigeria¹⁷. This forced local breweries to look at alternative indigenous cereals such as sorghum and maize as replacements for malted barley. Sorghum can be used in the lager brewing process as malt, or as a raw unmalted cereal in

the form of grits or whole grain¹⁷. It has been reported that sorghum grain has been used as an adjunct in Mexico and Latin America¹⁰. Vast quantities of the malted form are being used in Zimbabwe and South Africa in the production of African traditional opaque sorghum beer, while breweries in Nigeria are successfully producing lager beer from unmalted sorghum and unmalted maize²⁴.

Comprehensive reviews on brewing lager beer from sorghum, particularly malted sorghum have been compiled by Agu and Palmer¹ and Owuama³⁴. These show that remarkable progress has been made in investigating the factors that influence the production of sorghum malt for lager beer production. However, due to inherent problems such as insufficient diastatic power development, limited protein modification and also the need to supplement mashes with exogenous enzymes, together with variations in grains available, high malting losses, lack of malting capacity and high malting costs, it would therefore seem more favourable to mash with unmalted sorghum and commercial enzymes^{2,3,6,12,24,25}. Recent studies showed, that grits made from white waxy unmalted sorghum varieties, were suitable as an adjunct in lager beer production^{8,9,33}. MacFadden and Clayton²⁵ recommended the use of unmalted whole grain sorghum over sorghum grits, because the production of grits is expensive due to processing losses, increased energy costs and capital costs. In addition, sorghum grits are judged to produce very pale worts.

Different temperature/time programs have been proposed when mashing with unmalted sorghum. A proper liquefaction step, incorporating the high gelatinisation temperature of sorghum, resulting in a low viscosity mash is suggested^{23,25} regardless of the proportion of sorghum adjunct to barley malt. In all cases, this can only be achieved by the addition of a heat stable α -amylase¹⁷. When mashing with unmalted sorghum and malted barley, double mash systems have been proposed²⁵. These involve both a secondary sorghum mash (incorporating a proteolytic stand and a gelatinisation stand with the aid of commercial enzymes) and a malted barley primary mash (incorporating a proteolytic stand). The two mashes are then combined at 55–60°C and various temperature stands aimed at optimising temperature conditions for both the endogenous malt amylolytic enzymes and exogenous commercial enzymes (fungal α -amylase, cellulase and amyloglucosidase) are used. When mashing with 100% sorghum, a single infusion mash, which is being used industrially in the production of lager beer from unmalted sorghum and maize has been proposed^{17,21,24,25,30}. This has temperature stands at 50°C, 80–95°C and 60°C. Various cocktails of enzymes were suggested by MacFadden and Clayton²⁵.

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However, Little²⁴ suggested that a heat stable α -amylase, together with a protease and a fungal α -amylase are all that is needed to successfully produce a beer from sorghum. The 1988 ban on the importation of sorghum into Nigeria has since been lifted. This could lead to a return to a proportion of malted barley being used in the production of sorghum lager beer. Also, in South Africa, both malted and unmalted sorghum are being used in the production of traditional opaque beer, while malted barley alone is being used in the production of lager beers. In the South African lager industry, a commercial interest exists to substitute the imported malted barley for the locally grown sorghum crops.

This investigation aimed to assess the influences, that both the grist composition (unmalted sorghum and malted barley) and different commercial enzyme combinations (heat stable α -amylase, fungal α -amylase and a bacterial protease) have on the wort quality, when using a commercial type sorghum mashing program.

MATERIALS AND METHODS

Materials

Unmalted sorghum (Fara Fara variety, white in appearance, 9.75% moisture, 9.83% protein (dry wt)) originating from Nigeria was supplied by Quest International Ltd (Kilnagleary, Carrigaline, Co. Cork, Ireland). Malted barley (Cooper variety) was obtained from The Malting Company of Ireland (Togher, Cork, Ireland) (Table I). Mains water was used as the mashing liquor. The commercial enzyme preparations Hitempase 2XL, Bioprotease N100-L and Bioferm L (Table II) were kindly provided by Quest International Ltd (Kilnagleary, Carrigaline, Co. Cork, Ireland).

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 u/mL activities of the various enzymes are as follows:

Hitempase u/mL: One bacterial amylase unit is defined as the amount of enzyme, which will break down 5.26 mg of starch per hour under the conditions specified (0.1 M sodium acetate buffer, pH 6.0).

Table I. Malt specification.

| | | | |
|----------------------|-----------|------------------------------|----------|
| Moisture | 3.9% | Total Soluble Nitrogen (dry) | 0.73% |
| Extract (dry) | 79% | Free Amino N | 161 mg/L |
| Colour | 3.0 EBC | Kolbach Index | 41.2 |
| Diastatic Power | 205 WK | Friability | 96.3% |
| Total Nitrogen (dry) | 1.65% | Homogeneity | 98.5% |
| Protein (dry) | 10.31% dm | β -Glucan | 0.25% |
| Filtrate (30 min) | 193 mL | | |

Table II. Commercial enzyme preparations used in the present study.

| | Derivative Organism | Description | pH Optimum | Temperature Optima | Activity | Form |
|--------------------|-------------------------------|-------------------|------------|--------------------|------------------|--------|
| Hitempase 2XL | <i>Bacillus licheniformis</i> | α -Amylase | 4.0–8.0 | 90–95°C | 120,000 U/mL | Liquid |
| Bioprotease N-100L | <i>Bacillus subtilis</i> | Protease | 6.0 | 55°C | 100,000 NPU U/mL | Liquid |
| Bioferm L | <i>Aspergillus niger</i> | α -Amylase | 4.3–6.3 | 55–60°C | 600,000 U/mL | Liquid |

Bioprotease N100-L u/mL: One neutral protease unit (NPU) is the quantity of enzyme required to produce the equivalent of 1 μ g of tyrosine per minute from casein (calcium acetate buffer, pH 5.8 to 6.2).

