Importance of Endosperm Modification for Malt Wort Fermentability[1]

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ABSTRACT

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Malt wort fermentability is dependent on an adequate supply of the essential nutrients required by yeast. The barley and malt factors affecting this supply of nutrients are not well understood. This study used two doubled haploid populations (Arapiles × Franklin, TR251 × HB345), the latter with a hulless barley parent, to investigate effects of barley and malt quality on fermentability. Populations were grown and malted at different locations resulting in a broad range in malt quality with significant differences in extract, modification and enzyme levels. Fermentability, as indicated by apparent attenuation limit and ethanol levels, also showed significant differences among samples. Modification was the most important factor for good fermentability. There appeared to be several different mechanisms by which modification affected fermentability. High viscosities, slow starch release during mashing, increased glucose supply from better β-glucan breakdown and increased free amino nitrogen levels all affected fermentability. Effects of starch-degrading enzymes on fermentability became more significant in better modified malts with α-amylase showing stronger effects than diastatic power. The poorer fermentability of hulless barley malt was predominately due to low levels of α-amylase, although, free amino nitrogen also appeared to be an important factor.

Key words: Barley, hulless, β-glucan, ethanol, fermentable sugars, free amino nitrogen.

INTRODUCTION

Malt wort fermentability is a complex process dependent on several factors which complicates its measurement and makes it difficult to breed barley varieties with known fermentation potential. The fundamental requirement is to supply yeast with adequate nutrients, primarily fermentable sugars and free amino acids, but also micronutrients such as minerals and vitamins. Levels of the nutrients will ultimately depend on other factors, particularly levels of enzymes such as proteases, β-glucanases and starch-degrading enzymes. Fermentability is also strongly affected by conditions used in the brewhouse such as yeast strain, wort oxygenation, cellar temperature and tank pressure.

The complexity of malt and its fermentability has led to inconsistent conclusions on malt factors of greatest consequence to fermentability. For example, fermentable sugars, the most abundant nutrients in wort, have been shown by some to strongly affect fermentability[15] while others reported no effect[3,12,23]. Relationships between fermentability and levels of starch-degrading enzymes have also been controversial. Recently there has been some consensus on the importance of these enzymes but only when allowances were made for individual enzymes, such as α-amylase, beta-amylase and limit dextrinase versus diastatic power[11], and when allowances were made for differences in beta-amylase thermal stability[9,11,15]. Research has shown that positive effects of increased enzyme levels can be negated by other factors such as starch gelatinisation temperature[26], although, others have found this effect insignificant[11]. Gjertsen and Hartlev[13] also found enzyme levels had little effect on fermentability when enzymes were present at high levels, in which case modification was more important. Several fermentability studies[3,15] have shown a need for adequate modification. Bathgate et al.[2], though, found fermentability could be limited by over modification because of increased levels of soluble protein and reduced levels of fermentable sugars due to elevated malting losses. MacGregor[21] indicated starch granules readily gelatinize during mashing in malts with good β-glucan breakdown but with only adequate, not complete protein breakdown. Starch gelatinized at lower temperatures is more completely hydrolysed to fermentable sugars as starch-degrading enzymes are still active. Good modification also ensures adequate levels of amino acids for the yeast, although, in low gravity, or all malt worts, free amino nitrogen (FAN) is seldom limiting[24]. In worts produced where adjuncts are used (malt 70% fermentable material) or the extreme case of Japanese-style Happoshu (malt 25% fermentable material), FAN may well become limiting.

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Endosperm modification is affected by a range of barley characteristics including genetics, growing conditions as well as processing conditions in the malthouse. Each malting barley variety, while bred for rapid, efficient modification, has a varietal specific pattern and rate of modification. Barley growing conditions can also affect modification potential. High barley protein concentration resulting from drought, excess available nitrogen or other environmental factors can severely alter the modification properties of a barley sample[19]. Hard kernels, often referred to as steely kernels, have also been shown to modify more slowly than mealy type kernels[17]. Processing conditions in the malthouse also have a marked effect on modification[23].

Doubled haploid populations are excellent tools for studying complex traits[28] such as fermentability. The nature of such populations is a random assortment of genes, assuming no linkages, allowing for isolation of individual malt traits and a better possibility of studying effects on fermentability. In particular the effects of endosperm modification on fermentability could be studied independent of the effects of starch-degrading enzymes, provided there were no linkages between these two factors. The present study investigated the malt quality and fermentability properties of two doubled haploid populations; Arapiles × Franklin, an Australian population (Moody – personal communication), and TR251 × HB345, a Canadian hulless/covered population (Legge – personal communication). The parents of these populations offered the potential for a wide range in endosperm modification while different growing and processing conditions also increased the likelihood of a range in malt quality, thus, a good set of samples to study the complex nature of fermentability.

MATERIALS AND METHODS

Arapiles/Franklin population – Malting and malt analysis

A doubled haploid population (175 lines) from the cross Arapiles × Franklin, along with three replicates of the two parents, were grown at Charlick, South Australia in 2001. Arapiles and Franklin are two malting varieties with known malting potential and different beta-amylase alleles, with Franklin exhibiting the Sd1 and Arapiles the thermostable Sd2H form of the enzyme[8]. Samples (60 g) of the doubled haploid lines and parents were micromalted at the University of Adelaide using a Phoenix Automated Micromalting machine (Adelaide, SA, Australia) according to the following schedule: wet steep 7.5 h, air rest 8 h, wet steep 9 h (steeping at 15°C), germination 95 h (15°C), kiln 9 h @ 40°C, 4 h @ 60°C, 2 h @ 70°C, 4.5 h @ 80°C, 0.5 h @ 25°C.

Malt analysis of the Arapiles/Franklin samples was performed at the University of Adelaide according to Analytica EBC standard methods[10], Malt-4.5.1 (malt extract, a small version); Malt-4.16.1 (enzymatic wort β-glucan), Malt-4.8 (viscosity), Malt-4.9.2 (soluble protein) and Malt-4.10 (FAN). Diastatic power (DP) and α-amylase activity were measured spectrophotometrically using PAHBAH (4-hydroxybenzoic acid hydrazide), although, DP results were reported in °Lintner units[16].

