Influence of ammonia and lysine supplementation on yeast growth and fermentation with respect to gluten-free type brewing using unmalted sorghum grain

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Summary

Because gluten-free type brewing with unmalted sorghum does not provide adequate nitrogen for complete fermentation, wort supplementation with ammonia (as diammonium phosphate, DAP) or lysine on yeast performance was investigated. By Phenotype Microarray, under aerobic conditions, greater yeast growth was indicated with DAP than lysine both as single source and combined with sorghum wort amino acids. With sorghum fermentation, both DAP and lysine improved maltose and maltotriose uptake. However, DAP supplementation also maintained yeast numbers (24.0–21.3 x 10^6 cells/ml) whereas there was a decline with lysine supplementation. Lysine supplementation also resulted in adverse effects on yeast cell morphology. Neither DAP nor lysine supplementation resulted in evident genetic change to the yeast but the change in substrate from barley malt wort to unmalted sorghum wort slightly altered the yeast genetically. Therefore, ammonia as DAP has potential as a nitrogen supplement for improving yeast fermentation performance in sorghum gluten-free brewing.

Keywords: Ammonia, Lager yeast, Lysine Phenotype Microarray, Sorghum

Introduction

Sorghum is especially recognized for its potential in gluten-free beer brewing (Rubio-Flores and Serna-Saldivar, 2016). There are several reasons for this. Sorghum is only distantly botanically related to barley and its products have been shown to be safe when consumed by coeliacs (Ciacci *et al*., 2007). Also, there is considerable know-how regarding sorghum brewing technology both through traditional sorghum beer brewing in sub-Saharan Africa and more recently with lager and stout brewing using sorghum in countries such as Nigeria and Uganda (Alavi *et al.,* 2019).

The fermentation performance of yeast during brewing depends on the availability of nutrients, mainly nitrogen and carbon sources (Martínez-Moreno *et al.*, 2012), which are supplied by the cereal used. However, sorghum brewing fermentations can be especially problematic. Barley malt generally provides these in sufficient assimilable nitrogen for the yeast, while sorghum malts have been reported to produce adequate assimilable nitrogen when mashed with exogenous enzymes (Espinosa-Ramírez et al., 2013). In sorghum lager and stout brewing and gluten-free brewing generally, however, there is often a high ratio of raw (unmalted) grain to malt, or even just unmalted grain alone (Hager *et al.,* 2014; Alavi *et al.,* 2019). Unmalted cereal grain wort may provide insufficient amino acids and short peptides free amino nitrogen (FAN) for yeast fermentation. The levels of FAN in unmalted sorghum can be very low, only approx. 74 mg/L (Adetunji *et al.*, 2013). This is primarily because in unmalted grain there is less protein hydrolysis into peptides and amino acids, as occurs during malting. Also, with sorghum, in particular, the levels of endogenous proteolytic enzymes in the unmalted sorghum grain are low and the sorghum kafirin prolamin proteins are notably resistant to hydrolysis (Taylor *et al.,* 2013; Liu *et al.,* 2019) so that proteolysis is limited during mashing (Dlamini *et al.,* 2015).

Yeast fermentation rate can be improved fermentation time and fermented time reduced by the inclusion of nitrogen sources (Ugliano *et al.,* 2007; Chang *et al*., 2011). However, the extent of yeast growth supported by nitrogen sources depends on the type of nitrogen source (Martínez-Moreno *et al.*, 2012). The utilization of particular nitrogen sources is controlled through nitrogen catabolite repression (NCR) during fermentation. This allows early uptake of readily assimilable nitrogen sources such as the amino acids asparagine, glutamine, glutamine and ammonia through suppressing the uptake systems (permeases and other enzymes) of less preferred nitrogen sources (Fayyad-Kazan *et al.*, 2016). Further, Jiménez-Martí and Del Olmo (2008) reported that the addition of ammonia expresses most NCR-regulated genes in yeast grown in nitrogen depleted medium than other amino acids. Yeast nitrogen requirements during fermentation are also strain-dependent (Gutiérrez *et al.*, 2018). Also, of relevance is that yeast strains that consume a familiar nitrogen source generally have a good fermentation performance and shorter fermentation periods compared to those subjected to different nitrogen environments (Brice *et al.*, 2018).

The objectives of this present work were therefore to better understand how lager beer brewing yeast performs on unmalted sorghum grain wort with the aim of improving sorghum gluten-free alcoholic beverage fermentation. The work first studied the utilization of various free amino acids that are present in sorghum wort by brewing yeast under aerobic conditions, following their supplementation with lysine and ammonia, in the form of diammonium phosphate (DAP). Then, the effect of supplementing unmalted sorghum grain wort with the two selected nitrogen sources (ammonia and lysine) on the yeast and its fermentation performance under brewing-type conditions was investigated.