Bioferm L u/mL: One fungal α -amylase unit is the amount of enzyme which breaks down 5.26 mg of starch per hour under the conditions specified in this procedure (0.1 M acetate buffer, pH 5.0, 40°C).

Methods

Six grist combinations were assessed in this study:

- 100% malted barley,
- 20% unmalted sorghum + 80% malted barley,
- 40% unmalted sorghum + 60% malted barley,
- 60% unmalted sorghum + 40% malted barley,
- 80% unmalted sorghum + 20% malted barley, and
- 100% unmalted barley.

On each of the six grist combinations, five different enzyme combinations were assessed:

- No commercial enzyme added,
- Hitempase 2XL,
- Hitempase 2XL + Bioferm L,
- Hitempase 2XL + Bioprotease N-100L, and
- Hitempase 2XL + Bioferm L + Bioprotease N-100L.

All enzymes were added at a dose rate of 0.05% of total weight of the grist (% v/w) as recommended by the enzyme supplier. This corresponds to a specific enzyme activity of 60 bacterial amylase u/g of grain, 50 neutral protease u/g of grain and 300 fungal amylase u/g of grain for the enzymes Hitempase 2XL, Bioprotease N100L and Bioferm L respectively.

Milling

Unmalted sorghum (Fara Fara) and malted barley (Cooper) were milled using a Bühler Miag Laboratory scale disc mill (Bühler GmbH, Braunschweig, Germany) set at a fine grind setting of 0.2 mm^{16,26}.

Mashing

Milled unmalted sorghum and milled malted barley were weighed into a stainless steel mashing beaker (to give a total grist weight of 100 g) and mixed with mash-in liquor (300 mL) to give a liquor:grist ratio of 3:1. The pH of the mash was not adjusted. Mashing was carried out in a standard type European Brewery Convention mash bath (LB 8 Electronic) (Funke-Dr.N.Gerber Instruments GmbH, Leipzig, Germany). The enzymes Hitempase 2XL and Bioprotease N100L 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 mash was then cooled to 60°C in 10 min. The enzyme Bioferm L

was added at this point. After a 30 min temperature stand at 60°C, the temperature was increased to 78°C and held for 10 min. Mashing-off was performed at 78°C. The total weight of the mash was made up to 500 g with distilled water.

Filtration

All mashes were filtered into graduated cylinders through Schleicher & Schuell 597½ filter paper^{16,26} (Schleicher and Schuell GmbH, Dassel, Germany). Filterability was measured as the amount of filtrate (volume) recorded after a 30 min filtration.

Wort analyses

All analyses were carried out using the standard methods of the European Brewery Convention (EBC)¹⁶ and Mitteleuropäische Brautechnische Analysenkommission (MEBAK)²⁶. Using a Servo Chem Automatic Beer Analyser (SCABA) (Foss Tecator, Dublin), the specific gravity of the wort sample was measured. From this, the wort strength as °Plato (% w/w) was calculated. The extraction percentage of the grist during mashing was then calculated from the following formula:

$$\text{Extract} = P * (W + 400) / (100 - P) (\%)$$

$$\text{Extract (dry weight)}$$

$$= (\text{Extract} * 100) / (100 - W) (\%)$$

Where,

P = specific gravity of wort sample in °Plato (%w/w)

W = moisture in the grist (%)

400 = amount of water in the mash per 100 g of grist

Following the determination of the extract of the sample and the determination of the colour value¹⁶, the wort gravity was brought to a standardised gravity of 1.048 (12°P) by the addition of distilled water, and the remaining analyses were carried out. pH¹⁶ of the wort and the free amino nitrogen level¹⁶ using the ninhydrin colourimetric test were determined. Viscosity of the wort was measured using a falling ball viscometer²⁶ (Gebrüder Haake GmbH, Karlsruhe, Germany). Fermentability of the wort was carried out according to EBC²⁶. A commercial lager bottom-fermenting brewing yeast obtained from a local brewery was used in this experiment. Total soluble nitrogen of the wort was determined using the Kjeldahl method for nitrogen determination¹⁶. A Kjeltac distillation unit (Kjeltac Systems 1026, Tecator, Masons Technology Ltd, Dublin) was used in these determinations. The amount of nitrogen solubilisation was calculated from the following equation:

$$\text{Degree of Soluble Nitrogen}$$

$$= ((N * E) / (K * 1000)) / \text{TN}$$

Where,

N = Soluble nitrogen in wort (mg/L)

E = Extract of malt (wet weight) (%)

K = Gravity of wort (°Plato %w/v)

TN = % Nitrogen of grist (dry weight)