Covered/hulless population – Malting and malt analysis

A second doubled haploid population (53 covered and 54 hulless lines) was previously produced by anther culture techniques at Brandon Research Centre, Agriculture and Agri-Food Canada, from the cross TR251 × HB345. TR251 was a malt barley breeding line with good malt potential while HB345 was a hulless feed breeding line with good disease resistance and the heat stable beta-amylase gene (Legge – personal communication). The 107 doubled haploid lines, along with two replicates of each parent, were grown at Hamiota, Manitoba in 2002. Samples (500 g) of the lines and parents were micromalted at the Grain Research Laboratory in Winnipeg using a Phoenix Automated Micromalting machine (Adelaide, SA, Australia) according to the following schedule: wet steep 6 h, air rest 2 h, wet steep 4 h, air rest 12 h, wet steep 4 h, air rest 4 h, wet steep 4 h, air rest 4 h, wet steep 4 h (steeping at 13°C); germination 100 h (15°C), kiln 12 h @ 55°C, 6 h @ 65°C, 2 h @ 75°C, 4 h @ 85°C.

The covered/hulless samples were analysed at the Grain Research Laboratory using standard methods of the American Society of Brewing Chemists[1]; Malt 4 Extract, which is identical to EBC Congress extract, Malt 5a Nitrogen in laboratory wort, Wort-2b Extract by digital density meter, Wort 12 Free amino nitrogen, Wort 18 β-Glucan in Congress wort by fluorescence, and modified methods of Malt 6A Diastatic Power and Malt 7B α-Amylase.

Apparent attenuation limit

A small scale method for measuring apparent attenuation limit (AAL)[20] was used at the University of Adelaide to determine the fermentation properties of both the Arapiles/Franklin and the TR251/HB345 samples. The method brought 40 mL of EBC wort to a boil in a microwave followed by cooling, correction for evaporation losses and aeration through agitation. Resulting worts were incubated with 160 mg dried Mauribrew lager 497 yeast (Burns Philp, Toowoomba, Australia) at 25°C for 24 h with constant agitation. A DX-500 HPLC system (Dionex, Sunnyvale, CA, USA) at the University of Adelaide was used to measure levels of fermentable sugars in worts from both populations while ethanol levels in all the fermented worts were measured at South Australian Brewing Company according to Analytica EBC Beer-9.2.4[10].

Statistics

The Analysis of Variance (ANOVA) Procedure of the SAS Institute Inc.[25] was used to analyse the data. Duncan's Multiple Range test was used to determine significant differences among barley lines. Procedures of SAS Institute Inc.[25] were also used to calculate simple correlation coefficients among fermentability parameters and various malt quality parameters. The reverse multiple regression technique of the SAS Institute Inc.[25] was used to determine relationships between AAL or ethanol (dependent variables) and various malt parameters (independent variables) including; β-glucan, viscosity, FAN, DP, α-amylase, fermentable sugars and maltotetraose.

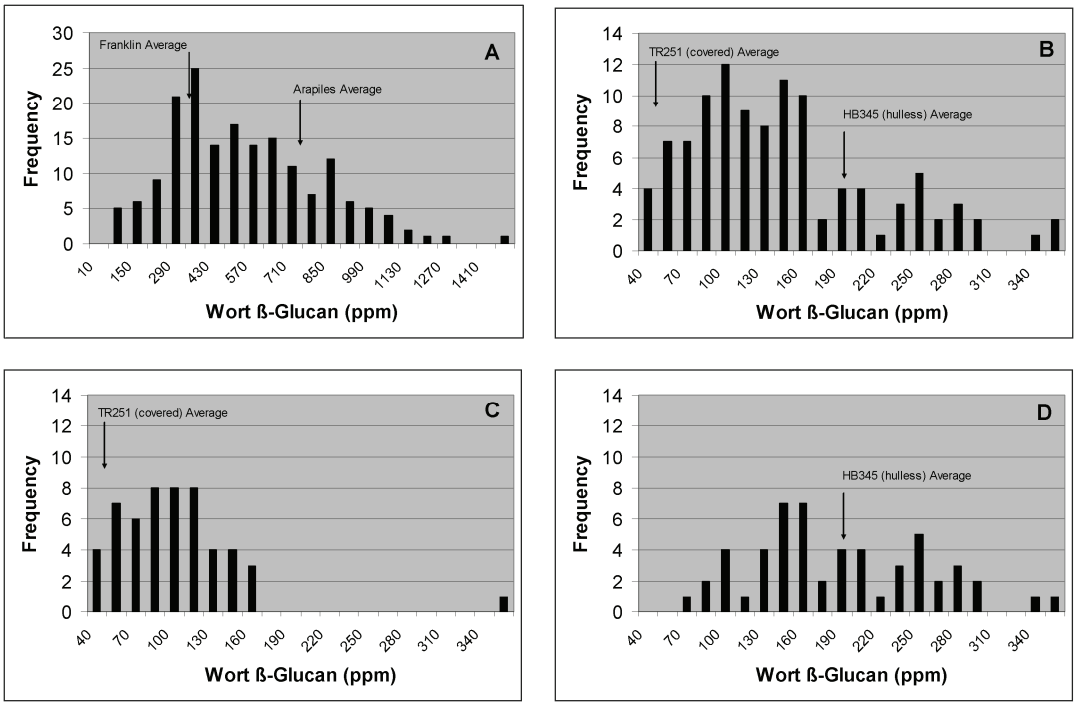

Fig. 1. Histograms of β-glucan levels in EBC worts made from: A) lines and parents of the Arapiles/Franklin population, B) lines and parents of the TR251/HB345 population, C) TR251/HB345 covered lines and parent, and D) TR251/HB345 hulless lines and parent.

RESULTS

The study investigated relationships of malting quality and fermentation properties for samples from two doubled haploid populations. Results showed a broad range in malt quality among the samples. The β-glucan levels in wort ranged from less than 100 ppm to greater than 1400 ppm (Fig. 1). Fig. 2 shows a range in DP from less than 20°L to greater than 300°L. Malt extract levels (Fig. 3) were exceptionally broad (77.5%–87.6%), predominantly because of the hulless samples which ranged from 82.7% to 87.6%. Fermentability varied considerably among the samples (Fig. 4) with AAL from less than 75% to greater than 85%. Fig. 5 shows a large range in ethanol production, 2.6% to 3.9%, which was partly due to higher levels produced by the hulless lines (3.1%–3.9%). As a result of significant differences between the hulless and covered lines within the TR251/HB345 population (Figs. 1 and 3, Table I), all statistical analyses treated the hulless and covered subsets as separate populations.