Materials and Methods

Sorghum grain and brewing yeast

Sorghum grain, variety Macia, a white tan-plant, low polyphenol, non-tannin type and lager beer yeast (*Saccharomyces pastorianus*) were both kindly provided by the South African Breweries (Pretoria, South Africa). The yeast had been used to ferment barley malt-based wort for six fermentation cycles. In this work, the yeast was then serially repitched for three further fermentation cycles in unmalted sorghum grain wort. The yeast, which was in the form of a slurry, was transported on ice and stored at 4°C until use, within 24 h.

Determination of nitrogen utilization by yeast by Phenotypic Microarray assay

Nitrogen utilization by yeast cells under aerobic growth conditions was determined by Phenotype Microarray (PM). Briefly, fresh yeast cells, obtained from the brewery, were sub-cultured onto plate count agar plates and incubated at 25°C for 4 days. Yeast suspensions (10^6–10^7 cfu/mL), determined using a turbidimeter, were inoculated into nitrogen source (individual amino acids) microplates (PM3) (Biolog, Haward, CA) at a volume of 100 µL/well. The PM plates were supplemented with DAP or lysine at a concentration of 50 mg nitrogen/L per supplement. They were then placed in an OmniLog reader (Biolog) and incubated at 25°C for 48 h. Data were collected every 15 min. Yeast growth was determined in OmniLog values (area under the curve) with values from the negative control (without any amino acid) being subtracted. Only absorbance values after 24 h and 48 h are presented here.

Laboratory brewing and fermentation

High gravity worts (approx. 16° Plato) were prepared from the unmalted sorghum as described by Adetunji *et al.* (2013). In brief, the mashing procedure comprised a protein rest in the presence of a protease and a thermostable α-amylase, after which the mash was heated to gelatinise and partially hydrolyse the starch. It was then cooled and a saccharification mashing step carried out with a fungal α-amylase. The unmalted sorghum wort contained 54 mg/ L of FAN, which increased to 102 and 106 mg/ L FAN after supplementation with lysine or DAP, respectively. The control was unmalted sorghum grain wort without nitrogen supplementation. Fermentations using a wort volume of 2 L were conducted in European Brewery Convention (EBC) cylindrical tubes (EBC Analytica Microbiologica, Method 2.5.4) (EBC, 1977) capped at the open end with cotton wool. Before fermentation, the wort was sterilised by boiling for 45 min.

The worts were inoculated with 14 g/L of yeast slurry, which gave an initial yeast count of approximately 23–25 x 10^6 cells/mL. Fermentations were carried out at 15°C and monitored daily over 7 days. Immediately after sampling, the samples were cooled on ice and centrifuged (8,000 g for 10 min at, 4°C) to separate yeast from wort. Yeast collection for subsequent fermentations involved discarding the trub-rich fraction at the bottom of the EBC tube, which constituted approx. 2% of the total yeast crop, and maintaining the remainder. The yeast was not acid washed because as it was not significantly bacterial contaminated. Before inoculation into fresh wort, yeast viability was determined by methylene blue staining and the slurry consistency was also measured. A total three fermentations of unmalted sorghum worts were carried out with serial repitching, designated fermentations 7, 8 and 9.

Specific gravity, sugar spectrum and yeast cell count

Wort specific gravity was measured by pycnometry following the EBC method 4.5.1 Extract of Malt: Congress Mash AM (EBC, 1998). Results were expressed in °P. The sugar profile was determined using an ultra-fast liquid chromatograph (Shimadzu, Kyoto, Japan) fitted with a refractive index detector (RID-10A) following the method by Phaweni *et al*. (1992). The unmalted sorghum worts for the sugar spectrum determination were from separate fermentations with different original gravities. The yeast cell count was determined by haemocytometry and expressed as cells/mL.

Yeast morphology

To examine cell morphology, yeast cells were fixed with 2.5% (v/v) glutaraldehyde in 0.075 M phosphate buffer (pH 7.4) for 30 min. The samples were carefully washed three times in buffer. They were then dehydrated in graded ethanol series (50, 70, 90 and 100%) and dried to the critical-point. After that, the cells were coated with carbon using an Emitech K550X sputter coater (Quorum Technologies, London) and examined with a scanning electron microscope (Zeiss Ultra Plus 55 FEG, Oberkochen, Germany).

Microsatellite analysis

DNA extraction

Yeast DNA was extracted with the PureLink™ Genomic DNA Mini kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. DNA concentration was determined using a nanodrop spectrophotometer (260/280 nm) (Genway Biotech, San Diego, CA). Only yeast cells from days 0 and 7 of fermentation

cycle 7 and day 7 yeast for both fermentation cycle 8 and 9 were used for this experiment.

