A Study of the Physical, Biochemical and Genetic Factors Influencing Malt Extract

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Introduction

Malt extract represents the soluble material extracted from malt during the mashing process. This includes simple sugars and amino acids for yeast nutrition during fermentation, and more complex carbohydrates and proteins, which add to the flavour, aroma and mouthfeel of the final product. Domestic and overseas maltsters and brewers use measurement of malt extract as a key assessment of the quality of the barley they are purchasing. Malt extract is therefore one of the most important parameters used by breeders to assess the quality of new barley varieties before release. The factors contributing to differences in extract potential between different varieties are however poorly understood.

In this study two complimentary methods were used to investigate factors influencing malt extract. The first was a simple correlation study conducted over two years to identify barley and malt characteristics associated with the expression of high malt extract. The second involved identifying and assessing chromosome regions (QTL) found to be associated with high malt extract.

There are two recommended methods for measuring malt extract. The first is the European Brewery Convention (EBC) method which uses a fine grind (0.2mm) and a ramping temperature programme starting at 45°C. This is a very gentle method which allows the hydrolytic enzymes time to act before destructive temperatures are reached. The fine grind also allows the enzymes easy access to the starch and ß-glucans. The second is the Institute of brewing (IOB) method, which uses a coarser grind (0.7mm), and a single temperature of 65°C. At this temperature enzymes are degraded rapidly. Both methods were assessed to identify differences between them.

Materials and Methods

Physical and Biochemical Factors

Twelve and eleven barley varieties were grown at two South Australian sites in 1997 and 1998, respectively. These included malting varieties from Canada (Harrington), Europe (Alexis and Chariot), Japan (Haruna nijo), Australian (Franklin, Schooner Sloop and Arapiles) and three Australian feed varieties (Chebec, Galleon and Barque). A second Canadian malting quality variety, Manley, was also included in 1997. The sites were Brinkworth and Tuckey in 1997 and Pinery and Maitland in 1998. Grain samples from three replicates of each variety at each site were screened on a 2.2mm sieve before being micromalted.

Fifty grams of each sample were malted in a Phoenix Automated Micromalting system at 17°C using the malting schedule described in the Waite Barley Quality Evaluation Laboratory Barley Quality Report (WBQEL, 1997). Micromalter grain moistures were adjusted to 45% at 24, 48 and 72 hours into germination. Hot water extract (HWE) was measured using small scale versions of the IOB and EBC methods (IOB Methods of Analysis, 1997; EBC Analytica, 1998). Diastatic power (DP), alpha amylase and beta amylase were measured as described in the WBQEL Barley Quality Report (1997). Malt proteins, grain proteins and soluble proteins were measured by the Kjeldahl method using a Kjeltec 1030 Auto Anaylzer. Calculations for protein levels were taken from either the EBC or IOB methods of analysis as appropriate (IOB Methods of Analysis, 1997: EBC Analytica, 1998). Barley and malt ß-Glucans, barley and malt starch, beta glucanase and limit dextrinase were measured by Megazyme assay kits. Malt and barley husk content was measured according to the EBC method (EBC Analytica, 1998). Barley and malt total pentose and arabinoxylans were measured using the phloroglucinol reaction (Lee, 1996). Grain hydration was measured at the end of steep and at 24, 48 and 72 hours into gemination, according to Landau *et al* (1995). Barley and malt 1000 corn weight were measured by the EBC method (EBC Analytica, 1998).

Genetic Factors
Forty-eight and sixty-four lines of the Galleon x Haruna nijo mapping population were grown at Charlick in 1996 and 1998 respectively. These were screened on a 2.2mm screen. Thirty grams of each sample was malted as described above at 15ºC. EBC extract and barley husk content was measured by the methods described above.

Statistical Analysis
Pearson product moment correlations between IOB and EBC HWE (mean of 3 replicates) and other barley and malt traits were undertaken, using the computer software package Jmp® (version 3.1.6, SAS Institute Inc.). The assumption of normality of each trait was visually assessed and appeared to be met. Scatter plots of each trait against both IOB and EBC extract were inspected to ensure no outliers were biasing the results.