The Arapiles/Franklin and TR251/HB345 populations had significantly different malt quality. Endosperm modification was especially different with the TR251/HB345 lines, on average, having significantly less wort β-glucan (Table I) and higher FAN than Arapiles/Franklin lines. The hulless lines from the TR251/HB345 population had significantly higher levels of β-glucan than covered lines from that population but FAN levels were not significantly different. The TR251/HB345 hulless lines had significantly higher malt extract (Table I) than either the Arapiles/Franklin lines or the TR251/HB345 covered lines, while the Arapiles/Franklin lines were significantly higher than the TR251/HB345 covered lines. The TR251/HB345 hulless and covered lines had similar DP levels with both having significantly higher DP than the Arapiles/Franklin lines. The TR251/HB345 covered lines had significantly higher levels of α-amylase than the TR251/HB345 hulless lines. Comparisons of α-amylase levels with the Arapiles/Franklin lines were not possible due to differences in units of measurement.

Fermentability results were also significantly different among the three populations (Table I). The TR251/HB345 covered lines showed highest AAL with the TR251/HB345 hulless lines having the lowest. However, the hulless lines produced significantly more ethanol than either hulled group while the TR251/HB345 covered lines produced significantly more ethanol than the Arapiles/Franklin lines.

Relationships between fermentability and malt quality were investigated with correlation analysis which indicated a significant effect of modification on fermentability (Tables II–IV). The β-glucan levels showed negative correlations with AAL for all three groups but especially for the Arapiles/Franklin population. The β-glucan was also negatively correlated with ethanol production for all three populations. Levels of FAN correlated significantly with both AAL and ethanol production in the Arapiles/Franklin population but only with AAL in the TR251/HB345 hulless subset and not at all with the TR251/HB345 covered samples.

Starch-degrading enzyme levels (DP, α-amylase) did show some effect on fermentability in all three populations (Tables II–IV). The effect of DP was very limited in the Arapiles/Franklin lines with α-amylase levels showing a greater effect. The importance of α-amylase was also evident in the TR251/HB345 hulless and covered

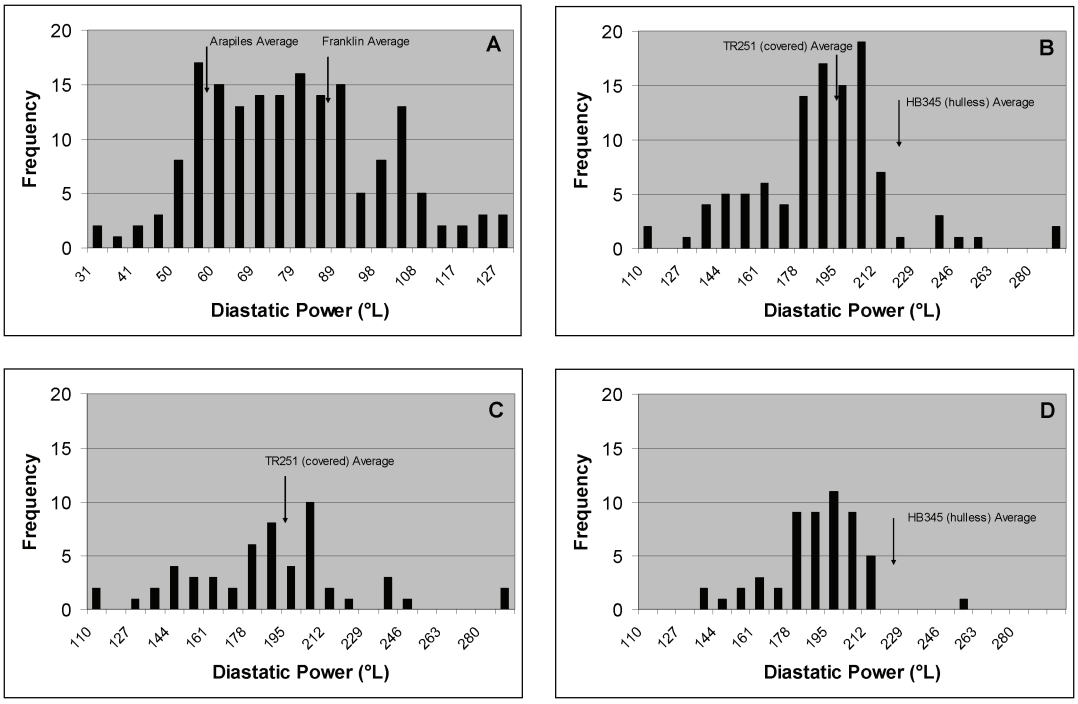

Fig. 2. Histograms of diastatic power (DP) levels in malt from: A) lines and parents of the Arapiles/Franklin population, B) lines and parents of the TR251/HB345 population, C) TR251/HB345 covered lines and parent, and D) TR251/HB345 hulless lines and parent.

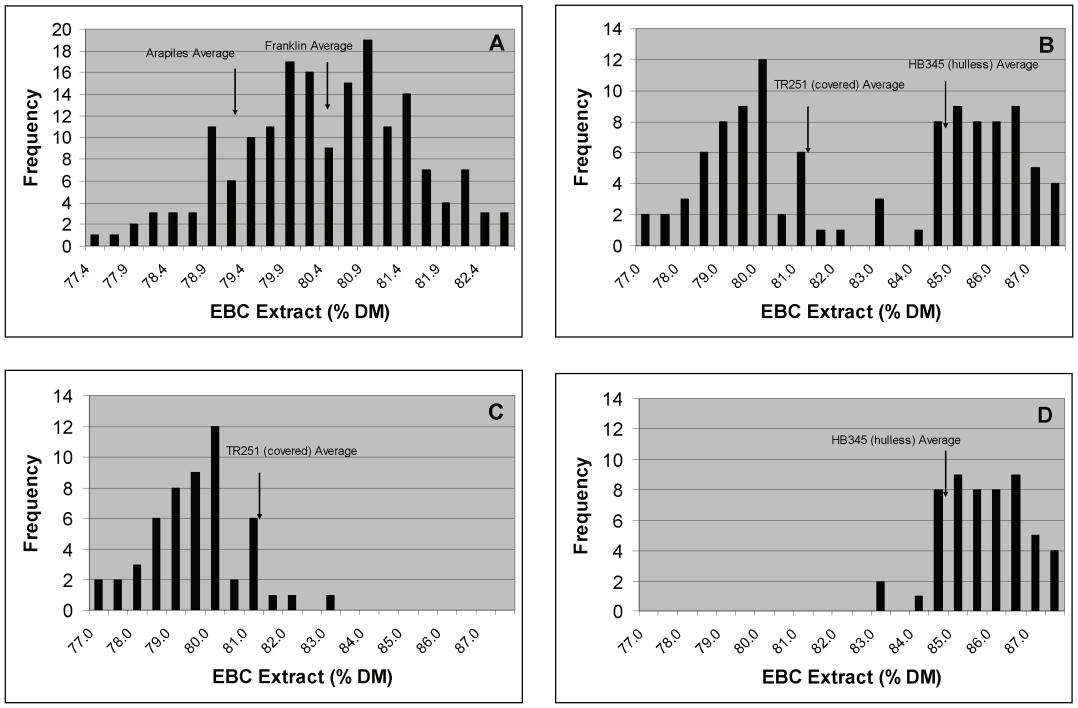

Fig. 3. Histograms of EBC extract levels for: A) lines and parents from the Arapiles/Franklin population, B) lines and parents from the TR251/HB345 population, C) TR251/HB345 covered lines and parent, and D) TR251/HB345 hulless lines and parent.

populations but DP also had a significant effect on AAL for these samples.