High molecular weight nitrogen

High molecular weight nitrogen was determined using a modified version of the MEBAK procedure²⁶. Into a 150 mL Erlenmeyer flask were placed, 20 g magnesium sulphate powder (MgSO₄·7H₂O), 0.325 mL of 5 N H₂SO₄ and 20 mL wort. The flask was placed in a water bath for 1 h at 35°C. The flask was gently stirred every 10 min. The contents of the flask were filtered through a black ribbon filter paper (Schleicher and Schuell GmbH, Dassel, Germany). The flask was rinsed 4 times with 5 mL of a MgSO₄ solution (350 g MgSO₄·7H₂O + 0.5 mL concentrated sulphuric acid in 500 mL H₂O, pH 1.6). The rinse was poured through the filter paper. The filter paper was rinsed 3 times with the MgSO₄ solution. When the filter paper was completely dry, it was placed in a Kjeldahl digestion tube and a Kjeldahl nitrogen determination¹⁶ was carried out. The high molecular weight nitrogen fraction could be calculated from the following equation:

$$\text{High Molecular Weight Nitrogen (mg/L)}$$

$$= (((2 * T) - B) / 2) * (1.4 * 1000 / 40)$$

Where,

T = Sample titration (mL)

B = Blank titration (mL)

Amino acid analysis

Amino acid analysis was carried out using a standard method²⁶ based on the principle of reversed phase chromatography with precolumn derivatisation and fluorescence detection. The apparatus used was a Beckman System Gold High Performance Liquid Chromatography unit (Beckman Coulter, Inc., Ca., USA). The column used in the method was a Spherimage ODS2, 5 µm, 250 * 4.6 mm ID vertex column (order no.: B115 Y742, serial no.: QB 135) (Wissenschaftliche Gerätebau, Berlin, Germany).

Experimental procedure/statistical analysis

Each experiment was repeated four times while each analysis was carried out in duplicate. The results quoted are the mean of the four repeated experiments together with standard deviations and error bars. Statistical data analysis (Analysis of Variance, ANOVA) was performed using Microsoft SPSS program. A probability level of p < 0.01 was used throughout this study.

RESULTS AND DISCUSSION

Mashing with 100% malted barley

Addition of enzymes to all malted barley mashes using the sorghum mashing program (together with added enzymes) resulted in an increase in the extraction of solubles. An extract of 86 ± 0.4% as opposed to the extract of the congress mash of 79% (Table I) was achieved. This reflected the power of the enzymes and mashing program in extracting solubles from the malted barley. A greater degree of nitrogen solubilisation together with free amino nitrogen production was observed. This reflected the longer

proteolytic stand together with the addition of the commercial enzyme Bioprotease (bacterial protease). Dale *et al.*¹² also observed similar results when exogenous enzymes were added to all malted barley mashes. Higher colour values and higher viscosity values were observed from the sorghum-mashing program. The higher colour values may be due to non-enzymatic browning caused by maillard reactions between amino acids and sugars at the higher mashing temperatures (95°C) of the sorghum program.

Mashing with grists comprised of unmalted sorghum (20–100%) and malted barley (80–0%)

Filterability rate. When mashing at sorghum levels $\geq 20\%$ without the addition of commercial enzymes, efficient filtration was not possible. At a sorghum level of 20% in the mash, only 50 ± 10 mL of filtrate was recorded after a 2 h period (Results not shown). However with the addition of commercial enzymes, Hitempase (heat stable α -amylase), Bioprotease and Bioferm (fungal α -amylase), the filterability rate was greatly increased (Fig. 1). The amyolytic hydrolysis of starch will only occur in mashing if the starch is effectively gelatinised^{5,13,35}. Agu and Palmer² found, that enzyme additions had no effect on wort filtration or extract development, when the starch remained ungelatinised. Liquefaction of the gelatinised starch by the addition of the commercial amylase enzymes was therefore found to be necessary for mash filtration. As shown in Fig. 1 as the amount of sorghum was increased, there was a significant decrease in the filterability rate, when the enzyme Bioferm was excluded from the system ($p < 0.01$). Dale *et al.*¹² found that when sorghum was included in the grist, filtration rates were lower than in all-malt mashes. At levels $\geq 60\%$ sorghum, filtration was not possible when only Hitempase and Bioprotease were added. Therefore, when Bioferm was added to the mash system, filterability was greatly enhanced. Agu and Palmer² found that Bioferm was not active on ungelatinised starch resulting in low extraction of the grist. At sorghum levels $\geq 20\%$ with additions of the three enzymes, filtration was not found to differ significantly regardless of the increase in sorghum content. Also at sorghum levels $\geq 80\%$ the inclusion of Bioprotease in the mash system was found to significantly increase the filtration rate at those adjunct levels.

Extract development. As the amount of sorghum was increased the extract significantly decreased ($p < 0.01$), (Fig. 2). This was in agreement with the results of Dale *et al.*

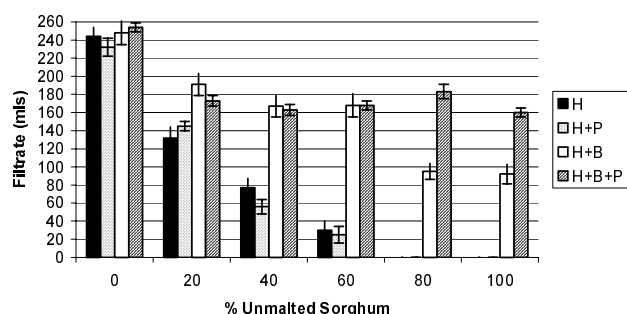


Fig. 1. The filtration rates of the different grist combinations under the influence of different enzyme combinations. H = Hitempase, P = Bioprotease, B = Bioferm.