Rep-PCR amplification

The microsatellite oligonucleotide primer $(GTG)_5$ was used. The PCR amplification was carried out in a DNA Engine peltier thermal cycler (Bio-Rad, Hercules, CA) using 2 µL DNA template, 0.4 µL dimethyl sulphoxide (4%), 0.28 µL $(GTG)_5$ primer (Integrated DNA Technologies, Coralville, IA), 10 µL Mastermix, 0.28 µL $(GTG)_5$ primer, 2 µL CoraLoad (Qiagen, Hilden, Germany) and 0.4 µL dimethyl sulphoxide (4%). The final volume of 20 µL was achieved with RNase free. The amplification conditions were as follows: initial denaturation at 95°C for 5 min; 30 cycles: denaturation (95°C for 30 s), annealing (40°C for 60 s), extension (65°C for 3 min) followed by a single final extension at 65°C for 8 min. The amplified PCR products were visualized following ethidium bromide staining of 1% agarose gel run at 100 V. The rep-PCR fingerprints were analysed using the BioNumerics software, version 7.10 (Applied Maths, East Flanders, Belgium). The $(GTG)_5$ fingerprints were analysed using Gel-Compar II version 6.5 (Applied Maths NV, Sint-Martens-Latem, Belgium) as described by Ntuli et al., 2017.

Statistical analyses

One-way ANOVA was used to determine the effect of specific gravity, yeast counts and pH on yeast fermentation performance. ANOVA was performed using Statistica software, version 12 (Tulsa, OK). The fermentation experiments were repeated twice, while the PM study was not repeated due to its high cost.

Results and discussion

Yeast utilization of nitrogen sources

PM assay was used to monitor the brewing yeast's nitrogen utilization under aerobic conditions. During the early stages of brewing fermentation, aerobic fermentation occurs, which is required for yeast synthesis of sterols and unsaturated fatty acids that are important for yeast multiplication and hence influence the rate and extent of fermentation (Briggs *et al.*, 2004). Lysine and ammonia were investigated as supplementary sources of assimilable nitrogen to those amino acids that are present in unmalted sorghum wort (Dlamini *et al.*, 2105). Lysine is classified as a group A amino acid in terms of yeast fermentation performance, meaning that it is one of the amino acids that is assimilated first during fermentation (Pierce, 1987). Although ammonia is a group C amino acid, which means it is must be taken up once Group A amino acids become limiting, it is preferentially used by yeast cells (Magasanik and Kaiser, 2002). Furthermore, both ammonia in the form of DAP and lysine are both commercially available as commodities and hence candidates as exogenous nitrogen supplements for yeast fermentation.

Concerning the effects of the individual amino acids, among the other group A amino acids, glutamine and asparagine, both found in significant amounts in sorghum wort (Dlamini *et al.* (2015), supported most the active respiration after 24 h and 48 h (Fig. 1) However, with lysine no yeast growth occurred when it was used as the sole nitrogen source. This could be due to lysine being deposited in the yeast cell vacuoles instead of the cytosol, which could have adversely affected its utilization (Karthikeyan *et al.*, 2008). Another factor is that lysine causes a feedback inhibition of the homocitrate synthase enzyme (regulated by LYS20 or LYS21) in the α-aminoadipate pathway for lysine biosynthesis in yeast cells (Ljungdahl and Daignan-Fornier, 2012).

Among the group B and C amino acids, valine, which is present in significant levels in sorghum wort (Dlamini *et al.*, 2015), and ammonia supported the best yeast growth. Proline, the sole member of Group D, supported yeast growth only after 48 h. The utilization of proline was probably to the fermentation being under aerobic conditions. Under aerobic conditions, mitochondrial oxidase is active, which is responsible for the metabolism of proline, (Wang and Brandriss, 1987). It is notable, however, that yeast growth in the presence of proline was very poor after 24 h. This suggests that it is the least preferred amino acid even under aerobic conditions.

Concerning the effects of ammonia and lysine supplementation, generally supplementation with ammonia supported greater yeast growth (bars marked with *) than the amino acids alone (Fig. 1). At 48 h enhanced yeast growth was obtained with DAP in combination with 16 of the 18 different sorghum wort amino acids studied. Specifically, ammonia supported the best growth when it was in combination with histidine (a group B amino acid), both at 24 and 48 h. Lei *et al.* (2013) reported that histidine stimulated yeast cell growth and increased fermentation rate and ethanol production, possibly through inhibiting the NCR system. Glycine, tyrosine and tryptophan (all Goup C amino acids) supplemented with ammonia also all resulted in high yeast growth after both 24 h and 48 h. This could be due to the fact that nitrogen sources are first converted to glutamate and glutamine by yeast before being utilized (Schure *et al.*, 2000). Ammonia can be used as the amino group donor in the direct synthesis of these two amino acids (Briggs *et al.*, 2004).