Simple sugars were correlated with fermentation parameters for all three populations (Tables II–IV). Maltotetraose, a non-fermentable sugar, showed the most significant effect on AAL, which was negative, for all three populations. It was also negatively correlated with ethanol production for the Arapiles/Franklin population and the TR251/HB345 hulless subset. Maltose, the most abundant fermentable sugar, showed a positive correlation with

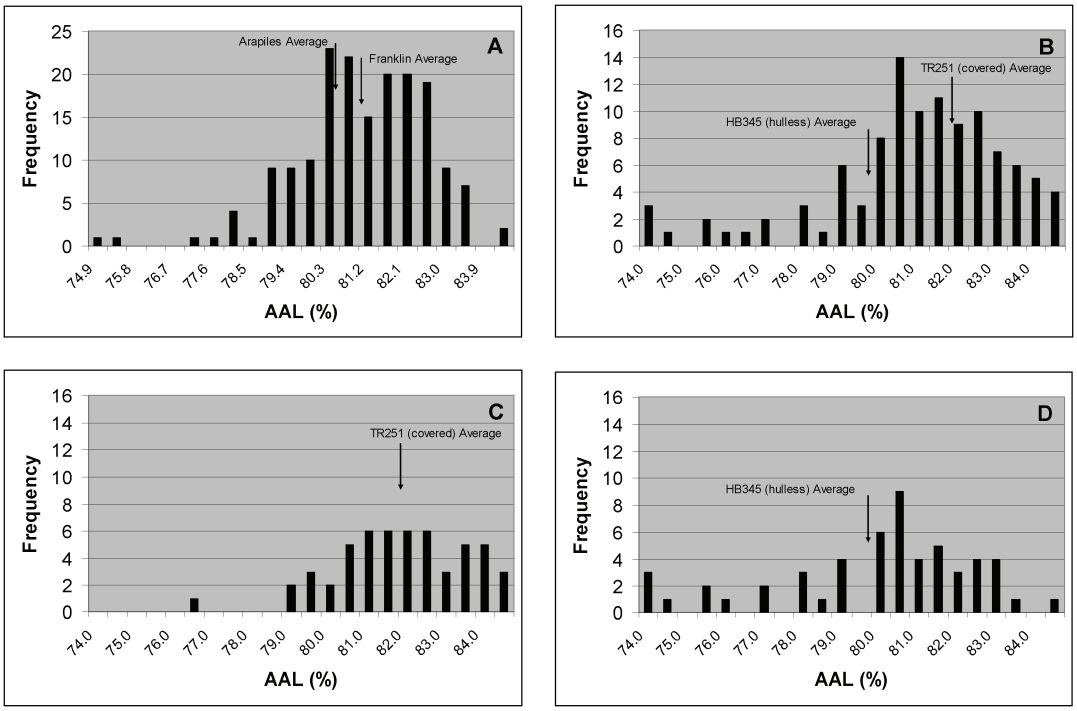

Fig. 4. Histograms of Apparent Attenuation Limits (AAL) for EBC wort made from: A) lines and parents of the Arapiles/Franklin population, B) lines and parents of the TR251/HB345 population, C) TR251/HB345 covered lines and parent, and D) TR251/HB345 hulless lines and parent.

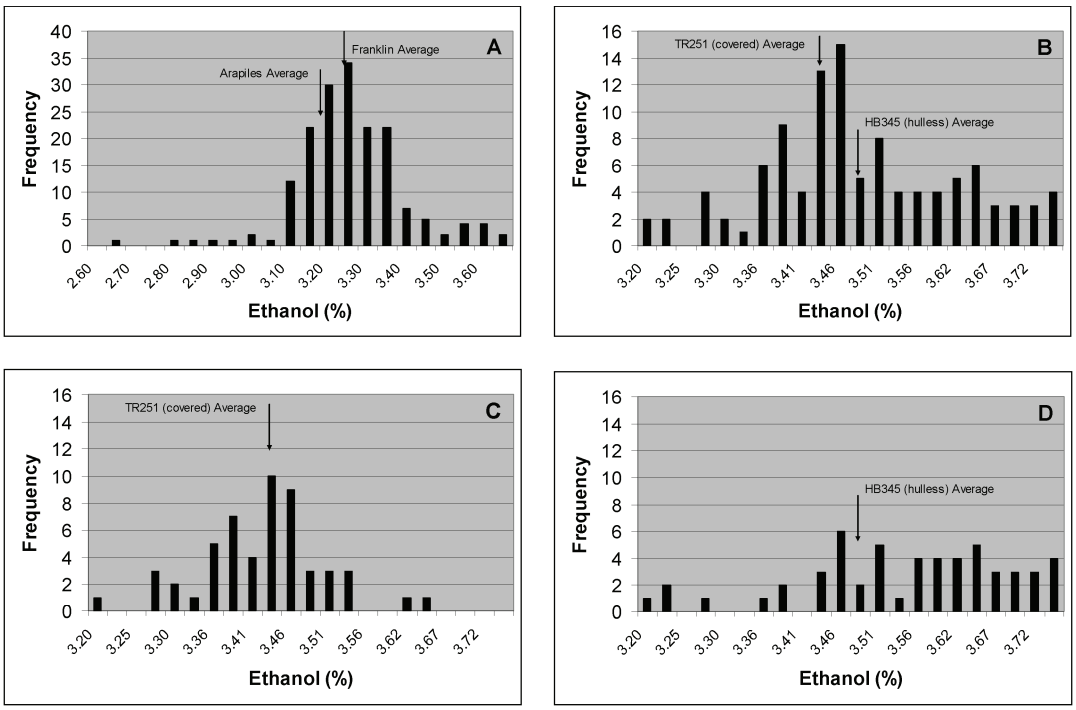

Fig. 5. Histograms of ethanol production from EBC extracts made from: A) lines and parents of the Arapiles/Franklin population, B) lines and parents of the TR251/HB345 population, C) TR251/HB345 covered lines and parent, and D) TR251/HB345 hulless lines and parent.

