Influence of High Protein Digestibility Sorghums on Free Amino Nitrogen (FAN) Production during Malting and Mashing

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ABSTRACT

In sorghum brewing obtaining sufficient Free Amino Nitrogen (FAN) for rapid and complete fermentation remains a problem due to the high proportions of unmalted sorghum used and the poor digestibility of wet-heat treated sorghum protein. Sorghum mutant lines with high protein digestibility (HPDS) have been developed through breeding. These HPDS have protein bodies with villi-like borders that apparently facilitate protease access. This work investigated FAN production from HPDS when malted and mashed, to assess their potential for use in sorghum brewing to improve wort FAN levels. When malted, HPDS contained substantially higher levels of FAN than normal protein digestibility sorghums (NPDS), 32 mg/100 g malt more. However, when the HPDS were mashed either as malt, or as grain or malt plus exogenous proteases, FAN production during mashing was not substantially higher than with NPDS subjected to the same treatments, only 6, 6-18 and 9-13 mg/100 g grain or malt, respectively. This is probably due to wet-heat induced cross-linking of the kafirin proteins reducing their susceptibility to proteolysis. Notwithstanding this, HPDS could be very useful for improving FAN levels in sorghum brewing if they are malted.

Key words: sorghum, malt, free amino nitrogen, mashing, exogenous proteases, protein digestibility

INTRODUCTION

In brewing, adequate levels of free amino acids and short peptides, collectively referred to as Free Amino Nitrogen (FAN) are required as a yeast nitrogen source for rapid and complete fermentation[13,17]. When brewing with sorghum this is a particular issue as the grists often comprise a low proportion of malt relative to unmalted adjunct or even 100% unmalted sorghum. Recent work on improving FAN production in sorghum brewing has focused on the use of exogenous proteases[7,12]. However, it appears that to achieve sufficient proteolysis of the sorghum proteins, excessively high levels of exogenous proteases may be required. This can be attributed to the poor digestibility of the sorghum kafirin storage protein[3], when it has been subjected to wet heat treatment, as occurs in the brewing process.

Weaver and co-workers at Purdue University identified mutant sorghum lines with improved protein digestibility[23]. These high protein digestibility sorghums (HPDS) were developed by crossing normal sorghum lines with a high-lysine sorghum mutant. More recently, research at Texas A&M University has focused on breeding to improve the agronomic and end-use quality of the HPDS, and determination of quantitative trait loci (QTLs) for HPD trait[24].

This work therefore investigated the FAN production from HPDS when malted and mashed , with the objective of assessing their potential for use in sorghum brewing to improve wort FAN levels.

MATERIALS AND METHODS

Materials

Sorghum cultivars. Normal protein digestibility sorghum (NPDS) parent (96GCPOB124) (Germinative Energy (GE) at 72 h 75%) (ICC), HPDS parent (P851171) (GE 92%) and HPDS progeny (04CS11278X 851171/96GCP0124) (GE 90%) were produced at the experimental farm of Texas A&M University, College Station, Texas, USA. An unrelated NPDS cultivar (NK8828) (GE 89%) from the Agricultural Research Council, Potchefstroom, South Africa was included as control. All the sorghum lines were white, tan-plant types.

Proteases. Commercial proteolytic enzymes Flavourzyme500 MG (an aminoprotease with 500 leucine aminopeptidase units/g) and Bioprotease P Conc. (an acid protease with 400,000 haemoglobin units/g) were kindly donated by Novozymes SA (Marlboro, South Africa) and Kerry Biosciences (Johannesburg, South Africa), respectively.

Malting

The sorghum grains were malted according to agreed standard laboratory methods[20]. Malt was dried at 50^{o}C, after which the external roots and shoots were removed by rubbing in a coarse mesh bag so that roots and shoots were broken off and fell through the mesh. The sorghum grain and malt were milled using a laboratory-scale hammer mill type(Falling Number AB, Huddinge, Sweden) fitted with a 1.6 mm opening screen. The flour was stored in ziplock-type polyethylene bags at approximately 6°C until required.

Mashing

Ten grams of milled grain or malt was weighed into a 250 ml pre-weighed Erlenmeyer flask. Twenty ml tap water pre-heated to approx. 55^{o}C and 1 ml calcium chloride solution (200 ppm) was added and the contents of the flask mixed thoroughly. The temperature of the mixture

was kept at 55°C in a shaking water bath. In those treatments where the addition of exogenous proteases was investigated, 1 ml enzyme solution prepared to give a ratio of 1:100 (w/w) enzyme to flour was added and then mixed. The contents were mashed at 55°C for 45 min at the natural pH of the mash, approx. pH 5.8. At the end of mashing, supernatant was removed for FAN analysis.

