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EARLY GENERATION SCREENING FOR MALTING QUALITY

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by

J S SWANSTON

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Early Generation Screening for Malting Quality

J.S. Swanston

Scottish Crop Research Institute, Mylnefield, Invergowrie, Dundee, DD2 5DA.

BACKGROUND

Early selection of the most promising crosses and concentration of resources on them is a highly efficient and cost effective strategy in barley breeding. Such selection has been successfully applied, for yield and other agronomic characters, at the Scottish Crop Research Institute (SCRI), but the requirements of both time and seed quantity, necessary for laboratory scale malting, have precluded such an approach for malting quality. Many rapid procedures to assess quality have been postulated, but no single test has been found to predict accurately the hot water extract obtained following malting and mashing.

Aspects of the physical structure of the endosperm are thought to have a strong influence on malting potential. Many tests have been developed to assess these characters, including measurement of the relative grain hardness of different cultivars (Allison et al., 1976). This has shown that no cultivars with hard endosperms will subsequently malt well, but soft grained types will not always yield good hot water extracts, as the test takes no account of the enzymes synthesised during malting which are necessary for endosperm breakdown. Consequently effective selection for quality has often followed selection for other characters, so breeders may expend considerable resources developing lines of limited commercial value. Procedures to facilitate selection at earlier stages of breeding programmes are necessary.

New breeding techniques, e.g. the production of doubled haploids from either Hordeum bulbosum or pollen culture, have facilitated the rapid

production of large numbers of inbred lines. These are of increasing importance as a means of producing both commercial cultivars and experimental lines for genetic studies. Assessment of the relative merits of such lines, for quality, may also be hampered by inadequate seed quantity.

Such problems could be overcome if laboratory scale malting was made faster or applicable to smaller samples, without loss of accuracy. Alternatively, a range of rapid testing procedures, which assess different aspects of quality on small seed samples may, in combination, offer a suitable means of predicting hot water extract. They may also facilitate a different approach to breeding by enabling a choice of parents, with complementary characteristics, and screening of progeny for a combination of desired features. A project was therefore initiated at SCRI, with the financial support of the Home Grown Cereals Authority (HGCA), to study early generation screening for malting quality.

EXPERIMENTAL PROCEDURES

Rapid Assessment of Hot Water Extract

Hot water extract (HWE) is generally regarded as the most important parameter of malting quality. It is assessed, in the Institute of Brewing's Recommended Methods of Analysis, by extracting 20 g of milled malt in 140 ml of water at 60°C, for 1 hour, cooling and, after making the suspension up to 200 ml, with additional water, filtering for a further hour. The specific gravity or refractive index of the resultant solution can then be used to calculate HWE.

The method is time consuming and not applicable where only small samples of malt are available. Consequently, a technique in which 0.5 g milled malt is extracted in 5 ml of water, in a centrifuge tube, was developed (Henry & McLean, 1985). In place of filtration, extracts are centrifuged for 5 mins. Results reported were lower than those obtained by

conventional analysis but an accurate prediction of a sample's true HWE could be obtained from a regression line, as a proportion of malts were extracted by both methods.

When the two methods were compared at SCRI (Table 1), not only were HWE figures for 3 cultivars noticably reduced, but variation between laboratory replicates was greatly increased. (The standard deviations were based on eight determinations). Increasing the quantity of both ground malt and hot water to 1 g/10 ml gave less variation than observed with 0.5 g samples but HWE results remained very low.

TABLE 1. Effect of sample size on hot water extract of centrifuged samples

Sample Size (g flour/ml water)	20 g/200 ml*		0.5 g/5 ml		1 g/10 ml	
Cultivar	Hot Water Extracts (L/Kg)					
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Triumph	317.7	3.8	274.6	16.6	262.7	6.8
Golden Promise	305.1	3.8	237.5	14.5	255.1	4.8
Koru	284.3	5.8	221.8	13.3	228.7	4.9

*Filtered sample - extraction by IOB recommended method

As malt milling produces a coarse grind with a wide variation in particle size, it was considered that very small samples could differ significantly in particle size distribution and this may have been the source of variation between replicates of 0.5 g. Additionally, the low HWE figure could be explained, especially in the 1 g samples by the "plug" of material which collected in the narrow base of the centrifuge tubes, reducing the efficiency of extraction. Both these hypotheses were tested. In Table 2, it is shown that using a finer grind of flour, with a more even

particle distribution, slightly increased HWE, but the standard deviation was still unacceptably high. Extracting in a wider vessel and transferring to a centrifuge tube produced only a slight increase in HWE (Table 3).