AAL and ethanol production for both the TR251/HB345 hulless and covered lines but the relationship was not significant for the Arapiles/Franklin population. Glucose was significantly correlated with both AAL and ethanol production for all populations.

Levels of simple sugars were affected by a number of malt parameters as indicated by correlation analysis (Tables II–IV). All three populations showed a significant negative correlation between β-glucan and glucose. The Arapiles/Franklin population also showed a significant

Table I. Malt quality of the Arapiles/Franklin population and the covered and hulless lines from the TR251/HB345 population.[a]

	EBC malt extract (%)	β-Glucan (ppm)	Viscosity (cps)	FAN (ppm)	DP (°L)	α-Amylase (DU)	AAL (%)	Ethanol (% w/w)
Arapiles/Franklin	80.2b ± 1.2[b]	502a ± 273	1.81a ± 0.23	110b ± 19	75b ± 21	na[c]	80.8b ± 1.5	3.23c ± 0.14
Covered TR251/HB345	79.3c ± 1.3	94c ± 55	1.44c ± 0.03	260a ± 26	182a ± 40	66.1a ± 9.5	81.6a ± 1.8	3.41b ± 0.08
Hulless TR251/HB345	85.4a ± 1.1	184b ± 74	1.54b ± 0.04	263a ± 28	183a ± 21	50.1b ± 15.2	79.7c ± 2.2	3.54a ± 0.15

[a] Column means followed by different letters differ significantly ($P < 0.01$) based on Duncan's Multiple Range Test.
[b] Average ± standard deviation.
[c] Values with compatible units not available.

Table II. Correlation coefficients among malt quality parameters of the Arapiles/Franklin population.[a]

	β-Glucan	Viscosity	FAN	DP	α-Amylase	AAL	Ethanol	Glucose	Maltose	Malto-tetraose
β-Glucan	1.000									
Viscosity	0.732***	1.000								
FAN	–0.305***	–0.436***	1.000							
DP	0.034	–0.189*	0.350***	1.000						
α-Amylase	–0.166*	–0.304***	0.414***	0.257***	1.000					
AAL	–0.724***	–0.718***	0.452***	0.186*	0.387***	1.000				
Ethanol	–0.529***	–0.486***	0.377***	–0.006	0.360***	0.692***	1.000			
Glucose	–0.219**	–0.346***	0.313***	0.165*	0.344***	0.368***	0.315***	1.000		
Maltose	–0.022	0.011	–0.090	0.003	–0.024	0.053	0.053	0.440***	1.000	
Maltotetraose	0.202**	0.275***	–0.201**	–0.145	–0.252***	–0.575***	–0.329***	0.106	0.293***	1.000

[a] *,**,*** Significantly different from zero at the 0.05, 0.01 and 0.001 levels of probability, respectively.

Table III. Correlation coefficients among malt quality parameters of the TR251/HB345 covered lines.[a]

	β-Glucan	Viscosity	FAN	DP	α-Amylase	AAL	Ethanol	Glucose	Maltose	Malto-tetraose
β-Glucan	1.000									
Viscosity	0.546***	1.000								
FAN	–0.442**	–0.213	1.000							
DP	–0.151	–0.522	–0.019	1.000						
α-Amylase	–0.087	–0.484***	–0.071	0.535***	1.000					
AAL	–0.283*	–0.651***	0.037	0.695***	0.635***	1.000				
Ethanol	–0.465***	–0.241	0.125	0.056	0.236	0.515***	1.000			
Glucose	–0.369**	–0.412**	–0.043	0.488***	0.327*	0.582***	0.486***	1.000		
Maltose	–0.179	–0.117	–0.311*	0.220	0.137	0.403**	0.523***	0.102	1.000	
Maltotetraose	0.081	0.326*	–0.098	–0.385**	–0.190	–0.411**	0.044	–0.113	–0.122	1.000

[a] *,**,*** Significantly different from zero at the 0.05, 0.01 and 0.001 levels of probability, respectively.

Table IV. Correlation coefficients among malt quality parameters of the TR251/HB345 hulless lines.[a]

	β-Glucan	Viscosity	FAN	DP	α-Amylase	AAL	Ethanol	Glucose	Maltose	Malto-tetraose
β-Glucan	1.000									
Viscosity	0.613***	1.000								
FAN	–0.573***	–0.512***	1.000							
DP	–0.333**	–0.504***	0.309*	1.000						
α-Amylase	–0.337*	–0.498***	0.337**	0.300*	1.000					
AAL	–0.431**	–0.446***	0.355**	0.628***	0.717***	1.000				
Ethanol	–0.471***	–0.302*	0.253	0.392**	0.619***	0.898***	1.000			
Glucose	–0.546***	–0.610***	0.268	0.348*	0.458***	0.378**	0.320*	1.000		
Maltose	–0.167	–0.152	–0.266	0.275*	0.166	0.456***	0.516***	0.350**	1.000	
Maltotetraose	0.259	0.200	–0.116	–0.549***	–0.455***	–0.803***	–0.718***	–0.119	–0.409**	1.000

[a] *,**,*** Significantly different from zero at the 0.05, 0.01 and 0.001 levels of probability, respectively.

positive correlation between β-glucan and maltotetraose. Starch-degrading enzymes showed some significant effects on simple sugars (Tables II–IV) with glucose levels significantly increased by both DP and α-amylase in all populations. Maltotetraose was significantly decreased by α-amylase with the TR251/HB345 hulless lines and the Arapiles/Franklin lines while DP lowered maltotetraose levels in the TR251/HB345 covered and hulless lines. Maltose was not affected by these enzymes.

Regression analysis provided further information on effects of malt quality on fermentability. The analysis used AAL (Table V) or ethanol production (Table VI) as dependent variables and β-glucan, FAN, viscosity, DP, the fermentable sugars, glucose and maltose, and the non-

Table V. Summary of regression analysis of effect of enzyme levels and modification parameters on Apparent Attenuation Limit (AAL) for the three populations studied.

