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Abstract

The potential to utilise low nitrogen barley for production of distilling quality malt was studied. This presents an opportunity to reduce the environmental impact of nitrogen fertiliser applications. Malting barley (cv. Octavia) was grown without the application of inorganic nitrogen fertiliser, to produce grain with a relatively low nitrogen concentration (1.16 %, dry weight basis). Following micro-malting trials, dextrinizing units (58 DU) obtained from low nitrogen malt were much higher than a typical specification of 45 DU for malt with a conventional nitrogen concentration (<1.5 %). A higher soluble nitrogen ratio (SNR), or index of modification (IoM), of 49 indicated greater modification of the low nitrogen barley, resulting in higher extract released into the wort. Additionally, much lower levels of β-glucan were found in low nitrogen malt wort (64 mg/L compared with over 100 mg/L in wort of conventional nitrogen malt). Low nitrogen malt also produced higher predicted spirit yields following wort fermentation and wash distillation. These findings indicate that lower nitrogen concentration barley can be processed without negatively impacting malt quality for distilling applications. The implication of these findings to help realise more environmentally sustainable production of barley for malting and use in distilling is also discussed briefly.

Keywords: barley, nitrogen, fertiliser, malting, mashing.

Introduction

Different sectors of the alcohol industry have different malt-quality requirements resulting in different barley varieties being bred for each, whether that be for malt distilling, grain (e.g. wheat) distilling or brewing. For malt spirit distillers, the optimal grain protein concentration is considered to be 9.4 % (equivalent to 6.25 x 1.5 % nitrogen (1)) and, as protein concentration is inversely related to grain starch concentration, represents the best balance for high starch concentration and potential alcohol yield (2). Therefore, these attributes are seen as critical determinants of alcohol yield following fermentation of the malted barley wort. Grain protein concentration is the cumulative result of factors such as barley variety, soil nitrogen availability, field topography, soil type, cropping history, sowing date, nitrogen fertiliser application rate and timings, plus environmental conditions (3,4,5).

Grain nitrogen, once hydrolysed to amino acids and short peptides, is important for nutrition of yeast during fermentation, and the enzymatic modification of the endosperm (6). The two main measures of enzyme activity, dextrinizing units (DU) and diastatic power (DP), reflect the activity of α-amylase and the combined activity of the starch degrading enzymes, respectively. The starch degrading enzymes consist of β-amylase, α-amylase, limit dextrinase and α-glucosidase; with DP providing a measure of mainly β-amylase (7). α-Amylase is synthesised in the aleurone layer during endosperm modification, whereas β-amylase is already formed and present in the endosperm and released during the malting process. In general, the greater the degree of endosperm modification, the greater the concentration of α-amylase synthesis and the more β-amylase released from the protein-starch matrix. With under-modification, there is the risk that β-amylase remains trapped in the matrix, where it is unable to breakdown starch fragments to maltose although it has been observed that temperatures of 55-60 °C during mashing can liberate bound β-amylase (8). Under-modification can also lead to wort separation and filtration issues due to cross linking of non-degraded cell wall components such as β-glucans (9).

Previous work (10, 11) has reviewed the impact of nitrogen concentration on malting performance and the properties of the wort produced. These studies showed that during the germination-phase of malting the endosperm of barley grain with lower nitrogen concentration modified more-rapidly. Consequently, while the extract quality was high, there was also lowered concentrations of soluble nitrogen, free amino nitrogen (FAN) and peptides – which are required to help optimise yeast growth and metabolism. Conversely, high nitrogen concentration was found to decrease malting performance and result in a reduced 'extract' quality, that is lower levels of saccharifiable sugars recovered in the wort solution (12, 13).

In this study, we report on the malt- and wort-qualities produced from barley grain with a low (1.16 %) nitrogen concentration compared to a typical malted barley specification and a commercially available malted barley. The low nitrogen concentration barley crop was grown without the application of inorganic nitrogenous fertiliser. Thus, these studies present an opportunity to test whether there is a relationship between the grain nitrogen concentration and alcohol yield potential. That is, if the latter is not compromised by the former, then we may speculate that less inorganic nitrogen fertiliser may be applied to lower the environmental impact of barley production without compromising malting qualities or alcohol yields.