A total of 278 RFLP marker loci, covering the majority of the barley genome (Langridge *et al.*, 1995) were used for simple and interval regression analysis, the latter by the method of Haley and Knott (1992). A minimum LOD threshold of 3.0 was used. Simple regression and interval analysis was performed with the computer program “Qgene” (Nelson, 1997).

Results and Discussion

Physical and Biochemical Factors
Table 1 shows the mean IOB and EBC extract results for each variety and site. Tuckey and Pinery are significantly lower (P<0.01) than Maitland and Tuckey for both types of extract.

Pearson correlations were calculated between malt extract and other traits. As suggested by Devore and Peck (1996), we used the following to classify correlation coefficients as strong (r=0.8-1.0), moderate (r=0.6-0.8), weak (r=0.4-0.6) and negligible (below r=0.4).

The correlations for both EBC and IOB extracts at each site are shown in Table 3. The traits measured can be divided into a number of sections:

- Physical traits such as screenings, 1000 corn weights and grain hydration
- Components of the barley and malt such as protein, husk, starch, β-glucan and arabinoxylan contents
- Enzyme levels such as diastatic power, α-amylase, β-amylase, β-glucosidase and limit dextrinase.

Table 1. IOB and EBC Hot water extract results for each variety (mean of 4 sites and 3 reps) and the mean for each site.

Variety	EBC HWE	IOB HWE	Site	EBC HWE	IOB HWE
Alexis	81.1	79.1	Pinery 98	79.3	76.2
Arapiles	79.7	76.7	Maitland 98	82.2	80.3
Barque	77.4	74.7	Tuckey 97	81.2	78.8
Chariot	81.0	78.8	Brinkworth 97	78.9	76.7
Chebec	79.8	77.5			
Franklin	81.6	79.0			
Galleon	78.9	74.3			
Harrington	82.5	80.2			
Haruna Nijo	81.9	80.1			
*Manley	80.2	78.6			
Schooner	80.2	78.3			
Sloop	80.0	78.9			
LSD	*2.06*	*2.73*			
SE	*0.30*	*0.40*			

*Brinkworth and Tuckey only

Physical traits

Screenings showed weak correlations with either IOB or EBC extract at a few sites, while malt and barley 1000 corn weights showed moderate correlations at most sites.

Grain hydration is a measure of the ability of water to filter through the endosperm of the grain during the malting process. A loosely packed, mealy grain will allow the water to spread evenly over the whole of the endosperm, therefore allowing even modification (Landau *et al.*, 1995). Correlations were found between grain hydration and both IOB and EBC extract at all sites and at all times except Maitland at the end of steep and at 24 hours into germination. The strongest relationship was at the Tuckey site with strong correlations at both 24 and 74 hours for EBC extract and 72 hours for IOB extract. 48 and 72 hours generally showed stronger correlations with both IOB and EBC extract than at steep and at 24 hours.

Table 2. Barley Protein levels (mean of 3 reps) and range for individual sites

Site	Barley Protein	
	Mean	**Range**
Pinery 98	11.8	10.3-13.8
Maitland 98	11.1	9.4-13.8
Brinkworth 97	12.5	10.9-15.2
Tuckey 97	9.1	7.1-10.5

Components of Barley and Malt

Of all of the malt and barley components, the only two traits that showed consistent relationships with either IOB or EBC extract were barley husk content and malt β-glucan levels. Barley husk content showed weak to moderate relationships with extract at all sites. The relationship with barley husk content was stronger for EBC extract than IOB extract at most sites. Malt β-glucan showed moderate to strong relationships with IOB extract at all sites. EBC extract showed a slightly weaker correlation at all sites with a moderate overall relationship.

A number of the other traits correlated with extract at single sites only and therefore will not be discussed.

Table 3. Correlations between malt extract measured by the EBC and IOB methods and other traits.