*al.*¹² whereby at sorghum levels $>50\%$ the extract recovery decreased as the percentage sorghum was further increased. At the lower sorghum levels (20–40%) there appeared to be no significant increase in extract due to the enzyme combination used. However at higher sorghum levels (60–100%) there was a noticeable increase in extract due to the enzyme combination used. At 60% sorghum, with the addition of Bioferm, the extract increased from $74 \pm 0.8\%$ to $78 \pm 0.67\%$. The addition of Bioprotease gave an increase in extract to $79 \pm 0.71\%$ and the addition of both Bioferm and Bioprotease increased the extract to $81 \pm 0.23\%$. At 100% sorghum (Hitempase + Bioferm) there was a sharp drop in extract from $76 \pm 0.45\%$ (80% Sorghum) to $70 \pm 0.23\%$ (100% Sorghum). This was due to the fact that at 100% sorghum there were no endogenous malt proteolytic enzymes present and hence extract development was far less. In contrast, when the exogenous enzyme Bioprotease was added at 100% sorghum, an extract of $76 \pm 0.45\%$ was achieved. Dale *et al.*¹² reported with regard to enzyme additions that enzyme preparations with 1,4 α -D-glucan glucanohydrolase and proteinase activities gave increases in extract, whereas enzyme preparation with just β -glucanase and cellulase activities gave no apparent increase in extract.

pH. As the percentage sorghum was increased, the pH of the wort increased accordingly (Table III) ($p < 0.01$). This confirmed the work of others such as Dale *et al.*¹² who also used a variety of sorghum originating from Nigeria. Demuyakor *et al.*¹⁴ however found that an increase in sorghum content gave a decrease in pH. They used a variety originating from Ghana. It would therefore appear that if there is no pre-adjustment of pH at mash-in, then the pH of the resultant wort is dependent on the variety of sorghum. At 100% sorghum the pH was quite high at 6.19 ± 0.01 in comparison to the 0% sorghum wort at a pH of 5.54 ± 0.02 ($p < 0.01$). It appeared that the type of enzyme combination had no effect on the final wort pH ($p > 0.01$).

Colour. As the amount of sorghum was increased, the colour of the resultant wort significantly decreased (Table III) ($p < 0.01$). This again confirmed the work of Dale *et al.*¹². Demuyakor *et al.*¹⁴ however stated the sorghum malt filtrate was darker than that for barley malt. However in that case the colour of the grain was a red sorghum variety. Colour of the resultant wort therefore is dependent on the sorghum cultivar. In addition, it could be observed that

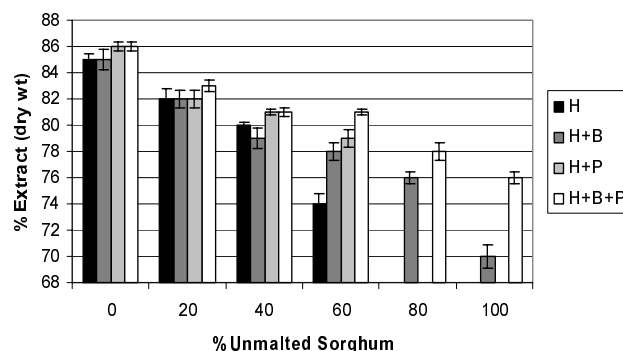


Fig. 2. The extract developed from mashes comprised of different grist combinations under the influence of different enzyme combinations. H = Hitempase, P = Bioprotease, B = Bioferm.

with the addition of the enzyme combination Hitempase and Bioprotease, the colour value of the wort increased (Table III). This may be because with the addition of Bioprotease, a greater amount of the available protein was solubilised and perhaps this yielded a darker colour due to melanoidin production at the mashing temperature of 95°C.

Viscosity. High viscosity of wort can be related to problems during wort as well as final beer filtration. The causative factors of high viscosity are usually undegraded starch and cell wall materials such as β -glucans and arabinoxylans. With regard to sorghum brewing Aisien and Muts⁴, stated, that addition of a β -glucanase caused no significant improvements during beer filtration. Demuyakor *et al.*¹⁴ found that β -glucan levels in sorghum malt extract were very low compared with barley malt extract. Etokakpan and Palmer¹⁵ observed that filtration of sorghum beer was not related to the β -glucan content but rather to its composition. For 0%, 20% and 40% sorghum mashes the viscosity of the resultant wort significantly decreased as the

amount of sorghum was increased (Table III) ($p < 0.01$). The viscosity also decreased in the presence of the commercial enzymes Hitempase and Bioprotease. It was interesting to note that at 80% sorghum (together with Hitempase, Bioferm and Bioprotease) the wort viscosity was quite low at 1.98 ± 0.08 mPas, whereas at 100% sorghum the wort viscosity increased to 2.4 ± 0.02 mPas. This could be due to the structure of the mash bed particularly since sorghum contains no husk fraction. It could also be due to the absence of malt endogenous enzymes resulting in poorer starch, proteolytic, glucan and arabinoxylan breakdown.