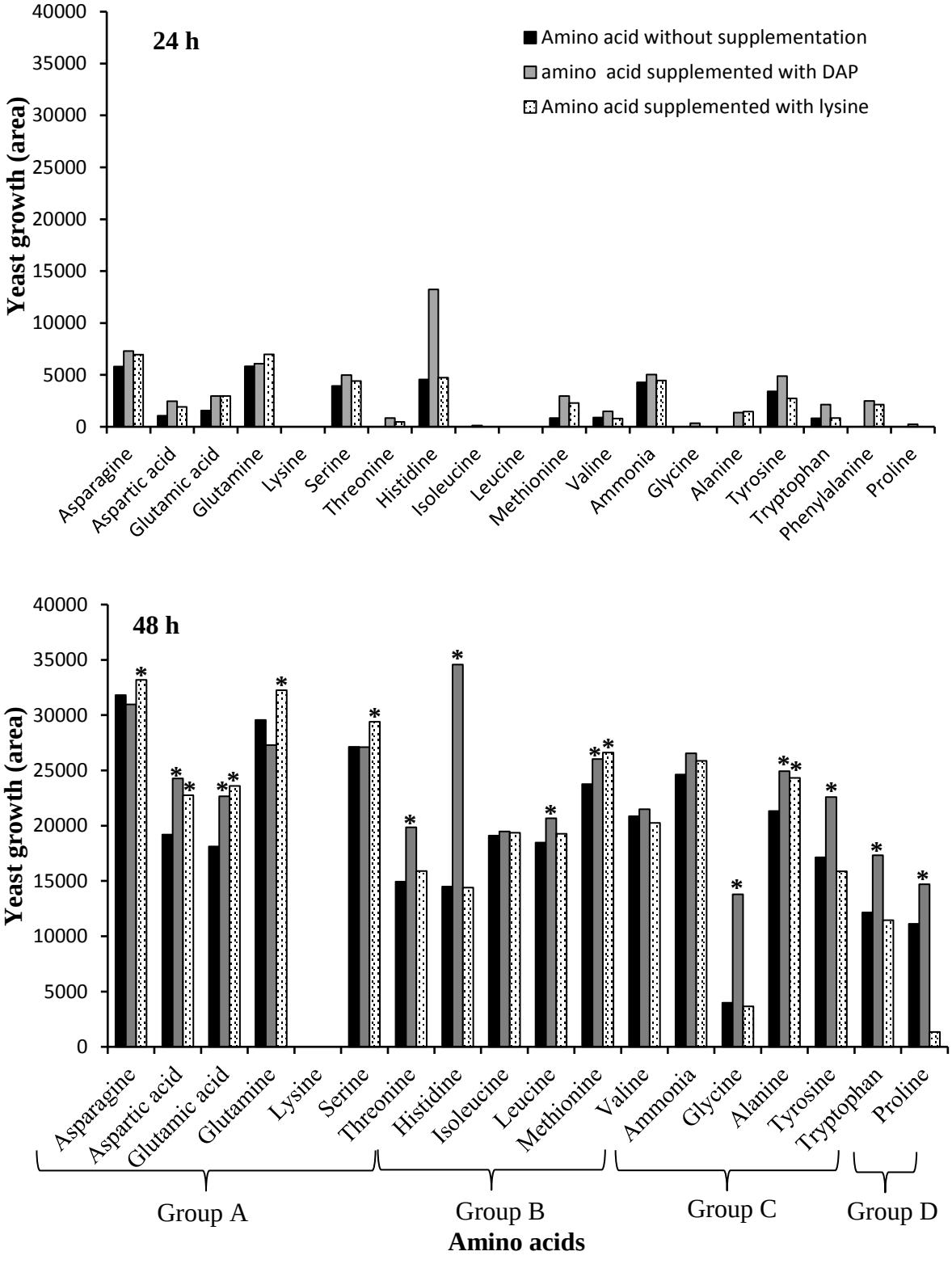

Fig. 1 Extent of growth of yeast cells under aerobic conditions on nitrogen source phenotype microarray plates supplemented with ammonia as DAP or lysine and incubated for 24 h and 48 h at 25°C. * Indicates increased yeast growth with lysine or DAP supplementation.

When lysine was used as a supplement to the other amino acids, yeast growth was generally supported and greater yeast growth was indicated with 7 of the other amino acids (bars marked with *) (Fig. 1). A study that investigated among others, yeast fermentation of wort supplemented with lysine and L-methionine reported that lysine supplemented fermentations were more rapid (completed in 48 h) than L-methionine supplemented fermentations (completed in 103 h) (Lekkas *et al*., 2007). The authors attributed this to an increase in suspended yeast cell concentration with lysine supplementation.

On the basis of this PM assay data, which indicated a considerable increase in yeast growth with ammonia supplementation and some increase with lysine supplementation, their effects on fermentation of actual unmalted sorghum grain wort were investigated.

Fermentation performance of serially repitched yeast

The controlled re-use (serial repitching) of yeast is a normal practice in brewing as it more convenient and economical than growing up a fresh batch of yeast for each fermentation. Here, the yeast had first been used to ferment barley malt-based wort for six fermentation cycles. It was then used to ferment unmalted sorghum grain wort for three further cycles.