	Model	r^2	ANOVA F value[a]
Arapiles/Franklin			
One-variable	AAL = 82.9 – 0.004 β-glucan	0.52	184.6
Two-variable	AAL = 84.9 – 1.76 maltotetraose – 0.004 β-glucan	0.71	209.5
Three-variable	AAL = 82.4 + 0.089 glucose – 1.93 maltotetraose – 0.003 β-glucan	0.79	212.5
TR251/HB345 covered			
One-variable	AAL = 76.0 + 0.031 DP	0.48	47.79
Two-variable	AAL = 106.9 + 0.022 DP – 20.4 viscosity	0.59	36.14
Three-variable	AAL = 100.2 + 0.058 maltose + 0.019 DP – 20.2 viscosity	0.65	30.96
TR251/HB345 hulless			
One-variable	AAL = 83.7 – 2.21 maltotetraose	0.64	94.05
Two-variable	AAL = 112 – 2.05 maltotetraose – 18.6 viscosity	0.73	68.89
Three-variable	AAL = 100.6 – 2.04 maltotetraose + 0.02 FAN – 13.7 viscosity	0.75	49.50

[a] All significant at $P < 0.0001$.

Table VI. Summary of regression analysis of enzyme levels and modification parameters on ethanol production for the population.

	Model	r^2	ANOVA F value[a]
Arapiles/Franklin			
One-variable	Ethanol = 3.37 – 0.001 β-glucan	0.28	65.19
Two-variable	Ethanol = 3.47 – 0.080 maltotetraose – 0.001 β-glucan	0.33	41.20
Three-variable	Ethanol = 3.27 + 0.007 glucose – 0.100 maltotetraose – 0.001 β-glucan	0.39	35.41
TR251/HB345 covered			
One-variable	Ethanol = 2.76 + 0.006 maltose	0.27	19.23
Two-variable	Ethanol = 2.90 + 0.005 maltose – 0.001 β-glucan	0.42	17.87
Three-variable	Ethanol = 2.78 + 0.003 glucose + 0.004 maltose – 0.001 β-glucan	0.45	13.47
TR251/HB345 hulless			
One-variable	Ethanol = 3.74 – 0.109 maltotetraose	0.52	55.46
Two-variable	Ethanol = 3.83 – 0.097 maltotetraose – 0.001 β-glucan	0.60	38.71
Three-variable	Ethanol = 3.32 + 0.004 maltose – 0.083 maltotetraose – 0.001 β-glucan	0.65	31.35

[a] All significant at $P < 0.0001$.

Table VII. Comparison of malt quality among the three β-glucan groups from the Arapiles/Franklin population.[a,b]

	Low β-glucan group (n = 59)		Medium β-glucan group (n = 58)		High β-glucan group (n = 59)	
Analysis	**Avg.**	**Std. Dev.**	**Avg.**	**Std. Dev.**	**Avg.**	**Std. Dev.**
β-Glucan (ppm)	228c	84	461b	74	817a	180
FAN (mg/L)	113a	20	114a	20	102b	13
Viscosity (cps)	1.65c	0.08	1.77b	0.13	2.01a	0.25
Diastatic power (°L)	72a	19	79a	23	73a	19
α-Amylase (units[c])	83.2a	18.2	83.8a	14.9	78.9a	15.3
EBC extract (%)	81.0a	1.0	80.3b	0.8	79.3c	1.3
AAL (%)	81.8a	1.2	81.3b	1.0	79.5c	1.3
Ethanol (% v/v)	3.30a	0.12	3.25b	0.13	3.16c	0.14
Glucose (mg/mL)	28.19a,b	4.94	29.17a	5.92	26.71b	3.63
Maltose (mg/mL)	131.1a	13.7	130.6a	13.6	131.3a	10.0
Maltotetraose (mg/mL)	1.22b	0.38	1.20b	0.34	1.41a	0.42

[a] Row averages followed by different letters differ significantly ($P < 0.01$) based on Duncan's Multiple Range Test.
[b] Malt yield data not available for Arapiles/Franklin samples.
[c] umoles maltose equivalents/minute/gram dry matter.

fermentable sugar, maltotetraose, as independent variables. Results supported the strong negative effect of β-glucan on both AAL and ethanol production for the Arapiles/Franklin population. The negative effect of maltotetraose was also evident for both AAL and ethanol production for this population. Regression analysis for the TR251/HB345 covered lines indicated a strong positive influence of DP, as well as its major product, maltose, on both AAL and ethanol. Modification appeared to have some effect on fermentability as indicated by significant negative effects of viscosity on AAL and β-glucan on ethanol production. In contrast, the TR251/HB345 hulless lines were more like the Arapiles/Franklin lines with modification having a stronger negative influence as indicated by the negative effect of maltotetraose levels on both AAL and ethanol production. AAL for the hulless lines was also negatively affected by viscosity while β-glucan negatively affected ethanol production. In a three-variable model FAN showed a positive effect on AAL for the hulless lines.

In order to further investigate how β-glucan affected fermentability and ethanol production, the three popula-

Table VIII. Comparison of malt quality among the three β-glucan groups from the TR251/HB345 hulless lines.[a]

	Low β-glucan group (n = 18)		Medium β-glucan group (n = 18)		High β-glucan group (n = 18)	
Analysis	**Avg.**	**Std. Dev.**	**Avg.**	**Std. Dev.**	**Avg.**	**Std. Dev.**
β-Glucan (ppm)	113c	24	172b	19	267a	57
FAN (mg/L)	276a	23	267a	30	244b	20
Viscosity (cps)	1.51b	0.03	1.54b	0.04	1.57a	0.03
Diastatic power (°L)	191a	19	180a	18	179a	24
α-Amylase (D. U.)	53.6a	15.4	51.9a	16.4	44.7a	12.8
EBC extract (%)	86.0a	1.1	85.5a	0.7	84.8b	1.1
AAL (%)	80.7a	2.4	79.8a,b	2.5	78.7b	2.9
Ethanol (% v/v)	3.61a	0.13	3.54a,b	0.14	3.48b	0.15
Glucose (mg/mL)	71.54a	5.83	71.47a	4.66	66.58b	6.17
Maltose (mg/mL)	124.8a	10.7	124.1a	9.5	123.3a	8.3
Maltotetraose (mg/mL)	1.58a	0.89	1.77a	0.80	2.09a	1.16
Malt yield (%)	86.9a	1.2	87.4a	1.9	88.0a	1.5

[a] Row averages followed by different letters differ significantly ($P < 0.01$) based on Duncan's Multiple Range Test.