Materials and Methods

Barley grain with low nitrogen concentration

Sample collection - Samples of barley cv. Octavia were obtained from field trials conducted at the James Hutton Institute, Invergowrie (UK, harvest 2015). The barley was grown at 100 % of the recommended seeding rate (185 kg/ha) with pre-emergence weed control, and no fertilisers or later weed control applied. Desiccant (glyphosate) was applied at maturity of the barley. Grain was cleaned and screened (> 2.5 mm mesh) prior to malting. The barley grown had a total nitrogen of 1.16 %, 2.3 % of screenings <2.5 mm, a thousand grain weight (TGW) of 50.5 g and a moisture content of 10.3%.

Malting - The barley samples were replicated thrice in a Steep Germination micro-malting machine (CLP) using the following malting regime: steeping 8 h wet at 12 °C, 15 h air at 16 ˚C, 8 h wet at 12 ˚C; germination 96 h at 14-16 ˚C; and kilning for 18 h at 70 ˚C.

Control distilling malt

Distilling malt (produced from barley cv. Concerto) for comparison of fermentation performance was obtained from Brewers Select Limited (UK). The malt had a total nitrogen concentration of 1.37 % (on a dry weight basis) which is at the lower end of nitrogen concentration for a typical distilling malt. The remaining malt quality indicator values were as follows - dextrinising units: 57, diastatic power: 64.0 ˚Lintner ("as is"), friability: 93 %, homogeneity: 98 %, soluble nitrogen ratio: 35.7, Extract (0.7 mm, "as is"): 79 %, fermentable extract (0.7 mm): 68 %, fermentability (0.7 mm): 86.5 %, β-glucan: 120 mg/L. This malted barley represents a commercially available malt distilling product of conventional nitrogen concentration.

Malt analyses

Malt analyses of the micro-malted low nitrogen concentration barley and control malt were carried out by a commercial malting company (courtesy of Bairds Malt Ltd, Arbroath, UK) using standard industry methods for good comparison with commercial practice. The parameters measured included: friability; homogeneity; whole corns; enzyme activity via measurement of diastatic power and dextrinising units; wort β-glucans; extract; fermentable extract; total nitrogen; soluble nitrogen; soluble nitrogen ratio (SNR); fermentability; free amino nitrogen and, predicted spirit yield (PSY). The use of a well-known commercial malting company for these analyses is important in removing laboratory variation in the assay of some parameters such as the enzymes utilised, including the substrates used to indicate presence of enzymes rather than activity.

Fermentation performance

Wort preparation and fermentation - Wort was prepared using the Analytica EBC method 4.6.1 (14). Mashes were prepared in duplicate for each of the three malt samples with low nitrogen concentration (totalling 6 mashes and fermentations). Three replicate mashes were completed for the control distilling malt. All were adjusted with distilled water to a specific gravity of 1.036. Yeast (Anchor Dry strain of *Saccharomyces*

cerevisiae, Lallemand Biofuels & Distilled Spirits) was pitched at the manufacturer's recommended pitching rate (1 g dried yeast/L, 1.85 x 10^{10} viable yeast cells/g) into a Duran bottle containing 200 mL of the wort, with a headspace of 340 mL, before fermenting at 30 °C, static, in a recirculating water bath (TXF200, Grant Instruments) as an incubator. Fermentation was monitored using the RF Gas Production System (ANKOM Technology) for 67 hours with a pressure reading recorded every 5 minutes. The resulting gas production data was analysed using the Neural Network Function of JMP 14.1.0 64-bit version (SAS Institute Inc.) to generate the equation that represents the gas production rate based on the resulting 804 data points obtained per fermentation. Based on this the inflection points were calculated to identify the time point of maximum gas production (Vmax, mL /h) and the initiation and termination of the exponential gas production phase. The JMP script can be found in Appendix 1.

Wort and wash analysis - The pH (HI 208 pH meter, Hanna) and density (DMA35, Anton Parr) of the wort and the wash (fermented wort) were measured. The yeast cell count including viability (Neubauer haemocytometer) of the wash were measured according to EBC method 3.1.1.1 Haemocytometry and 3.2.1.1 Methylene Blue/Violet Stain (14).