There was no variation in fermentation parameters with serial repitching. This is line with the findings of Bühligen *et al*. (2013) on that bottom-fermenting *Saccharomyces pastorianus* strains can be used repeatedly without negatively affecting vital physiological characteristics. Hence, mean data values for the two fermentations are given in Table 1 and Figure 2. Concerning the effects of ammonia and lysine supplementation on yeast growth on the unmalted sorghum grain wort, there was a

significant ($p \leq 0.05$) reduction in yeast counts after 7 days fermentation except with ammonia supplementation. Further, ammonia supplemented wort had considerably higher yeast counts ($p \leq 0.05$) after 7 days fermentation than the control and lysine supplemented worts (Table 1). These did not differ from each other ($p > 0.05$). These findings for fermentation of the sorghum grain wort are in generally agreement with the PM growth data under aerobic conditions (Fig. 1). The lack of yeast growth with lysine supplementation during actual sorghum fermentation is also consistent with the work of Kemsaward *et al.* (2015). These authors classified lysine as a bad nitrogen source based on fermentation parameters such as growth rate and viability of *S. cerevisiae* in the stationary phase wine fermentation.

Table 1 Effects of ammonia (DAP) and lysine supplementation on yeast counts after 7 days of sorghum grain wort fermentation at 15°C

Treatment	**Day 0**	**Day 7**
	(x 10^6 cells/mL)	
Control (no supplementation)	25.5^{c} (2.8)	16.8^{a} (1.8)
DAP	24.0^{bc} (2.1)	21.3^{b} (1.1)
Lysine	26.5^{c} (2.8)	15.0^{a} (3.5)

Figures in parentheses represent standard deviations; values with different letter superscripts are significantly different (n = 2 independent experiments) ($p \leq 0.05$)

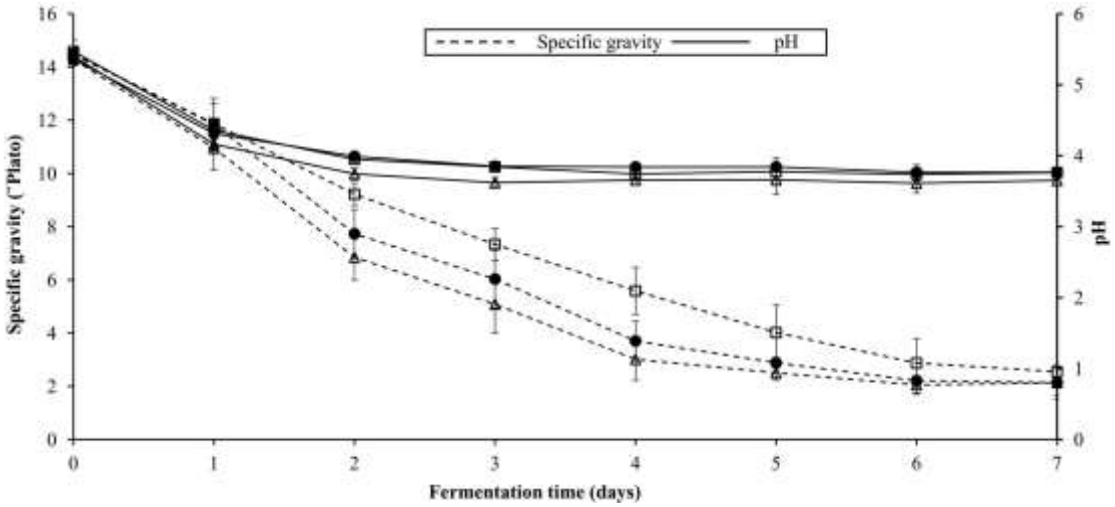

Fig. 2 Effects of nitrogen supplementation with ammonia as DAP and lysine on wort specific gravity and pH during fermentation of unmalted sorghum grain wort at 15°C for up to 7 days. No supplementation: squares; Lysine supplemented: closed circles; DAP supplemented: triangles. Error bars indicate standard deviations (n=3)

Supplementation with DAP improved both the rate and amounts of maltose and maltotriose taken up by the yeast from the sorghum wort (Table 2). In contrast, lysine supplementation only increased the amounts of maltose and maltotriose taken up (Table 3). The rate of glucose and fructose uptake was similar in both the DAP supplemented and control (unsupplemented) worts (Table 2). However, with lysine supplementation, however, glucose uptake was slower (approx. 19% taken up) after 1 day compared to the unsupplemented wort (approx. 54% taken up) (Table 3). The slow utilization of glucose was not surprising since lysine has been classified as a "bad" nitrogen source with respect to glucose consumption (Kemsawasd *et al.*, 2015).

Table 2 Effect of ammonia (DAP) supplementation on sugar uptake during fermentation of sorghum grain wort at 15°C (g/ 100 ml)

Treatment	Day	Maltose	Maltotriose	Glucose	Fructose
Control	0	4.73^{c} (0.01)	2.80^{e} (0.00)	0.60^{b} (0.01)	0.09^{b} (0.00)
	1	2.70^{d} (0.00)	2.19^{d} (0.00)	0.01^{a} (0.00)	0.01^{a} (0.00)
	2	2.17c (0.02)	2.09^{c} (0.00)	0.01^{a} (0.00)	0.01^{a} (0.00)
DAP	0	5.63^{f} (0.10)	3.65^{f} (0.01)	1.04^{c} (0.01)	0.24^{c} (0.01)
	1	0.62^{b} (0.01)	1.55^{b} (0.03)	0.02^{a} (0.00)	0.01^{a} (0.00)
	2	0.17^{a} (0.01)	1.43^{a} (0.00)	0.02^{a} (0.00)	0.01^{a} (0.00)