Analyses

Grain physical characteristics. Three replicates of 300 sorghum kernels were each used to estimate grain hardness and 1,000 kernel weight using a Single Kernel Characterization System, SKCS 4100 (Perten Instruments, Springfield, IL). Grain endosperm texture was estimated by visual examination of longitudinal sectioned half kernels[18].

Germinative Energy (GE). Determined according to ICC Draft Standard 174[9].

Transmission electron microscopy (TEM). Sorghum grains were sectioned and then prepared for and viewed by TEM as described[12]. Protein bodies in the sub-aleurone layers of the normal and high protein digestibility sorghums were compared.

Protein (N x 6.25). Determined by a Dumas procedure[1].

Lysine. Determined by acid hydrolysis of the protein into its constituent amino acids, followed by reverse-phase HPLC[2], using fluorescence detection. Human serum albumin was used as control and norvaline and sarcosine were used as internal standards.

***In vitro* protein digestibility.** Determined on the sorghum grain and malt samples using the pepsin method of Hamaker et al.[8], where the solubilisation of the protein is measured after incubation at 37°C and pH 2.0 for 2 hours. After incubation, the digest is centrifuged, the supernatant discarded, and the quantity of protein the insoluble residue is determined. Protein

digestibility is calculated protein solubilized (total protein in grain or malt minus residual protein) divided by total protein, and expressed as a percentage.

FAN. Determined by the European Brewery Convention ninhydrin assay using glycine as standard[5] and expressed as mg FAN/100 g sorghum grain or malt.

Statistical analysis

All experiments were performed at least twice and closely agreeing replicate results were obtained. The data were analysed using one way analysis of variance and the means separated using Fischer's Least Significant Difference test at $p<0.05$.

RESULTS AND DISCUSSION

The grain properties of the HPDS types were considerably different from those of the NPDS types. The NPDS were much harder, had much higher 1000 kernel weight (Table I), and had mainly corneous type endosperm (Fig. 1). In contrast, not only were the HPDS softer and low 1000 kernel weight (Table I), their endosperm was also completely floury (Fig. 1). This confirms earlier reports the HPD trait in maize and sorghum tends to be associated with soft endosperm[22].

With regard to endosperm ultrastructure, TEM revealed that the surfaces of the protein bodies of the HPDS were irregular, with villi-like margins (indicated by arrows), compared to the protein bodies of the NPDS, which were essentially spherical with smooth surfaces (Fig. 2). It has been proposed that these invaginations in the surface of the protein bodies of the HPDS mutants apparently offer easier accessibility to proteolytic enzymes for digestion of the storage protein in the interior of the protein bodies[15], which is mainly alpha-kafirin[16].

With regard to protein related properties, while the protein content of the grains of two HPDS cultivars was in the same range as the NPDS, they had at least 70% higher lysine content (Table I), resulting from their high-lysine mutant origin[23]. As expected, the protein digestibilities of the HPDS were also higher than the NPDS, some 26-42% higher. This was higher than the original description of the high digestibility types where in uncooked digestibility of 10-15% improvement was reported[23]. With all the sorghums, malting improved protein digestibility on average 25%. This can be attributed primarily to modification of the sorghum endosperm protein body and protein matrix by the endogenous proteases during germination[21]. Like the raw grain, the malts of the HPDS also had higher protein digestibility than the NPDS. However, the percentage increase in protein digestibility with malting in HPDS was considerably less than with the NPDS. This indicates that protein modification which occurred during malting had a greater effect on protein digestibility than the high protein digestibility trait.

The level of FAN in the HPDS raw grains was similar to that in NPDS (Table II). This indicates that despite the higher lysine content and digestibility of these HPDS, the proportion of nitrogen as protein as opposed to amino acids and peptides was the same as in NPDS. As expected, there was a very large increase in FAN, on average 65 mg/100 g sorghum in all the sorghums with malting, which was as a result of the action of the endogenous proteolytic enzymes[6,11,14]. The malts of the HPDS contained substantially higher levels of FAN than those of NPDS, on average 32 mg FAN /100 g (51%) more. This was presumably because of the higher digestibility of the protein bodies (Fig. 2). However, the quantity of FAN produced when all sorghum malt types were mashed was low, on average only 12 mg/100 g sorghum, in line with earlier reports[7,19]. Further, with the HPDS malts, FAN production during mashing was only slightly higher than that from the NPDS, on average 6 mg/100 g more. To investigate this

further, both raw grain and malt of HPDS and NPDS were mashed with exogenous protease enzymes.