Soluble nitrogen. From Table IV and Fig. 3 it is clear that as the amount sorghum was increased the amount of soluble nitrogen significantly decreased (from 1045 ± 5 mg/L 20% sorghum H + P + B to 290 ± 5 mg/L 100% sorghum H + P + B) ($p < 0.01$). The degree of soluble nitrogen decreased from 46 (20% Sorghum H + P + B) to 10 (100% Sorghum H + P + B). These results are in agree-

Table III. The analytical properties of worts separated from mashes comprised of different grist combinations under the influence of different enzyme combinations.

| % Sorghum | 0 | 20 | 40 | 60 | 80 | 100 |
|---------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Viscosity (mPas) | | | | | | |
| H | 2.47 \pm 0.03 | 2.33 \pm 0.02 | 2.25 \pm 0.03 | 2.05 \pm 0.01 | x | x |
| H + B | 2.35 \pm 0.03 | 2.3 \pm 0.01 | 2.19 \pm 0.02 | 2.12 \pm 0.03 | 2.15 \pm 0.04 | 2.2 \pm 0.03 |
| H + P | 2.36 \pm 0.02 | 2.27 \pm 0.02 | 2.18 \pm 0.02 | 2.21 \pm 0.02 | x | x |
| H + B + P | 2.35 \pm 0.03 | 2.2 \pm 0.02 | 2.14 \pm 0.01 | 2.08 \pm 0.01 | 1.98 \pm 0.08 | 2.4 \pm 0.03 |
| Colour (EBC) | | | | | | |
| H | 8.5 \pm 0.12 | 6.55 \pm 0.07 | 5.85 \pm 0.06 | 5.34 \pm 0.12 | x | x |
| H + B | 9.99 \pm 0.14 | 6.37 \pm 0.12 | 5.65 \pm 0.10 | 5.26 \pm 0.04 | 4.7 \pm 0.12 | 3.72 \pm 0.12 |
| H + P | 9.05 \pm 0.08 | 8.06 \pm 0.15 | 7.15 \pm 0.05 | 6.12 \pm 0.10 | x | x |
| H + B + P | 11.37 \pm 0.2 | 7.9 \pm 0.11 | 5.94 \pm 0.08 | 5.1 \pm 0.10 | 4.0 \pm 0.02 | 4.18 \pm 0.11 |
| pH | | | | | | |
| H | 5.56 \pm 0.02 | 5.69 \pm 0.01 | 5.78 \pm 0.03 | 5.92 \pm 0.02 | x | x |
| H + B | 5.54 \pm 0.01 | 5.64 \pm 0.02 | 5.73 \pm 0.01 | 5.86 \pm 0.01 | 6.01 \pm 0.01 | 6.15 \pm 0.01 |
| H + P | 5.54 \pm 0.02 | 5.67 \pm 0.03 | 5.82 \pm 0.04 | 5.95 \pm 0.03 | | |
| H + B + P | 5.54 \pm 0.02 | 5.64 \pm 0.02 | 5.75 \pm 0.01 | 5.86 \pm 0.01 | 6.02 \pm 0.03 | 6.19 \pm 0.02 |
| % Apparent Fermentability | | | | | | |
| H + B + P | 83.74 \pm 0.4 | 80.24 \pm 0.6 | 76.3 \pm 0.2 | 72.6 \pm 0.4 | 72.12 \pm 0.6 | 34.48 \pm 2.1 |

H = Hitempase, B = Bioferm, P = Bioprotease, x = filtration impossible, no worts obtained.

Table IV. The nitrogen fractions of worts separated from mashes comprised of different grist combinations under the influence of different enzyme combinations.

| % Sorghum | 0 | 20 | 40 | 60 | 80 | 100 |
|---------------------------------------|---------------|---------------|--------------|--------------|--------------|--------------|
| Total soluble nitrogen (mg/L) | | | | | | |
| H | 1028 \pm 36 | 820 \pm 24 | 647 \pm 23 | 514 \pm 14 | x | x |
| H + B | 1036 \pm 21 | 848 \pm 32 | 653 \pm 15 | 506 \pm 18 | 352 \pm 15 | 196 \pm 21 |
| H + P | 1176 \pm 15 | 1077 \pm 35 | 835 \pm 20 | 663 \pm 30 | x | x |
| H + B + P | 1151 \pm 28 | 1045 \pm 41 | 807 \pm 14 | 627 \pm 27 | 473 \pm 31 | 290 \pm 47 |
| High molecular weight nitrogen (mg/L) | | | | | | |
| H | 204 \pm 11 | 161 \pm 3 | 136 \pm 12 | 154 \pm 4 | x | x |
| H + B | 209 \pm 6 | 169 \pm 6 | 152 \pm 11 | 129 \pm 6 | 95 \pm 2 | 69 \pm 7 |
| H + P | 210 \pm 7 | 191 \pm 11 | 166 \pm 5 | 153 \pm 3 | x | x |
| H + B + P | 209 \pm 7 | 181 \pm 8 | 145 \pm 4 | 145 \pm 7 | 113 \pm 12 | 59 \pm 6 |
| Free amino nitrogen (mg/L) | | | | | | |
| H | 201 \pm 10 | 168 \pm 2 | 121 \pm 13 | 90 \pm 6 | x | x |
| H + B | 229 \pm 9 | 194 \pm 6 | 143 \pm 12 | 104 \pm 7 | 65 \pm 7 | 38 \pm 5 |
| H + P | 254 \pm 15 | 211 \pm 7 | 169 \pm 21 | 115 \pm 2 | x | x |
| H + B + P | 266 \pm 23 | 242 \pm 4 | 190 \pm 12 | 147 \pm 1 | 101 \pm 12 | 61 \pm 2 |
| Degree of soluble nitrogen* | | | | | | |
| H | 41 | 31 | 24 | 18 | x | x |
| H + B | 41 | 32 | 24 | 18 | 12 | 6 |
| H + P | 47 | 41 | 31 | 24 | x | x |
| H + B + P | 46 | 40 | 30 | 23 | 17 | 10 |