Figures in parentheses represent standard deviations; values with different letter superscripts in a column are significantly different (n = 2 independent experiments) ($p \leq 0.05$)

Table 3 The effect of lysine supplementation on sugar uptake during fermentation of sorghum grain wort at 15°C (g/ 100 ml)

Treatment	Day	Maltose	Maltotriose	Glucose	Fructose
Control	0	9.21^{d} (0.12)	4.63^{d} (0.07)	1.09^{d} (0.03)	0.25^{b} (0.02)
	1	3.97^{b} (0.25)	3.63^{c} (0.09)	0.50^{b} (0.02)	0.01^{a} (0.00)
	2	2.94^{b} (0.08)	2.75^{b} (0.01)	0.38^{a} (0.04)	0.01^{a} (0.00)
Lysine	0	9.05^{d} (0.12)	4.76^{d} (0.00)	1.19^{d} (0.03)	0.24^{b} (0.02)
	1	4.16^{c} (0.19)	3.43^{c} (0.00)	0.96^{c} (0.02)	0.02^{a} (0.00)
	2	1.85^{a} (0.03)	1.53^{a} (0.04)	0.34^{a} (0.02)	ND

Figures in parentheses represent standard deviations; values with different letter superscripts in a column are significantly different (n = 2 independent experiments) ($p \leq 0.05$)

Figure 2 shows that there was more rapid reduction in wort specific gravity (measure of sugar utilisation of the yeast) with the ammonia supplemented worts than with the lysine supplemented worts. However, the ammonia and lysine supplemented worts both reached the same final gravity of 2.1°P after 7 days fermentation. In contrast, the unsupplemented wort showed the slowest reduction in specific gravity and had the highest final gravity (2.6°P). The rapid reduction in wort specific gravity with ammonia supplementation is in agreement with the work of Torrea *et al.* (2011). These authors

found much more rapid sugar consumption (in 8 days) with grape must supplemented with ammonia (320 mg N/L) compared to the control (160 mg N/L) where complete sugar consumption only occurred after 13 days fermentation.

With the unsupplemented sorghum wort, the slow reduction in specific gravity over the 7 days fermentation period (Fig. 2), is a clear indication of nitrogen limitation during fermentation. The unmalted sorghum grain wort FAN level was only approx. 50 mg/L, compared to a requirement of at least 115 mg/L in sorghum worts with such high fermentable sugar levels (Pickerell, 1986).

During alcoholic fermentation, the pH changes as the substrate sugars are metabolised by yeast to ethanol and other compounds (Liu *et al.*, 2015). A rapid pH reduction that occurs during the early stages of fermentation is indicative of good yeast performance. Figure 2 shows that ammonia supplementation supported a significantly ($p \leq 0.05$) more rapid reduction in pH after 3 days than the lysine supplemented and unsupplemented worts. The relatively low final pH with ammonia supplementation can be attributed to the high levels of phosphate anion that remain when yeast utilizes ammonium cations (Ugliano *et al.*, 2007).

Yeast morphology during serial fermentation

Scanning electron microscopy was used to determine if serial fermentation on unmalted sorghum grain wort caused changes in yeast cell morphology, as a result of nitrogen limitation or more generally due to the change in substrate from barley malt wort (Fig. 3). Yeast cells from the ammonia supplemented worts were least affected by serial fermentation. In general, fermentation cycle 9, day 7, yeast cells did not differ greatly from the day 0 cells of fermentation cycle 7. A few wrinkled cells were observed after cycles 8 and 9 but the majority of the cells had a normal oval-shaped slightly