HPLC analysis - D-(+)-glucose (>= 99.5 %, Sigma Life Science), D-(+)-maltose monohydrate (>= 99.0 %, Sigma Life Science), maltotriose (93.0 %, Acros Organics), glycerol (95-98 %, Sigma-Aldrich) and ethanol (>= 99.0 %, Fisher Scientific) concentrations were measured by High Performance Liquid Chromatography (HPLC). The HPLC system consisted of a SP8800 ternary HPLC Pump (Spectra-Physics) equipped with a SpectraSERIES AS100 Autosampler Pump (Spectra-Physics) and a SP6040XR RI detector controller Pump (Spectra-Physics). Analyses were carried out with a ROA-Organic Acid H+ Ion Exclusion column (Phenomenex Inc) with the dimensions 300 x 7.8 mm. The detector was set at 40 °C. The mobile phase was isocratic and consisted of water with 0.0025 M sulphuric acid (95-98 % A.C.S. reagent; 1 L; Sigma-Aldrich) with a flowrate of 0.6 mL/min. Before analysing samples, the system was calibrated by running a calibration line of the analytes at concentration between 20 g/L to 0.3125 g/L. Xylose (99+%) (Acros Organics) at a concentration of 7.5 g/L was used as an internal standard. Samples were centrifuged at 1.500 x g for 2 min (Centrifuge 5702, Eppendorf) in a 5702/R A-4-38 rotor (Eppendorf) and filtered through a 0.45 µL syringe filter (Merck Millipore). Before injecting, samples were diluted to be in the calibrated range based on the measured gravity. Data acquisition was performed using CSW32 version 1.3.4 software.

Statistical Analysis

Unless stated otherwise *t*-tests were conducted using Minitab 19. One sample *t*-tests were carried out to compare malting trials to a single value from a typical malted barley specification and independent *t*-tests were performed to compare test and control malt fermentation data.

Results & Discussion

Malting Performance

The α-amylase activity of the low-nitrogen concentration malt was significantly higher than the minimum stated within the typical malt specification (DU, Table 1). As α-amylase is synthesised during the malting process a high level of this enzyme synthesis, measured as DU, indicates that the malting process has proceeded as expected. In contrast, β-amylase is released from the latent form during the malting process. The combined activity of the DP enzymes, mainly measured as β-amylase (Table 1), was significantly lower ($p<0.01$) in the low-nitrogen concentration malt. This suggests that a lower level of β-amylase was present in the protein matrix and likely as a result of the lower nitrogen concentration. In general, β-amylase is considered the most important DP enzyme due to its involvement in the release of maltose sugars. Also, its activity positively correlates with DP and with protein concentration, in particular the hordein fraction (15, 16, 17). However, the fact that the low-nitrogen concentration malt was well modified was evident on comparison of the test malt parameters to that of a typical malt specification (Table 1). This confirms conclusions of previous studies that specifications for enzymes such as α-amylase and β-amylase in malt are best set at a range of values rather than as single values (18, 19). Furthermore, measurements of friability and homogeneity (Table 1) showed that the low nitrogen-concentration malt performed as well (homogeneity) or better (friability, $p<0.05$) when compared to a typical malt specification. This indicates that the soft mealy endosperm of the barley with lower nitrogen was sufficiently modified in an even, consistent manner to produce a more friable fraction of malt. The higher soluble nitrogen ratio (SNR, soluble-/total-nitrogen), also known as index of modification (IoM) obtained from the lower nitrogen malted barley further confirmed that the test malt was well modified. Significant protease activity will hydrolyse the protein matrix of the endosperm. Indeed, the index of modification (49 %) achieved for the lower nitrogen barley appear to suggest over-modification considering malt is judged adequately with a maximum IoM of 40 % (20). In this regard it is important to note that low nitrogen malt is associated with a greater percentage of mealy grains (i.e. flour-like), whereas higher nitrogen concentration is associated with an increase in steely (i.e. hard) grains (11, 12). Mealiness and steeliness will affect the malting performance of barley during malting. Thus, the modification indicators of friability, homogeneity, whole corns and SNR are consistent with the understanding that low nitrogen-concentration grains are more likely to have a more-mealy endosperm structure and a faster malting rate (11), typically leading to better endosperm modification: as confirmed by the PSY results.