With both HPDS and NPDS raw grain there was greater FAN production during mashing with Bioprotease P Conc than with Flavourzyme, on average 62 and 35 mg/100 g sorghum (Table III). This can be attributed to the greater activity of the Bioprotease P Conc enzyme preparation (see under Proteases). However, the difference in FAN production between the HPDS and NPDS was low, on average 18 and 6 mg/100 g sorghum, for Bioprotease P Conc and Flavourzyme respectively. The same was found when malts of the HPDS and NPDS were mashed with the exogenous protease enzymes (Table IV). In this case, the difference in FAN production was 13 and 9 mg/100 g, respectively. These results are in agreement with those for sorghum malts mashed on their own (Table II) and show that under a wide range of conditions FAN production from HPDS during mashing was only slightly higher than from NPDS. It is probable that the wet-heat treatment of mashing still caused intermolecular disulphide-bonded cross-linking of the kafirin proteins[4,12] in these HPDS, which reduces the susceptibility of kafirin to hydrolysis[3].

Notwithstanding this, Table IV shows that the combination of using HPDS, malting these sorghums and applying exogenous proteases resulted in substantial wort FAN being obtained from sorghum, in this case 121-161 mg/100 g sorghum. Such levels meet the recommended adequate wort FAN levels of 130-150 mg/L for support of optimal yeast growth and rapid fermentation[13,17].

CONCLUSIONS

HPDS have substantially higher protein digestibility compared to NPDS when measured by the pepsin (endoprotease-type) assay. They produce malt with substantially higher levels of FAN. However, they yield only slightly higher FAN when mashed. This is probably as a result of wet-heat induced cross-linking of the kafirin proteins, which reduces their susceptibility to proteolysis.

Notwithstanding this, HPDS could be very useful for improving FAN levels in sorghum brewing if they are malted. Also, to further increase FAN production when mashing with HPDS malts, it is recommended that the various lines are screened to select those with the highest protease activity.

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LEGENDS TO TABLES

Table I. Grain characteristics and effects of malting on protein digestibilities of normal (NPDS) and high protein digestibility sorghum (HPDS) cultivars.

Table II. Effects of malting and mashing with normal (NPDS) and high protein digestibility sorghum (HPDS) cultivars on FAN (mg/100 g sorghum).

Table III. Effect of exogenous proteases on FAN production when mashing with unmalted normal (NPDS) and high protein digestibility sorghum (HPDS) cultivars (mg/100 g sorghum).

Table IV. Effect of exogenous proteases on FAN production when mashing with malted normal (NPDS) and high protein digestibility sorghum (HPDS) cultivars (mg/100 g malt).

LEGENDS TO FIGURES

Fig. 1. Endosperm texture of sorghum types. (A). NPDS parent, (B) NPDS, (C) HPDS parent, and (D) HPDS progeny.

Fig. 2. Transmission electron micrographs of protein bodies of different sorghum types. (a) NPDS (control), and (b) HPDS (progeny). PB – Protein Bodies, Arrows - Villi-like margins of protein bodies.

Table I. Grain characteristics and effects of malting on protein digestibilities of normal (NPDS) and high protein digestibility sorghum (HPDS) cultivars.

	Raw grain						Malt		
Sorghum cultivar	**Single kernel hardness**	**Thousand kernel weight**	**Endosperm texture[1]**	**Protein (% dry basis)**	**Lysine (g/100 g protein)**	**Protein digestibility (%)**	**Protein (% dry basis)**	**Protein digestibility (%)**	**Increase in protein digestibility with malting (%)**
HPDS parent	33.5	21.6	5	10.1	3.9	65.8^{b}±4.1	8.7	78.8^{c}±0.6	19.8
HPDS progeny	20.3	18.8	5	10.9	3.4	73.7^{b}±3.1	9.0	82.9^{b}±1.6	12.4
Means HPDS	**26.9**	**20.2**	**5**	**10.5**	**3.7**	**69.8**	**8.9**	**80.9**	**16.1**
NPDS parent	79.2	27.1	2	13.8	2.0	51.5^{a} ±1.5	12.1	67.5^{a}±1.6	31.1
NPDS control	77.8	25,3	2	7.7	1.6	52.2^{a}±2.3	6.9	71.8^{b}±1.0	37.5
Means NPDS	**78.5**	**26.2**	**2**	**10.4**	**1.8**	**51.9**	**9.5**	**69.7**	**34.3**
Grand means	**52.7**	**23.2**	**3.5**	**10.6**	**2.7**	**60.9**	**9.2**	**75.3**	**25.2**

[1]Where 1 and 2 are corneous, 3 and 4 are intermediate, and 5 is floury.