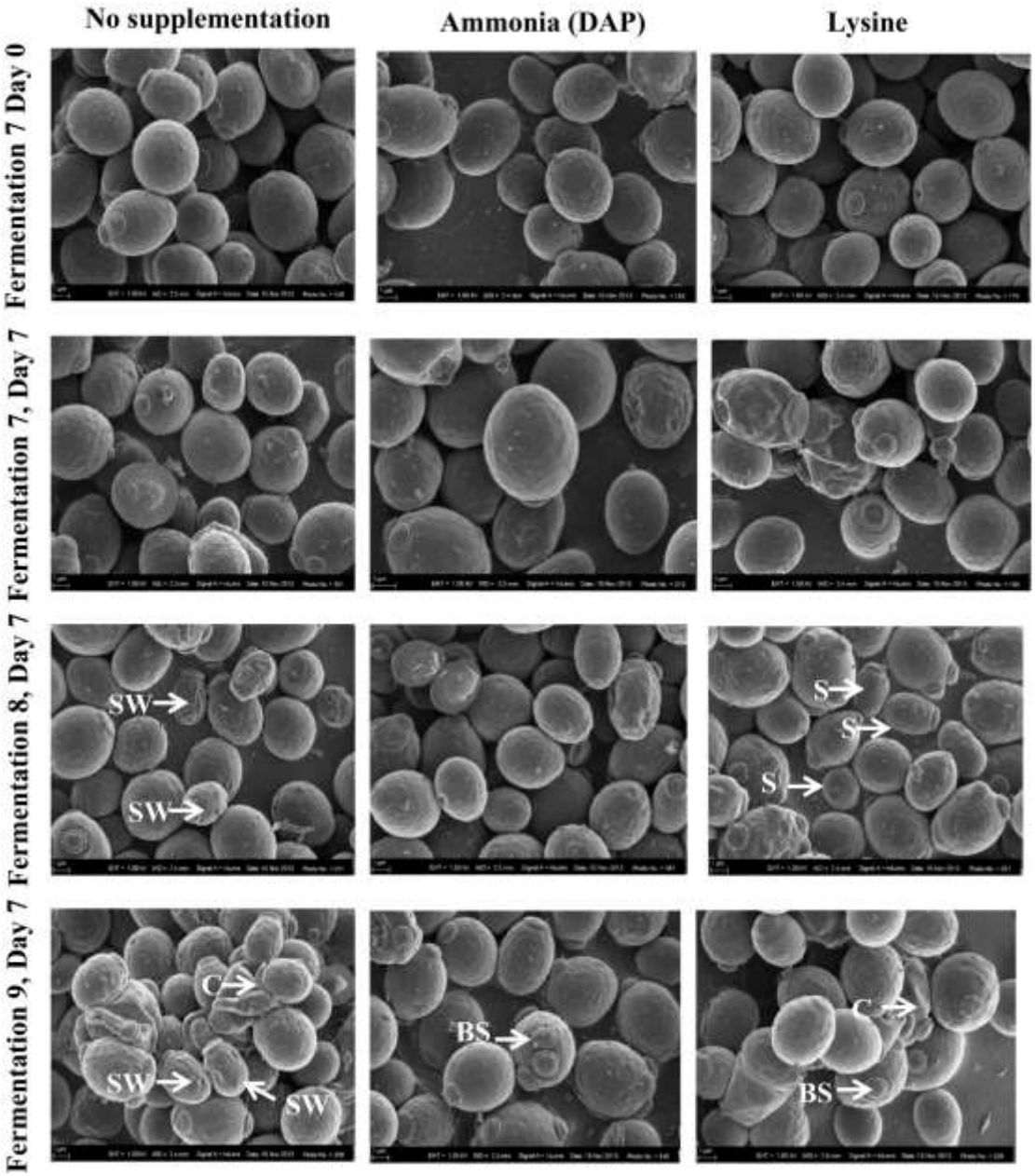

Fig. 3 Scanning electron micrographs showing the effect of serial repitching on yeast cell morphology during fermentation of unmalted sorghum grain wort supplemented with ammonia as DAP and lysine at 15°C for up to 7 days. S: small sized cells; SW: small wrinkled cells; C: collapsed cells; BS: bud scars

smooth surface, similar to those from fermentation cycle 7. In contrast, the yeast cells from the unsupplemented worts of fermentation cycles 8 and 9 and also those of the lysine supplemented worts (but to a lesser extent) were of different sizes compared to day 0 cells of fermentation cycle 7. They were notably small sized cells with polar bud

scars and they appeared uniformly rough because of wrinkling of the cell wall. At the end of fermentation cycle 9, some of the cells had also collapsed. This finding is in general agreement with the lower final yeast counts with the unsupplemented and lysine supplemented worts (Table 1).

The morphology of the yeasts cells from the unsupplemented and lysine supplemented worts indicates that the cells were undergoing autolysis. The steps of yeast cell autolysis include destruction of the cell membrane, loss of cell turgor that results in a decrease in cell size and subsequently wrinkled or folded yeast cells (Martınez-Rodrıguez *et al.*, 2001). Overall, the results indicate that nitrogen limitation caused considerable damage to the yeast, resulting in substantial cell autolysis. Moench *et al.* (1995) reported that exposure of yeast cells to excess stress or repeated exposure to low-level stress, as here, can cause autolysis. Such damage is consistent with poorer yeast fermentation performance on the unsupplemented and lysine supplemented worts (Fig. 2).