Table IX. Comparison of malt quality among the three β-glucan groups from the TR251/HB345 covered lines.[a]

	Low β-glucan group (n = 18)		Medium β-glucan group (n = 17)		High β-glucan group (n = 18)	
Analysis	**Avg.**	**Std. Dev.**	**Avg.**	**Std. Dev.**	**Avg.**	**Std. Dev.**
β-Glucan (ppm)	52c	13	87b	10	145a	67
FAN (mg/L)	277a	23	255b	26	249b	18
Viscosity (cps)	1.42b	0.03	1.43b	0.02	1.45a	0.04
Diastatic power (°L)	189a	10	178a	10	182a	8
α-Amylase (D. U.)	68.2	10.2	65.0	10.2	65.2	8.4
EBC extract (%)	79.7a	1.5	79.2a	0.8	78.9a	1.3
AAL (%)	82.1a	2.1	81.6a	1.2	81.2a	1.8
Ethanol (% v/v)	3.44a	0.09	3.42a,b	0.05	3.37b	0.09
Glucose (mg/mL)	67.19a	6.09	62.17b	4.23	63.30b	5.81
Maltose (mg/mL)	114.9b	7.6	121.4a	5.7	115.9b	8.5
Maltotetraose (mg/mL)	0.95a	0.54	0.81a	0.30	0.82a	0.76
Malt yield (%)	90.7a	1.4	91.7a	1.2	91.2a	1.2

[a] Row averages followed by different letters differ significantly ($P < 0.01$) based on Duncan's Multiple Range Test.

tions studied were each divided into three approximately equal groups of lines with high, medium and low levels of wort β-glucan. The resulting Arapiles/Franklin groups had significantly different fermentability with the high β-glucan group having significantly lower AAL and ethanol production than the medium β-glucan group which in turn was significantly poorer than the low β-glucan group (Table VII). Differences among the TR251/HB345 hulless groups (Table VIII) were not as obvious with AAL and ethanol production differences only being significant between the high and low β-glucan groups. There were no significant differences in AAL among the TR251/HB345 covered β-glucan groups (Table IX) but the high β-glucan group did produce significantly less ethanol than the low group.

There were several other significant differences among the β-glucan groups. FAN and glucose levels were significantly lower in the high β-glucan versus the low β-glucan groups for all three populations. The high β-glucan groups also had significantly higher viscosity in each of the three populations. Malt extract was consistently lower in high β-glucan groups with differences being significant in the TR251/HB345 hulless and Arapiles/Franklin populations. The high β-glucan groups for the TR251/HB345 hulless and covered lines had significantly higher levels of maltotetraose. Neither DP nor α-amylase levels differed significantly among β-glucan groups for any of the populations.

Table X. Comparison of β-glucan and FAN effects on AAL within the β-glucan groups of Arapiles/Franklin population.[a]

Arapiles/Franklin	β-Glucan	FAN	Glucose
Low β-glucan	–0.397**	0.545***	0.291
Med β-glucan	–0.298*	0.309*	0.390**
High β-glucan	–0.581***	0.299*	0.395**

[a] *,**,*** Significantly different from zero at the 0.05, 0.01 and 0.001 levels of probability, respectively.

Correlation analysis within the β-glucan groups of Arapiles/Franklin indicated that the effect of β-glucan on AAL was reduced at lower levels of β-glucan (Table X). Each of the β-glucan groups, for this population, showed a significant negative correlation between AAL and β-glucan level but the correlation was only highly significant in the high β-glucan group. The β-glucan tended to have a negative effect on AAL in high β-glucan groups for TR251/HB345 covered and hulless, but not significantly. FAN was not correlated with AAL in any of the TR251/HB345 covered or hulless β-glucan groups but all three Arapiles/Franklin groups showed some relationship, with the low β-glucan group showing a highly significant positive correlation between FAN and AAL. Glucose levels had a significant positive effect on AAL in the high β-glucan groups of all populations. This relationship was also significant in the medium β-glucan group of the Arapiles/Franklin lines and the low β-glucan group of the TR251/HB345 covered lines.

DISCUSSION

The samples investigated in the present study showed a broad range in malt quality with many factors having significant effects on fermentability, emphasising the complexity of malt wort fermentability. Adequate endosperm modification was the most important factor for good fermentability. Other positive factors such as levels of starch-degrading enzymes only appeared to effect fermentability when malts were adequately modified. Fermentable sugars did show some significant effects on fermentability, even in under-modified malts. Levels of FAN also appeared to limit fermentability in under-modified malts.

A broad range in malt quality was expected among the samples studied. The two populations were grown under different growing environments. The Australian environment offered a longer growing season with ample time for starch deposition. As a result the Australian samples had significantly higher levels of malt extract than the covered Canadian lines (Table I) but significantly less DP than either the covered or hulless Canadian lines. Furthermore, the two populations were malted under considerably different conditions. The shorter Australian schedule had been developed to maximize differences among lines in a breeding program while the Canadian schedule was much longer having been designed specifically for hulless barley[5]. The Australian lines, therefore, were less modified as indicated by significantly higher levels of wort β-glucan and significantly lower levels of FAN (Table I). The Canadian population was well modified with very low levels of β-glucan, especially in the covered lines, and more than adequate FAN in both sets of lines.

The samples showed interesting differences in fermentability both in terms of AAL and ethanol production. The TR251/HB345 covered samples had significantly higher AAL than either the TR251/HB345 hulless or the Arapiles/Franklin samples with the TR251/HB345 hulless samples being the poorest. However, the hulless samples produced significantly more ethanol than either of the other two groups with the Arapiles/Franklin producing significantly less than the TR251/HB345 covered samples. Different results for AAL and ethanol were not unexpected given that AAL is a measure of the degree to which a wort will ferment while ethanol levels are a measure of a fermentation end product. As a result, positive aspects of the hulless samples, e.g., higher malt extract, resulted in significantly more ethanol despite significantly poorer AAL. In contrast, the positive aspect of significantly higher malt extract in the Arapiles/Franklin samples was not high enough to overcome the inferior AAL resulting from under modification and, thus, the covered TR251/HB345 samples produced more ethanol.

Correlation and regression analysis suggested positive attributes of malt quality, such as levels of starch degrading enzymes, only improved fermentability when a malt was well modified. The significantly lower levels of starch-degrading activity in the under-modified Arapiles/Franklin population showed less effect on AAL than might be expected. In contrast, the Canadian samples, with better modification and significantly higher levels of enzymes, showed a much stronger relationship between enzymes and AAL. The right mixture of DP and α-amylase was also emphasized in the hulless samples where α-amylase levels were more limiting to fermentability than DP. Significantly lower levels of α-amylase (Table I) could explain inferior fermentability often observed with hulless malt[6,11]. Lower levels α-amylase likely resulted from the processing of hulless malt[6], although, it was possible that lower levels were due to a linkage between the α-amylase (amy2) and the hulless (n or nu) genes as they are located only 9 cM (approximately) apart on chromosome 1 (7H)[18]. The Arapiles/Franklin population also supported the importance of α-amylase[11] as fermentability in this population was also more significantly limited by α-amylase than DP.