All results are means of at least two experiments. Standard deviations are in parentheses. Means with different letter superscripts within the columns differ significantly ($p<0.05$)

Table II. Effects of malting and mashing with normal (NPDS) and high protein digestibility sorghum (HPDS) cultivars on FAN (mg/100 g sorghum).

Sorghum cultivar	Grain FAN	Malt FAN	FAN produced during malting	FAN at end of mashing	FAN produced during mashing
HPDS parent	12.1^{b} ±0.4[1]	83.3^{c}±2.4	71.2	96.1^{c} ±3.3	12.8
HPDS progeny	15.5^{d}±0.7	103.4^{d}±2.6	87.9	120.1^{d} ±2.4	16.7
Means HPDS	**13.8**	**93.4**	**79.6 (577)[1]**	**108.1**	**14.8 (16)**
NPDS parent	10.6^{a}±0.8	73.1^{b}±2.5	62.5	83.8^{b}±2.2	10.8
NPDS control	14.2^{bc}±0.7	50.4^{a} ±1.5	36.2	57.6^{a}±0.8	7.2
Means NPDS	**12.4**	**61.8**	**49.4 (398)**	**70.7**	**9.0 (15)**
Grand Means	**13.1**	**77.6**	**64.5**	**89.4**	**11.9**

[1]All results are means of two experiments with standard deviations. Means with different letter superscripts within the columns differ significantly ($p<0.05$)

[2]Percentage increase in brackets

Table III. Effect of exogenous proteases on FAN production when mashing with unmalted normal (NPDS) and high protein digestibility sorghum (HPDS) cultivars (mg/100 g sorghum).

Sorghum cultivar	**FAN at end of mashing**		**FAN produced**	
	Flavourzyme	**Bioprotease**	**Flavourzyme**	**Bioprotease**
HPDS parent	51.2^{b}±1.3^{1}	80.6^{c}±1.7	39.1	68.5
HPDS progeny	53.4^{b} ±2.4	89.9^{d} ±1.1	37.9	74. 4
Mean HPDS	**52.3**	**83.8**	**38.5 (279)[2]**	**71.5 (518)**
NPDS parent	45.0^{a} ±2.2	63.5^{a}±2.1	34.4	52.9
NPDS control	44.3^{a}±1.0	67.8^{b}±2.5	30.1	53.5
Mean NPDS	**44.7**	**65.7**	**32.3 (260)**	**53.2 (429)**
Grand means	**48.5 ±4.5**	**75.5 ±12.1**	**35.4**	**62.3**

[1]All results are means of two experiments with standard deviations. Means with different letter superscripts within the columns differ significantly ($p<0.05$)

[2]Percentage increase in brackets compared to the average FAN levels in the grains

Table IV. Effect of exogenous proteases on FAN production when mashing with malted normal (NPDS) and high protein digestibility sorghum (HPDS) cultivars (mg/100 g malt).

Sorghum cultivar	**FAN at end of mashing**		**FAN produced**	
	Flavourzyme	**Bioprotease**	**Flavourzyme**	**Bioprotease**
HPDS parent	112.7[c]±1.9[1]	148.1[b]±4.1	29.4	64.9
HPDS progeny	129.0[d]±1.2	174.4[c]±2.6	25.6	71.0
Mean HPDS	**120.9**	**161.3**	**27.5 (29)[2]**	**68.0 (73)**
NPDS parent	91.9[b]±1.8	135.1[b]± 4.1	18.8	62.0
NPDS control	69.5[a]±2.4	97.6[a] ±2.1	19.1	47.2
Mean NPDS	**80.7**	**116.4**	**19.0 (31)**	**54.6 (88)**
Grand Means	**100.8 ±25.8**	**138.8 ±32.0**	**23.2**	**61.2**

[1]All results are means of two experiments with standard deviations in brackets. Means with different letter superscripts within the columns differ significantly ($p<0.05$)

[2]Percentage increase in brackets compared to the average FAN levels in the malts