H = Hitempase, B = Bioferm, P = Bioprotease, x = filtration impossible, no worts obtained, * = calculated from total soluble nitrogen mean values.

ment with the results of Dale *et al.*¹². The decrease in nitrogen solubilisation as a result of the increase in the amount of adjunct is not unexpected. It is expected, that addition of adjunct (without commercial enzyme) will result in a linear dilution of the barley malt soluble nitrogen. Referring to Table V (Pt1) it is clear that the addition of the enzyme Bioferm gave no increase in nitrogen solubilisation. However the addition of Bioprotease gave increases of 29–31% total soluble nitrogen (TSN) when levels of 20–60% sorghum were used (Pt2) ($p > 0.01$). When Bioferm was added (Pt3) this increase was reduced slightly perhaps due to some negative inhibition of the protease enzyme. When all three enzymes were included in the mashing experiments at levels of 20–60% unmalted sorghum the increase in TSN was 24%. At higher sorghum levels (80, 100%) the increase in TSN was 34% and 48% respectively ($p > 0.01$).

High molecular weight nitrogen. As the percentage sorghum increased there were significant decreases in the high molecular weight nitrogen fraction (HMWN). However, enzyme addition was found to have no significant effect on the HMWN values ($p > 0.01$). High molecular weight nitrogen has been associated with foam stability^{20,29}. The inclusion of sorghum at increased levels lowers the amount of HMWN. Therefore reduced foam stability may be anticipated when high levels of sorghum are used. For normal 12°P wort a HMWN value of 200–240 mg/L is required for adequate foam stability²⁶.

Free amino nitrogen. From Table IV and Fig. 3, it can be observed that as the amount of sorghum was increased, the free amino nitrogen significantly decreased from a maximum of 242 ± 4 mg/L (20% Sorghum-H + B + P) to a minimum of 61 ± 2 mg/L (100% Sorghum- H + B + P) ($p < 0.01$). A FAN content of 130–150 mg/L is regarded as being sufficient for adequate yeast metabolism³¹. With the inclusion of malt at 40%, a FAN content of 147 ± 1 mg/L was attained. However, Bajomo and Young⁵ successfully produced a beer with no fermentation defects from a 100% sorghum wort containing as little as 51 mg/L FAN. They did this over five consecutive fermentations using yeast cropped from the preceding fermentation. In contrast malted barley worts in which the FAN was diluted to a level similar to the sorghum wort, but with which the gravity was maintained by the addition of glucose, upon fermentation, fermentation difficulties were experienced. Referring to Table V, it can be seen that the addition of Bioferm at sorghum levels of 20–60% gave increases in

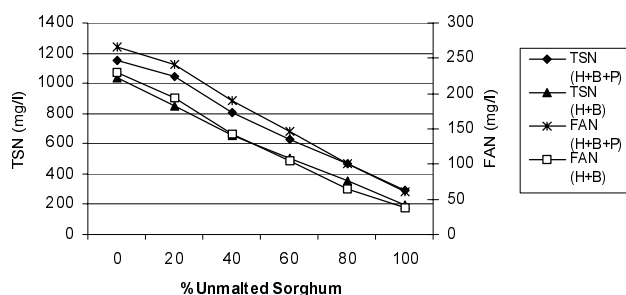


Fig. 3. The influence of Bioprotease on total soluble nitrogen values (TSN) and free amino nitrogen values (FAN). H = Hitempase, P = Bioprotease, B = Bioferm.

FAN of 15.5–18.2% (Pt5). The addition of the protease also gave a significant increase in FAN (Pt6), while the use of Bioferm and Bioprotease in combination gave a further increase in FAN (Pt7). The effect of the addition of the Bioprotease enzyme can be seen in Pt8. It is seen that the % increase in FAN is dependent on the amount of sorghum adjunct. Increasing the amount of adjunct gives a corresponding decrease in FAN levels. The increase in FAN levels observed with the addition of Bioferm may be due to an amino peptidase side activity of this enzyme or also to the fact that perhaps the amylolytic action of the enzymes makes the protein more accessible to the amino peptidase ability of the Bioprotease enzyme.

Attenuation limit. The apparent attenuation limit shown in Table III showed, that as the amount sorghum was increased the attenuation limit decreased accordingly. At 0% sorghum, the apparent attenuation value was $83.7 \pm 0.4\%$. At 80% sorghum, a value of $72.1 \pm 0.6\%$ was achieved. However, at 100% sorghum a value of $34.4 \pm 2.1\%$ was attained. This shows that with the inclusion of malted barley at a level of 20% per weight of grist the apparent attenuation limit was doubled. Bajomo and Young⁷ reported increases in the attenuation limit, when producing worts from 100% unmalted sorghum by the addition of various exogenous enzymes. When their mashes were supplemented with an enzyme preparation containing amylolytic activity, proteolytic activity, cellulolytic activity, and β -glucanase activity, an attenuation limit of 58.8% was achieved. When mashes were supplemented with an amyloglucosidase, an attenuation limit of 78.6% was attained. This therefore suggested that when brewing with 100% unmalted sorghum the addition of an amyloglucosidase is necessary for adequate attenuation.