Yeast microsatellite analysis during serial fermentation

A PCR-fingerprinting method based on the microsatellite primer $(GTG)_5$ was used to examine whether the yeast underwent genetic changes as a result in the change in substrate from barley malt wort to unmalted sorghum wort and as result of ammonia or lysine supplementation. The $(GTG)_5$ microsatellite primer method was used as it has been widely applied for the discrimination of *Saccharomyces* yeast species and for differentiation between strains within species (Kállaia *et al.*, 2019). The $(GTG)_5$ analysis showed five different bands, ranging from 400 to 2500 bp for all the treatments (Fig. 4). Cluster analysis of the genomic fingerprints, based on the presence or absence of major bands showed two main groups, with a similarity value of 63%. The first group

consisted of all the fermentation cycle 7 treatments (no supplementation, supplementation with ammonia and supplementation with lysine), while the second group showed a 100% homology of fermentation 8 and 9 treatments. This indicates that the change of substrate from barley malt to unmalted sorghum resulted in a slight genetic change to the yeast. Fermentation 8 lysine supplemented cells after 7 days fermentation showed a shift in one of the bands. Apart from this minor difference there was no difference in genomic fingerprint between the treatments. The shift in one of the bands that occurred with fermentation 8 lysine supplemented cells was not significant as the BioNumerics software showed a 100% homology within the groups. The fact that yeasts over the three fermentation cycles with unsupplemented unmalted sorghum wort or with ammonia or lysine supplemented worts showed no evident genetic changes is a positive finding. It suggests that lager yeast developed for fermentation of barley malt wort can readily ferment unmalted sorghum wort with genetic adaptation to this substrate.

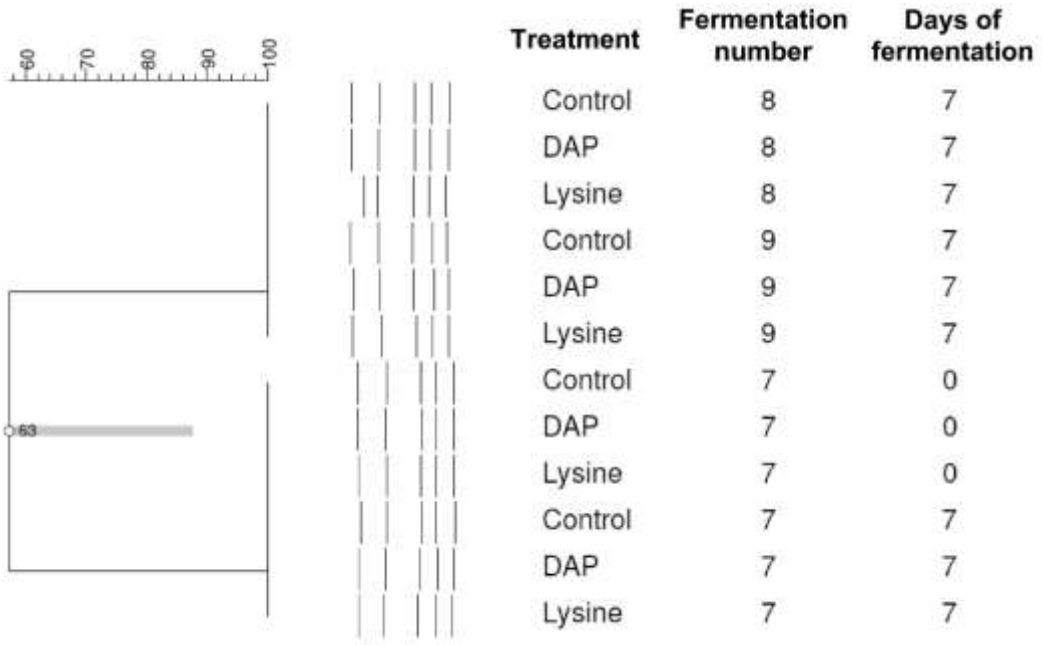

Fig. 4 Tree plot and diagram representation of the $(GTG)_5$ amplification patterns as produced after fermentation of sorghum wort supplemented with ammonia (DAP) and lysine

Conclusions

The study demonstrates that both the amount of assimilable nitrogen and type of nitrogen strongly influence yeast growth and yeast fermentation performance when brewing with unmalted sorghum grain wort. Yeast fermentation performance is adversely affected when unmalted sorghum wort is the sole source of nitrogen. This clearly affects yeast functioning, resulting in collapse and autolysis of cells. Ammonia in the form of diammonium phosphate increases yeast growth under aerobic conditions and improves fermentation performance. This could be because ammonia is one of the most preferred nitrogen sources by yeast and that it regulates permease and other enzyme activities associated with uptake of less preferred nitrogen sources, and also functions as a substrate for protein synthesis. However, lysine supplementation is not as effective and results in similar adverse changes in yeast morphology to those observed without supplementation. Supplementation with ammonia or lysine does not cause any genetic change but the change in substrate from barley malt wort to unmalted sorghum wort appears to alter the yeast genetically. Therefore ammonia in the form of diammonium phosphate has potential as a nitrogen supplement for improving yeast performance in gluten-free fermented alcoholic beverages using unmalted sorghum. The findings of this work may encourage the use of unmalted sorghum to produce more affordable gluten-free fermented alcoholic beverages. Further work, however, is required to establish the effect of ammonia addition on ethanol yield and on the flavour profile of such fermented alcoholic beverages.

Acknowledgment

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Ethical approval

Ethical approval was not required as the work did not involve studies with human participants or animals.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest: The authors of this paper, Bhekisisa Dlamini, John Taylor and Elna Buys all declare that there is no conflict of interest. Further, they declare that they have read and approved the manuscript for submission.

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