Predicted Spirit Yield

To maximise the production of alcohol the malting process must produce a malt that has a high extract with a high degree of fermentability, resulting in a high spirit yields when processed within a distillery. Malts made from the test barley and control commercial barley were mashed in a similar way. Analysis of the wort indicated that the low nitrogen malted barley gave both higher extract results (81 %) and fermentable extract result (69 %) when compared to extract results (79 %) and fermentable extract (68%) obtained from the control malt. Again, on fermentation of the wort, the test malt also gave a higher average PSY (419 L/t) when compared to the PSY (410 L/t) obtained from the control malt (Table 1). These

results are consistent with reported data which found barley grain with a low nitrogen-concentration led to a higher yield of extract as a result of increased carbohydrate content and improved endosperm modification when compared to a barley with a higher nitrogen concentration (10). An important observation worth mentioning is that the lower nitrogen malted barley produced similar glucose to maltose ratio of 1:6 usually associated with elite malted barley (21). The glucose to maltose ratio found in the wort of the test malt is comparable to a ratio of 1:8 obtained from the control malt (Table 2a). This is important because a wort where glucose is in excess to maltose, some yeast strains may lose their ability to ferment maltose (22). Despite having greater maltotriose and elevated glycerol at the end of fermentation, ethanol levels did not differ between the low nitrogen-concentration and the control malts (Table 2b).

FAN is important in yeast growth and performance during fermentation therefore impacts fermentation rate and the efficient flow of fermented wort (wash) from fermenters. Although the low nitrogen-concentration malt had a significantly lower FAN concentration (115.7 mg/L) than that of a typical malt (150-180 mg/L, Table 1), it was found to ferment at a faster rate (Figure 1, Table 3). It is not clear at present if the higher SNR (49) or IoM (49 %) found in the malt made from the low nitrogen barley resulted in over-modification and, if the control malt was modified to a comparable IoM, whether this difference in enzyme and fermentation performance would persist. Over-modification of malt will cause some losses of hydrolysed soluble nitrogen materials through the embryo and then to the rootlets and shoots. Notwithstanding, in the early 1970s Meilgaard (23) showed that yeast required approximately 100 mg/L of FAN for efficient fermentation to occur. Additional studies are in agreement with the results presented here (23, 24). In addition, it was also observed that barley varieties such as Chariot and Optic can produce reduced levels of FAN products, yet support similar yeast performance, indicating that specific peptides rather than total-FAN *per se* determine fermentation efficiency (25, 26). It is also important to note that both Chariot and Optic were extensively used in distilling for a long period of time. Therefore, the low nitrogen malt studied is likely to have produced peptides that were readily assimilated by yeast during fermentation bringing into question the value of FAN as a parameter to dictate malt quality and, therefore, market value. At the end of fermentation, no statistically significant differences between the low nitrogen-concentration and control malt were found for yeast cell count and viability (Table 4).

Processability

Despite the low nitrogen-concentration malt demonstrating a high PSY, incomplete breakdown of cell wall material or proteins can result in decreased extraction efficiency due to the restricted access of hydrolytic enzymes to their substrates. This can lead to an increased extract viscosity, separation and/or wort run-off issues, and insufficient FAN for yeast growth during fermentation. Beta-glucan is a critical cell wall component known to impact upon the ease of processing negatively. Although previous studies found a weak relationship between total nitrogen concentration and glucan content (9, 12, 27) similar processing issues can also be due to insufficient breakdown of cell wall material. The low nitrogen malt produced wort that contained much lower β-glucan concentration (64.3 mg/L, Table 1) compared to the concentrations 100 -300 mg/L usually associated with typical commercial malt (28). This observation further showed that adequate breakdown of β-glucan was achieved during the malting process. This observation is very important because

β-glucanase is not active during the mashing process (27). Again, the high friability score (as seen for the low nitrogen test malt), is a direct measure of cell wall breakdown and further confirms the faster filtration rate observed when the wort of the mashed malt made from the low nitrogen barley (results not shown). Even with a well modified malt a narrow range of modification within the batch is still important. A high homogeneity value and low whole grain percentage confirms a uniform modification and, therefore, processability.