Assimilable nitrogen composition of the extracts. The amino acid composition of the wort FAN is important because it is a nutritional requirement of yeast. Jones and Pierce¹⁸ classified amino acids into four groups based on their rate of uptake by yeast during fermentation. Group A amino acids are almost immediately absorbed from the

Table V. Percentage increase in total soluble nitrogen and free amino nitrogen levels due to addition of Bioprotease and Bioferm enzymes alone or in combination

| Total soluble nitrogen (TSN) (% increase) | | | | | | |
|---|------|------|------|------|------|------|
| % S | 0 | 20 | 40 | 60 | 80 | 100 |
| Pt 1 | 0.8 | 3.4 | 0.9 | -2 | — | — |
| Pt 2 | 14.4 | 31 | 29 | 29 | — | — |
| Pt 3 | 11.9 | 27.4 | 17.2 | 22 | — | — |
| Pt 4 | 11 | 23 | 24 | 24 | 34 | 48 |
| Free amino nitrogen (FAN) (% increase) | | | | | | |
| Pt 5 | 13.9 | 15.5 | 18.2 | 15.5 | — | — |
| Pt 6 | 26.4 | 25.6 | 39.7 | 27.8 | — | — |
| Pt 7 | 32.3 | 44 | 57 | 63 | — | — |
| Pt 8 | 16.2 | 24.7 | 32.8 | 41.3 | 55.4 | 60.5 |

S = Sorghum, H = Hitempase, B = Bioferm, P = Bioprotease

$$\text{Pt1} = 100 - [\text{TSN}(\text{H} + \text{B})] / [\text{TSN}(\text{H})] * 100$$

$$\text{Pt2} = 100 - [\text{TSN}(\text{H} + \text{P})] / [\text{TSN}(\text{H})] * 100$$

$$\text{Pt3} = 100 - [\text{TSN}(\text{H} + \text{B} + \text{P})] / [\text{TSN}(\text{H})] * 100$$

$$\text{Pt4} = 100 - [\text{TSN}(\text{H} + \text{B} + \text{P})] / [\text{TSN}(\text{H} + \text{B})] * 100$$

$$\text{Pt5} = 100 - [\text{FAN}(\text{H} + \text{B})] / [\text{FAN}(\text{H})] * 100$$

$$\text{Pt6} = 100 - [\text{FAN}(\text{H} + \text{P})] / [\text{FAN}(\text{H})] * 100$$

$$\text{Pt7} = 100 - [\text{FAN}(\text{H} + \text{B} + \text{P})] / [\text{FAN}(\text{H})] * 100$$

$$\text{Pt8} = 100 - [\text{FAN}(\text{H} + \text{B} + \text{P})] / [\text{FAN}(\text{H} + \text{B})] * 100$$

Table VI. The amino acid spectrum of worts (nmol/mL) separated from mashes containing unmalted sorghum and commercial enzymes Hitempase, Bioprotease, and Bioferm.

| % Sorghum | 0 | 20 | 40 | 60 | 80 | 100 | 100 (No P) | Typical Wort* |
|--------------------------------------|------|------|------|------|-----|-----|---------------|------------------|
| Aspartic Acid (A,1) | 729 | 235 | 575 | 594 | 544 | 360 | 521 | 301–751 |
| Glutamic Acid (A,1) | 859 | 927 | 955 | 846 | 759 | 394 | 303 | 238–884 |
| Asparagine (A) | 476 | 455 | 469 | 413 | 307 | 326 | 397 | 303–1362 |
| Serine (A,1) | 1017 | 222 | 655 | 599 | 105 | ND | 103 | 381–1332 |
| Glutamine (A,1) | 492 | 403 | 299 | 160 | ND | 0 | 0 | 68–753 |
| Histidine (B,3) | 514 | 360 | 417 | 271 | ND | ND | ND | 129–773 |
| Glycine (C,2) | 686 | 623 | 616 | 429 | 338 | 178 | 160 | 266–799 |
| Threonine (A,1) | 856 | 766 | 675 | 458 | 300 | 104 | 83 | 336–923 |
| Alanine (C,2) | 1814 | 1630 | 1562 | 1182 | 970 | 609 | 414 | 673–2245 |
| Arginine (A,3)/Amino Butyric | 2928 | 2191 | 2089 | 1308 | 588 | 602 | 575 | 344–2409 |
| Tyrosine (C,2) | 834 | 717 | 636 | 451 | 282 | 97 | 0 | 331–1104 |
| Valine (B,2)/Methionine (B) | 1045 | 925 | 645 | 549 | 354 | 113 | 53 | 817–2262 |
| Tryptophan (C) | 206 | 167 | 148 | 104 | 0 | 0 | 0 | 0–196 |
| Isoleucine (B,2)/Phenylalanine (C,2) | 1014 | 870 | 762 | 536 | 326 | 97 | 49 | 744–2476 |
| Leucine (B,3) | 1990 | 1689 | 1530 | 1099 | 680 | 252 | 89 | 762–2287 |

Results represent the absolute mean value of one wort sample, P = Bioprotease, ND = Not determined, 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 values for worts produced from 100% malted barley (modified from MEBAK).