Shading r=0.8-1.0, Shading r=0.6-0.8, Shading r=0.4-0.6, - missing data

***Trait**	Pinery 98 n=11		Maitland 98 n=11		Brinkworth 97 n=12		Tuckey 97 n=12	
	EBC	IOB	EBC	IOB	EBC	IOB	EBC	IOB
Screenings	0.57	0.45	0.39	0.35	0.04	-0.13	0.42	0.41
B1000 Corn Wt	-0.90	-0.78	-0.77	-0.76	-0.52	-0.28	-0.74	-0.65
M1000 Corn Wt	-0.92	-0.81	-0.39	-0.35	-0.67	-0.57	-0.72	-0.87
G Protein	-0.52	-0.51	-0.18	-0.18	0.21	0.19	0.00	-0.04
M Protein	-0.57	-0.53	-0.16	-0.17	0.11	0.13	0.02	0.01
B Husk	-0.66	-0.60	-0.42	-0.44	-0.84	-0.64	-0.60	-0.48
M Husk	0.16	0.21	0.13	0.04	-0.64	-0.71	0.20	0.40
B β-Glucan	0.14	0.09	-0.22	-0.32	0.09	-0.22	0.33	0.12
M β-Glucan	-0.78	-0.93	-0.80	-0.86	-0.48	-0.66	-0.58	-0.90
B Starch	0.33	0.24	0.29	0.33	0.28	0.07	0.71	0.43
M Starch	0.33	0.50	-0.20	-0.15	0.10	0.06	-0.76	-0.69
B Pentose	-0.23	-0.23	0.22	0.19	-0.03	0.35	-0.12	-0.30
M Pentose	-0.60	-0.50	-	-	0.00	0.14	0.21	0.43
B Arabinoxylan	-0.42	-0.30	0.24	0.22	0.01	0.30	0.29	-0.04
M Arabinoxylan	-0.65	-0.78	-	-	-0.07	-0.03	0.19	0.11
M β-Glucanase	0.91	0.92	0.87	0.89	0.29	0.35	0.56	0.79
Limit Dextrinase	0.64	0.74	0.37	0.45	0.75	0.79	0.76	0.84
Diastatic Power	0.66	0.71	0.68	0.64	0.19	0.15	0.58	0.60
α-amylase	0.87	0.82	0.64	0.56	0.39	0.46	0.75	0.85
β-amylase	0.22	0.34	0.44	0.47	0.15	0.09	0.50	0.50
G Hydration St	0.41	0.45	0.00	-0.05	0.52	0.40	0.75	0.55
G Hydration 24	0.46	0.51	0.37	0.35	0.53	0.41	0.83	0.68
G Hydration 48	0.45	0.50	0.56	0.53	0.65	0.52	0.77	0.71
G Hydration 72	0.87	0.79	0.53	0.50	0.57	0.50	0.91	0.84

* M = malt, B = barley, G = Grain

Enzyme levels

In general, positive correlations were found between both IOB and EBC extract and levels of measured enzymes at all sites except Brinkworth. Brinkworth in 1997 was characterised by very dry conditions through October (SARDI Crop Harvest Report, 1997/1998), which may have contributed to higher grain protein levels in a number of the late maturing overseas varieties (data not shown). As can be seen in Table 2 the mean grain protein and range at Brinkworth, in 1997, was generally higher than at the other 3 sites. This may have contributed to the differing relationships.

In general the traits that have the strongest and most consistent relationships with malt extract are 1000 corn weight, barley husk content, malt β-glucan levels, hydrolytic enzyme levels (except β-amylase) and grain hydration scores (Table 3).

Genetic Factors

The relationship between malt extract and factors contributing to malt extract were further investigated using QTL mapping techniques. Figure 1 shows the interval map for barley chromosome 2H for Galleon x Haruna nijo. As can be seen a region on chromosome 2H was found to be associated with both malt extract and husk content. At this locus Haruna nijo alleles confer high malt extract and low husk content.