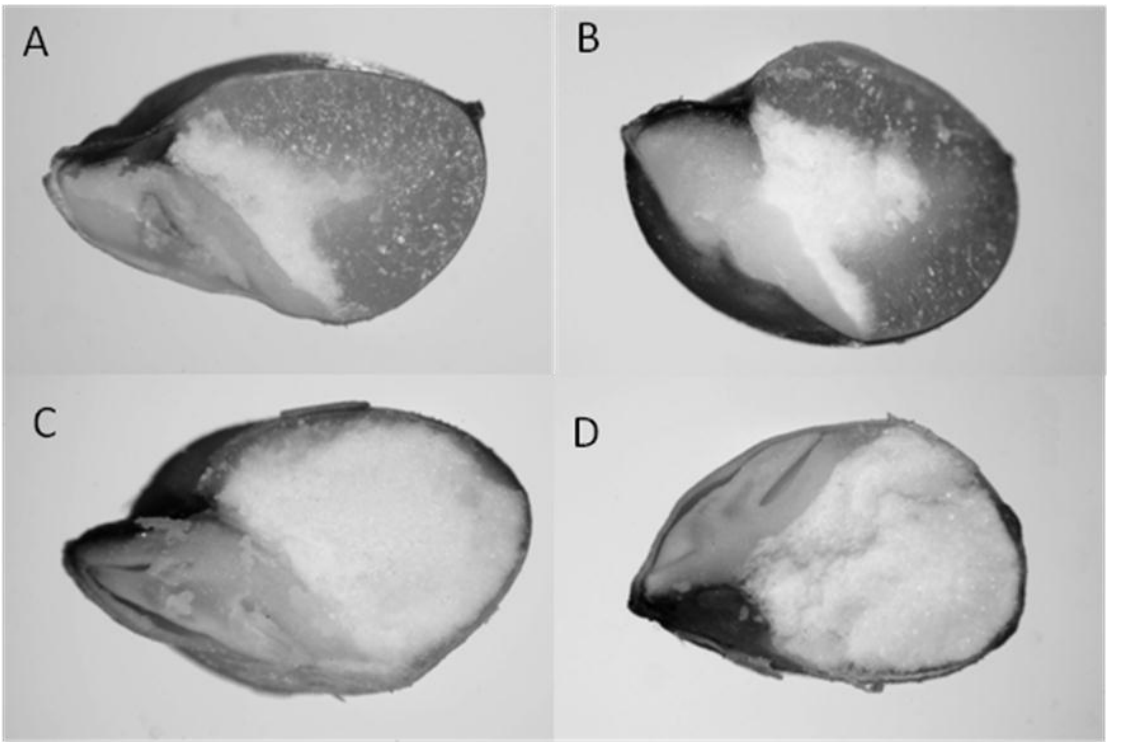

Fig. 1

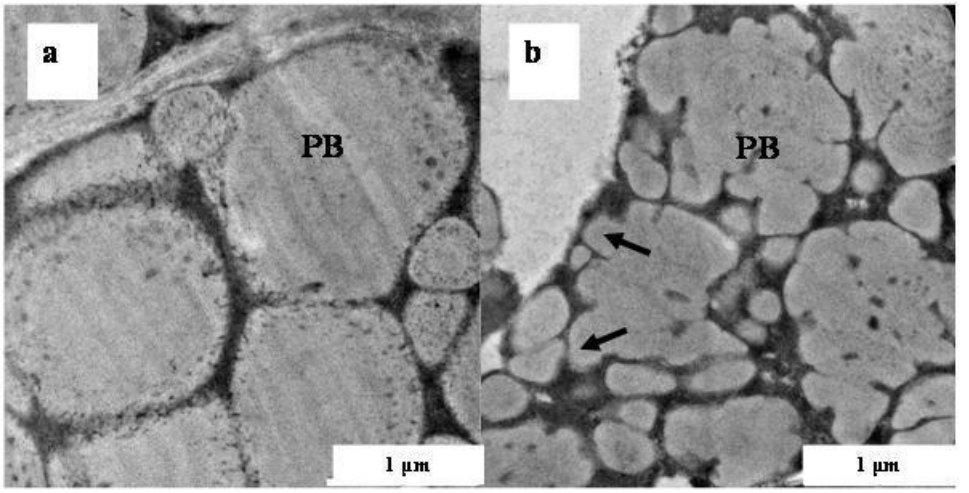

Fig. 2