wort. Group B amino acids are not absorbed immediately but are absorbed gradually during fermentation. Group C amino acids are only absorbed after the removal of Group A amino acids, while the group D imino acid Proline (not determined in this experiment) is only slightly absorbed under the anaerobic conditions of fermentation. They also arranged the amino acids into 3 classes depending on their levels of importance¹⁹. The initial concentration of class 1 amino acids in wort is not important since their carbon skeletons can be created by yeasts during normal metabolic activity. It is therefore not necessary for these amino acids to be present in wort. The initial concentration of the class 2 amino acids in wort are very important. Yeasts can in part find replacements, but class 2 amino acids are of such importance that changes in the concentration of this class of amino acids may considerably affect the quality of the final beer. The Class 3 amino acids are deemed critical since their skeletons are derived entirely from exogenous parent amino acids and there is little contribution from sugar synthesis. Deficiencies of these amino acids in the wort may result in major changes in the nitrogen metabolism of yeast and thus affect the final beer flavour quality.

Table VI shows the amino acid composition in nmol/mL. As the percentage sorghum was increased the quantities of all amino acids decreased, showing the overall resistance of the sorghum proteins to degradation. This was in agreement with previous studies done by Bajomo and Young⁷. In comparison to a typical wort composition, worts containing high proportions of sorghum were particularly low in the amino acids, serine (Class 1), glutamine (Class 1), tyrosine (Class 2), valine/methionine (Class 2), tryptophan (Class 3), isoleucine/phenylalanine (Class 2), leucine (Class 3). However, they were adequate in terms of the amino acids aspartic acid (Class 1), glutamic acid (Class 1), asparagine, alanine (Class 2), and arginine/aminobutyric (Class 3). The increase in leucine (Class 3) was from 89–252 nmol/mL. Interestingly, when Bioprotease was excluded from the mash, a higher amount of aspartic acid (Class 1) and glutamic acid (Class 1) was produced. However, for all other amino acids, the addition of Bioprotease increased their levels.

CONCLUSIONS

The results from the laboratory scale mashing experiments demonstrated the problems encountered in the production of wort suitable for the brewing of lager-type beer from grists containing unmalted sorghum and malted barley.

As previously stated, the mashing program was designed to cater for the high gelatinisation temperature of sorghum and the optimum temperatures of the added commercial enzymes. This mashing program in combination with the commercial enzymes was more effective in terms of extract development, nitrogen solubilisation, peptide degradation and filtration rates than the control mash in the absence of any enzymes when brewing with malted barley. Therefore, the mashing program was deemed suitable when mashing with grists containing malted barley at varying percentages. This mashing regime also meant that only one mash vessel was needed throughout mashing in comparison to the double mash system proposed by MacFadden and Clayton²⁵. This is a useful consideration for brewers, meaning less cost with regard to equipment and time and better utilisation of the existing vessels. One negative aspect of the mash program is the need for mash cooling. Industrially this could be done by the addition of a proportion of cold water to bring the temperature from 95°C to 60°C. Alternatively an external mash cooler would have to be employed.

The heat stable α -amylase Hitempase was necessary for liquefaction of the gelatinised sorghum starch and for efficient extraction. For acceptable filtration rates the inclusion of the fungal α -amylase Bioferm in the system was necessary when mashing with high levels of unmalted sorghum. With regard to extract development, the inclusion of both Bioferm and Bioprotease in mashes containing 100% unmalted sorghum gave results comparable to the control mash. Bioprotease gave increases in the amount of nitrogen solubilisation and peptide hydrolysis, while Bioferm had a positive effect on free amino nitrogen production.

Wort filtration rates encountered in the trials may not represent the true filtration behaviour of mashes during

brewhouse processing at industrial level. Due to the absence of husk in sorghum, lautering problems would be expected. However, the absence of husk would not present a problem, when a mash filter is used as the method of wort separation³². However, at high unmalted sorghum levels undegraded glucans and arabinoxylans, might give rise to filtration difficulties when using a mash filter for wort separation, since it is known that glucans and arabinoxylans increase the water-binding capacity of the spent grains.

The lower amounts of nitrogenous substances soluble in the worts containing high levels of sorghum may result in longer fermentation times and the production of beers of a different flavour and aroma profile. For example some of the factors that influence diacetyl formation are the concentrations of amino acids, valine, isoleucine and leucine²⁷. The lower levels of high molecular weight nitrogen produced from grists containing high levels of unmalted sorghum suggest, that foam stability problems may be encountered, when beers are produced from these worts. Dale *et al.*¹² suggested the addition of foam stabilising agents, while Agu and Palmer² had success in improving the foaming potential of unmalted sorghum worts by the late addition of a proportion of gelatinised sorghum grist.

The predominant use of sorghum is irreversible in Nigeria. However, with the lifting of the ban on the importation of malted barley, it seems useful to look at the addition of malted barley in small quantities to grist containing unmalted sorghum. Therefore, the addition of just 20% malted barley, led to a 100% increase in attenuation limit, 63% in total soluble nitrogen, 91% in high molecular weight nitrogen and 64% in free amino nitrogen when compared to grists containing 100% unmalted sorghum. The overall conclusion is that addition of malted barley to unmalted sorghum mashes in small quantities, together with the addition of exogenous enzymes, improves the potential for brewing high quality beers from unmalted sorghum. Also in countries where sorghum is the indigenous locally grown material and malted barley is the predominant cereal used for beer production, the inclusion of sorghum in mashes may represent a cheaper alternative without negatively affecting the end product.

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