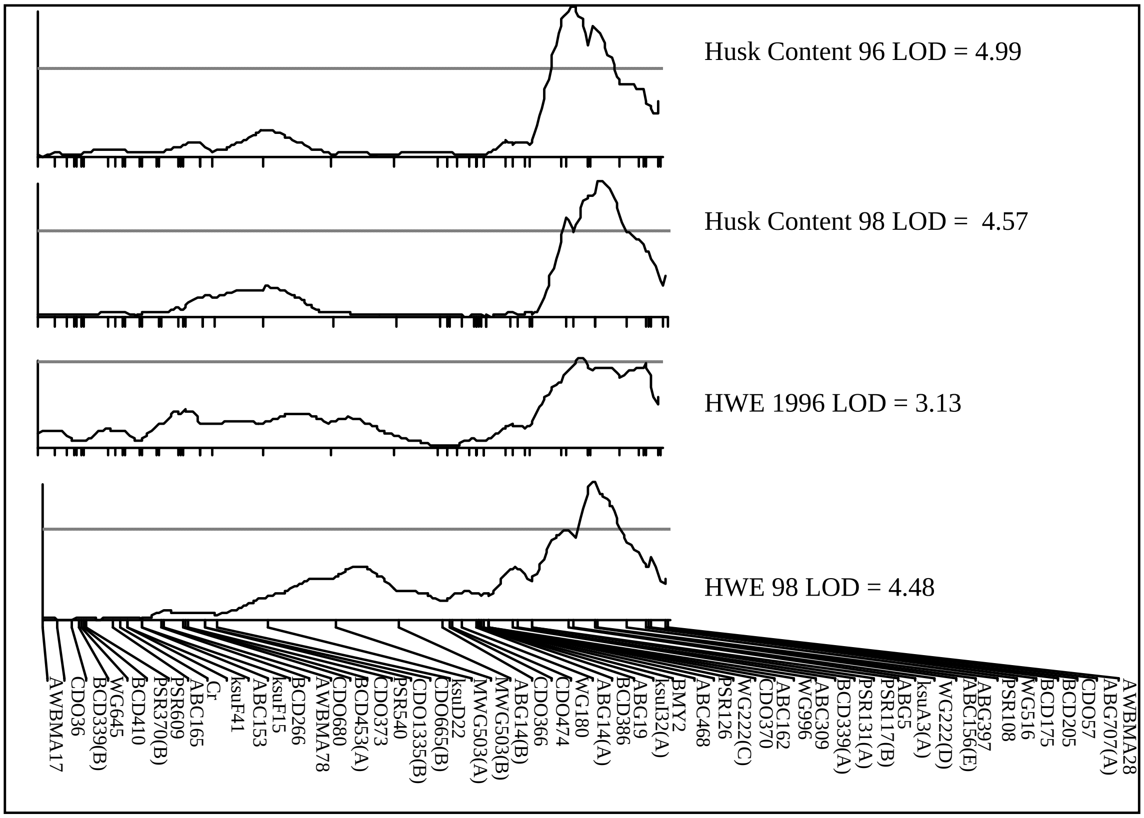

Figure 1. Interval map of barley chromosome 2H for the population Galleon / Haruna nijo showing RFLP markers.

Summary

Correlation analysis showed that malt extract is associated with 1000 corn weight, barley husk content, malt β-glucans, hydrolytic enzymes and grain hydration scores. However, the size of these relationships varied at different sites indicating they were influenced by environmental factors. It is planned to reassess the same data set using statistical methods, such as multi-environment trait analysis, to gain a greater understanding of the interactions between malt extract, its components and the environment.

IOB extracts showed stronger relationships with malt β-glucans and limit dextrinase levels. This was reversed with EBC extracts showing stronger relationships with barley 1000 corn weights, barley husk content and grain hydration scores at 48 and 72 hours. In general, however, similar associations were found between IOB and EBC extracts and other traits. The relationship between extract and barley husk content was confirmed using QTL mapping.

This study is a preliminary study only. In the future other traits such as grain hardness and starch components will be investigated.

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