The effect of under modification on fermentability is not well understood even though it is often referred to in the literature[2,11,13–15,22]. Results from the present study suggested several factors were involved in restricting fermentability. Significant differences in correlation coefficients suggested viscosity and β-glucan affected AAL independently with viscosity having the stronger effect (Tables III and IV). High viscosities have been implicated in human nutrition as a means of restricting enzyme movement in the intestine and thus limiting enzyme/substrate interaction[4]. A similar effect may occur during mashing with high viscosities restricting enzyme movement and, thus, reducing starch hydrolysis and fermentability.

The β-glucan likely affected fermentability independently of viscosity by restricting the release of starch granules from under-modified endosperm. In this case higher temperatures would have been required to release the starch and starch-degrading enzymes, beta-amylase in particular, would have become limiting due to heat lability and inactivation. Starch hydrolysis would have been incomplete and fermentability would have been reduced[21]. The restriction of starch release by unmodified endosperm structure was likely more of an effect with the Arapiles/Franklin lines. This population had significantly higher levels of β-glucan (Table I) suggesting a greater proportion of under-modified endosperm and potential for problems with starch release. This population was also the only one where viscosity/AAL correlation coefficients were not significantly higher than the β-glucan/AAL coefficients (Table II). As well, fermentability was significantly improved in the Arapiles/Franklin lines with the heat stable beta-amylase gene[7] which was not the case for the heat stable gene in the TR251/HB345 population[6]. Heat stable beta-amylase appeared to increase fermentability in samples where starch release was delayed due to under-modified endosperm but not necessarily in samples where fermentability was restricted more by high viscosity.

Glucose levels were significantly lower in samples with higher levels of β-glucan levels for all three sets of samples (Tables I–III). The levels of this fermentable sugar, in turn, showed strong positive correlation with both AAL and ethanol supporting the concept of Molina-Cano et al.[22] that β-glucan had been completely hydrolysed to fermentable extract (glucose). In the present study fermentability continued to improve as β-glucan levels fell to levels generally considered insignificant to malt quality (<100 ppm). Increased breakdown of β-glucan possibly contributed more glucose for fermentation.

Bathgate et al.[2] did warn that over modification can lead to poorer fermentability due to increased production of unfermentable soluble protein and the loss of fermentable extract as a result of increased malt losses. However, the present study found no significant differences in malting losses as β-glucan decreased among the groups, yet AAL and ethanol levels were significantly improved (Table IX). Therefore, there could be opportunity for breeders to develop barley lines with the ability to degrade β-glucan to glucose while avoiding excessive metabolization of the glucose by the germinating kernel.

Soluble β-glucan in wort may have also restricted fermentability in the present study. Symons and Brennan[27] have shown that soluble β-glucan can increase starch gelatinization temperature and others have shown that fermentability can be restricted by higher gelatinization temperatures[26]. Further research is required on how factors affect gelatinization temperature, including levels of β-glucan, and how this may limit fermentability.

FAN was another aspect of malt modification that had interesting effects on fermentability. FAN is generally considered adequate unless high adjunct brewing or high gravity brewing is practiced[24]. The present study used low gravity Congress extracts (ca. 8° Plato) and, therefore, FAN was not expected to be limiting. However, in the under-modified Arapiles/Franklin lines where FAN levels averaged 110 ppm, FAN was found to have a significant positive effect on both AAL and ethanol. (Table II). It could be argued that the FAN/AAL relationship was just the effect of β-glucan due to a β-glucan/FAN correlation. However, Table X shows that within the Arapiles/Franklin β-glucan groups, the FAN/AAL relationship was most significant in the low β-glucan group. FAN may have become more limiting as β-glucan levels dropped and became less of a restriction to fermentability.

The TR251/HB345 hulless population was even more interesting showing a significant positive effect of FAN on AAL (Table IV and IX) despite an average FAN level greater than 250 ppm. In contrast, the TR251/HB345 covered population with a similar average FAN level, showed no FAN/AAL relationship. Hulless malt has been previously shown to have significantly lower levels, compared to covered malt, of some individual amino acids[6]. Fermentability may have been restricted by limiting amounts of these amino acids supporting a need for research on the importance of individual amino acids versus the more general FAN analysis.

The study showed that fermentability can be affected by a number of factors which makes screening of breeding lines for fermentability difficult. Fermentability can be monitored by barley breeders with direct methods like apparent attenuation limit or ethanol production but these methods are very time consuming and production of consistent results can be difficult. The two methods are also dependent on different factors and, therefore, can supply contradictory information further complicating decisions for breeders. There is the possibility of screening early generation lines for fermentability potential with molecular markers. Past work[7] has shown that quantitative trait loci (QTL) exist for fermentability on Chromosome 1H, in conjunction with modification, and on chromosome 4H, in conjunction with heat stable beta-amylase. However, to completely understand the fermentability potential of lines further along in the program, this study emphasised a need for overall knowledge of the malting potential of the line. The study suggested continued emphasis on parameters related to endosperm modification, with renewed interest in wort viscosity, as well as levels of the starch-degrading enzyme, alpha-amylase. The study did investigate barley grown at different locations but different populations were grown at these locations and, therefore, genotype by environmental interactions were not effectively monitored. Correlations from the present study, though, would support further work on the effect of such interactions on fermentability.

CONCLUSIONS

Fermentability of a malt wort was found to be a complex process that would best be predicted by considering all aspects of a malt's quality, which supports past research[3,13]. Adequate modification was essential if other more positive attributes, such as levels of starch-degrading enzymes, were to exhibit their positive effect on fermentability. The degree of modification required to maximize fermentability, as indicated by levels of wort β-glucan, was lower than expected with improvements in fermentability still possible as levels of β-glucan receded below 100 ppm. The β-glucan appeared to affect fermentability in a number of ways including restriction of enzyme movement due to high viscosities during mashing, late release of starch from under-modified endosperm as well as increased levels of glucose due to complete β-glucan hydrolysis.

Differences in results from ethanol production versus AAL would suggest brewers could control fermentability in different ways depending on their objective. Maximum ethanol, or beer production, can best be achieved with a well modified malt exhibiting high levels of enzymes. In contrast, those brewers striving for a less fermented product would be better off considering under-modified malts with less concern for levels of starch-degrading enzymes.

Hulless barley malt continues to show potential to produce greater economic return in terms of ethanol production. However, the efficiency with which hulless malt extract is fermented could be improved. Levels of α-amylase were a significant limiting factor, as they were with covered malts. Therefore, improvements in α-amylase levels, either with increased stability during kilning of hulless malt or possibly the unlinking of α-amylase and hulless genes, would be beneficial.

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