Sustainability & Economic Viability

Raw ingredients account for a significant portion of a spirit's carbon footprint. Lienhardt et al. (29) demonstrated that a gin made from pea based neutral spirit had a lower environmental footprint across 12 of the 14 environmental impact categories they assessed than one made from wheat. Examining the greenhouse gas (GHG) footprints of the production life cycle for a selection of spirits from the world's largest producer (Diageo), it was found that the key brand Johnnie Walker Whisky had 29 % of its GHG footprint assigned to production of raw commodity (i.e. harvested grain) compared to another four different spirits (Captain Morgan (rum) 28 %; Smirnoff (vodka), 25%; Tanqueray (gin), 25 %) (30), and likely to contain the highest malted barley content. Reducing inorganic fertiliser use for raw material production is a potential option for reducing the GHG footprint. The 244 kha of Scottish spring barley sown in 2017 (31) required the application of 27 kt of nitrogen (110 kg/ha, usually as ammonium nitrate, 34.5% nitrogen. 2014-2018, 5-year mean) to achieve the desired yields (32). Accompanying this fertiliser use is a GHG footprint via the liberation of an estimated 1 t CO_2e/ha (ammonium nitrate releases 9.14 kg CO_2e/kg nutrient) (33). This is concomitant with pollution of soil and waterways too, via diffuse pollution of excess fertiliser-nitrogen which is leached. Growing this crop without the use of such fertiliser would not only avoid these emissions but also have a financial saving of around £20 million (based on average ammonium nitrate price £258/tonne, in August 2019) (34). The reduction in inorganic nitrogen use can, however, impact yields of cereal crop negatively, reducing the tonnage obtained per hectare. Therefore, the implications of the findings presented here is not necessarily that less nitrogenous fertiliser should be applied per se, rather that barley with a grain nitrogen concentration which is lower than industry recommended thresholds should not be rejected as a distilling feedstock. Further work is required to determine the balance between yield and inorganic fertiliser use and alternative cropping systems which rely on a higher level of organic nitrogen provision as provided, for example, by biological nitrogen fixation by legumes. Reliance upon organic legume-supported (i.e. organic nitrogen dependant) cropping need not compromise yield, and Iannetta et al. (35) has reported that this approach maximises productivity whilst minimising inorganic nitrogen use. Also, considering the environmental and social impacts of excessive nitrogen applications, it is an approach which is unlikely to remain acceptable in the longer term. This is especially true when use of 'best nitrogen management practices', as described by Good and Beatty (36), indicate that it is possible to reduce global applications rates by 20%, mainly in more 'developed' regions of the world, where high-input inorganic practices are the convention.

Conclusion

This study compared the malting and fermentability characteristics of a barley cultivar (Octavia) grown without added nitrogenous fertilisers. Octavia is of particular commercial relevance and credibility to major whisky producers. Concerto, another commercially significant barley variety, was cultivated more conventionally and its resultant higher nitrogen malt was judged to be a suitable comparator for distilling applications. It is salient to note that while the malting barley cultivar tested here is one of the main varieties in use by industrial distillers in the UK, and that the malting regime itself presents the major factors influencing the success of the malting process, future work might confirm this null hypothesis: that malting barley genotype does impact on these conclusions made here. In Scotland, the short and variable growing season can prevent spring sown barley from meeting the strict specifications that define malting quality barley grain. The results presented here indicate that barley grain with a low nitrogen concentration can produce malt of an acceptable quality for spirit production and, therefore, could be used to extend the acceptable range of barley nitrogen concentration. An important message from this study is that a higher nitrogen concentration in barley does not strictly equate to high enzyme production as, whilst all enzymes are proteins, not all proteins are enzymes. This study supports this theory as the low protein concentration barley produced malt with a very high α-amylase compared to the control malt. Furthermore, as lower nitrogen facilitates modification the malting process can be expedited, saving time, malting losses and energy (10). The study also showed that inorganic nitrogen fertiliser applications could be lowered to help reduce the environmental impacts of excessive inorganic nitrogen usage for spring barley production – assuming conventional yields can be preserved. Alternatively, the economic loss and/or environmental benefits accrued by avoiding excessive nitrogen-fertiliser use may be remunerated somehow and distributed fairly among the responsible corporate supply-chain actors (37). Equally, it may also be that any reduction in yield and quality due to low inorganic fertiliser nitrogen use can be overcome with the development of new barley cultivars which are better suited to low-input agricultural systems.

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Declaration of interest

The authors declare there are no conflicts of interest.

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Appendix 1 - Supplementary Data – Script for data analysis of gas production data collected by the ANKOM RF Gas Production System with JMP

```
dt = Current Data Table();
obj = Neural(
	Y( :Name( "MW 1g/L" ) ),
	X( :Time ),
	Informative Missing( 0 ),
	Validation Method( "Holdback", 0.3333 ),
	Fit( NTanH( 15 ) )
);
obj << (Fit[1] << Save Profile Formulas);
Rsquare = Report( obj )[Number Col Box( 2 )][1];
New Column( "Rsquare", Numeric, "Continuous", Format( "Fixed Dec", 12, 7 ) );
For( x = 1, x <= N Rows( dt ), x++,
	dt:Rsquare[x] = Rsquare
);
obj << close window;
Column( dt, 3 ) << set name( "Predicted" );
New Column( "First Derivative",
	Numeric,
	"Continuous",
	Format( "Fixed Dec", 12, 2 ),
	Formula( Eval( Derivative( Eval( :Predicted << get formula ), :Time ) ) )
);
New Column( "y",
	Numeric,
	"Continuous",
	Format( "Fixed Dec", 12, 2 ),
	Formula(
		Col Maximum(
			If( :First Derivative == Col Maximum( :First Derivative ),
				:Name( "MW 1g/L" ),
				Empty()
			)
		)
	)
);
New Column( "b",
	Numeric,
	"Continuous",
	Format( "Fixed Dec", 12, 2 ),
	Formula(
		Col Maximum(
			If( :First Derivative == Col Maximum( :First Derivative ),
				:y - :First Derivative * :Time,
				Empty()
			)
		)
	)
);
New Column( "Lag (h)",
	Numeric,
	"Continuous",
	Format( "Fixed Dec", 12, 1 ),
	Formula( -:b / Col Maximum( :First Derivative ) )
);
New Column( "Vmax (mbar/h)",
	Numeric,
```

```
		"Continuous",
		Format( "Fixed Dec", 12, 2 ),
		Formula(
			Col Maximum(
				If( :First Derivative == Col Maximum( :First Derivative ),
					:First Derivative,
					Empty()
				)
			)
		),
		Set Selected
	);
	New Column( "TC (h)",
		Numeric,
		"Continuous",
		Format( "Fixed Dec", 12, 2 ),
		Formula( Col Minimum( If( :First Derivative < 20 & :Time > 20, :Time ) ) ),
		Set Property(
			"Response Limits",
			{Goal( Maximize ), Importance( 1 ), Show Limits( 0 )}
		)
	);
```

Table 1: Functional attributes of low nitrogen-concentration malt compared to a typical commercial distilling malted barley specification ([a]20, [b]39, [c]28). Means and standard deviation shown. T-test conducted using the typical malt specification limit or, where a range is given, the midpoint. Statistical significance levels set as: NS, non-significant p>0.05, * p<0.05, ** p<0.01, *** p<0.001.

Malt quality indicators*	Low Nitrogen Malt		Typical Malt Specification	Statistical Significance
	Mean	SD		
Total Nitrogen (%, dry weight basis)	1.01	0.08	< 1.5 [a, b]	*
Dextrinising Units	58.00	4.36	> 45 [a]	*
Diastatic Power (°Lintner, "as is")	56.67	1.15	65-75 [a]	**
Predicted Spirit Yield (Litres of alcohol (LA)/tonne, 0.7 mm, "as is")	419.00	2.00	410.00 [b]	*
Friability (%)	98.33	0.58	> 96.00 [a]	*
Homogeneity (%)	99.33	0.58	> 98.00 [a]	NS
Whole Corns	0.40	0.20	Not specified	-
Soluble Nitrogen Ratio	49.10	2.43	< 40 [a]	*
Extract (%, 0.7 mm, "as is")	81.27	0.31	> 78 [a]	**
Fermentable Extract (%, 0.7 mm)	69.17	0.35	> 68 [a]	*
Fermentability (%, 0.7 mm)	85.13	0.21	87-88 [a]	**
Free Amino Nitrogen (mg/L)	115.67	2.08	150-180 [a]	**
β-glucan (mg/L)	64.33	6.35	100-300 [c]	**

Table 2: Composition of low nitrogen malt (1.01 % nitrogen) compared to distilling malt (1.37 % nitrogen) in a) wort prior to fermentation and b) wash post fermentation. Mean results and standard deviation shown. Analysis of variance conducted with statistical significance levels set as: NS, non-significant p>0.05, * p<0.05, ** p<0.01, *** p<0.001.

a)

	Low Nitrogen Malt (g/L)		**Commercial Malt** (g/L)		**Statistical Significance**
	Mean	**SD**	**Mean**	**SD**	
Maltotriose	15.08	0.402	12.27	0.208	***
Maltose	50.25	0.917	49.45	0.534	NS
Glucose	8.68	0.144	6.43	0.120	***
Total Sugars	74.01	1.33	67.875	0.797	***
Glycerol	0	-	0	-	-
Ethanol	0	-	0	-	-

b)

	Low Nitrogen Malt (g/L)		**Commercial Malt** (g/L)		**Statistical Significance**
	Mean	**SD**	**Mean**	**SD**	
Maltotriose	1.83	0.214	1.04	0.027	***
Maltose	0	-	0	-	-
Glucose	0	-	0	-	-
Glycerol	1.77	0.028	1.62	0.008	***
Ethanol	45.50	0.918	43.84	1.441	NS

Table 3: Maximum gas production (Vmax, mL/h) during fermentation and the initiation and termination of the exponential gas production phase. Analysis of variance conducted with statistical significance levels set as: NS, non-significant p>0.05, * p<0.05, ** p<0.01, *** p<0.001.

		Low Nitrogen Malt		Commercial Malt		Statistical Significance
		Mean	**SD**	**Mean**	**SD**	
Exponential Phase (h)	Start	5.5	0.19	6.7	0.10	***
	End	41.91	3.40	44.42	1.04	NS
Max gas production (Vmax, mL/h)		200.1	11.4	203.8	5.41	NS

Table 4: Suspended yeast cell counts and viability after 67 h fermentation of low nitrogen-concentration malt (1.01 % nitrogen) compared to distilling malt (1.37 % nitrogen). Mean results and standard deviation shown. Analysis of variance conducted with statistical significance levels set as – NS, non-significant $p>0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

	Low Nitrogen Malt		**Commercial Distilling Malt**		**Statistical Significance**
	Mean	**SD**	**Mean**	**SD**	
Cells/mL	8.6×10^6	8.0×10^5	8.4×10^6	1.3×10^5	NS
Viability (%)	70.9	2.9	67.1	2.5	NS

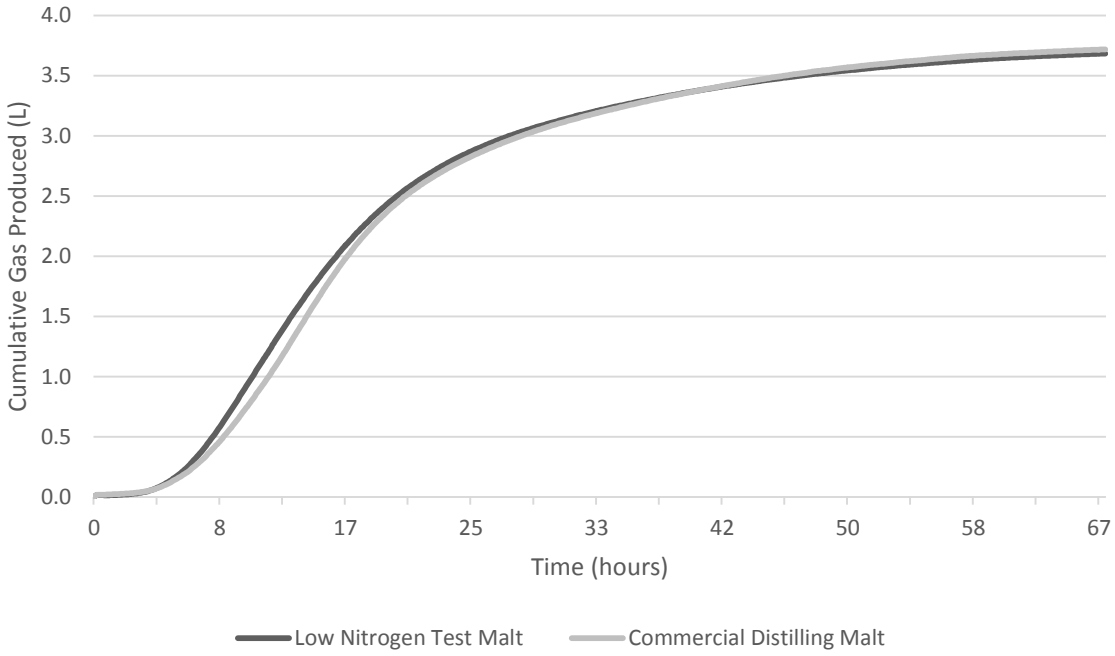

Figure 1: Fermentation gas production (CO_2) in L as an indicator of fermentation rate.