TABLE 2. Effect of particle size on hot water extract

Sample	Sample Size	Hot water Extracts			
	(g flour/ ml/water)	Fine Grind		Coarse Grind	
		Mean	S.D.	Mean	S.D.
IOB Check Malt	0.5/5	272.0	10.4	258.8	16.1

TABLE 3. Effect of mashing container on hot water extract

Cultivar	Sample Size	Hot water Extracts			
	(g flour/ ml/water)	Centrifuge tubes		Wide-necked jars	
		Mean	S.D.	Mean	S.D.
Triumph	1/10	256.2	9.0	265.4	6.8
Golden Promise	1/10	244.7	9.9	257.9	4.8
Koru	1/10	210.8	5.9	231.4	5.0

It was therefore decided to compare the effects of filtration and centrifugation. Using 0.5 g/5 ml extracts, 3 cultivars were compared (Table 4). It was possible therefore to assign the low HWE values observed earlier to the effects of centrifugation, but variation was still too great. In a final experiment, sample size was increased to one-tenth of the standard method and samples were filtered for 15 mins. Results presented in Table 5 indicate that such a technique would yield acceptable results but with a considerable saving in both time and sample size requirements.

Table 4. Effect of centrifugation on hot water extract.

Cultivar	Sample Size (g flour/ ml/water)	Hot water Extracts			
		Centrifuged samples		Filtered Samples	
		Mean	S.D.	Mean	S.D.
Triumph	0.5/5	274.6	16.6	316.0	7.6
Golden Promise	0.5/5	237.5	14.5	303.6	13.4
Koru	0.5/5	221.8	13.3	269.6	8.2
IOB Check Malt	0.5/5	260.1	9.8	332.1	10.7

Table 5. Hot water extract results from ten-fold "scaling down" of extraction process.

Cultivar	Sample Size (g flour/ml water)	Hot Water Extracts	
		Mean	S.D.
Triumph	2/20	314.9	1.1
Golden Promise	2/20	298.7	1.7
Koru	2/20	277.7	1.7

The milling energy of malted barley

As differences in endosperm structure between cultivars may be assessed by relative grain hardness, relative differences in hardness, following malting might indicate the extent of breakdown, or modification, of the endosperm occurring during malting, in different cultivars. As this would incorporate effects of both endosperm structure and enzyme activity, it could provide a more accurate prediction of malting performance. Initial assessments were made on 32 entries from a spring barley trial sown in April 1986. 60 g samples were malted and hot water extracts were carried out as described by Taylor & Swanston (1987). In addition, 5 g samples of both malted and unmalted barley were assessed for milling energy using an upgraded version of the comparamill (Allison et al., 1979).

Results presented in Table 6 confirmed the hypothesis that the milling energy of malted barley would provide an accurate prediction of hot water extract (HWE), as the correlation between the two characters was very much higher than that observed between HWE and grain milling energy. The correlation between the respective milling energies of grain and malt was similar to that observed between grain milling energy and HWE, indicating that much of the variation in both HWE and malt milling energy cannot be explained simply in terms of endosperm structure, although this remains a highly significant contributory factor. Assessments of further trials of both spring and winter barley have given very similar results.

TABLE 6. Correlations between malt and grain milling energy and hot water extract in a spring barley trial.

	Hot Water Extract	Grain Milling Energy
Malt Milling Energy	-0.921^{***}	0.498^{**}
Grain Milling Energy	-0.521^{**}	

$^{**}0.001<p<0.01$ $^{***}p<0.001$

Hot water extract is not dependent entirely on extent of modification, as inadequate enzyme levels could reduce the efficiency of starch breakdown during a brewery mashing process. Malt milling energy does not assess starch breakdown, so a rapid assay, for α-amylase activity, on the flour saved after milling the malt, was developed. 600 mg of flour were extracted as outlined by Smith (1970) and β-amylase activity was destroyed by incubation with Mercuric Chloride for 30 mins (O'Connell et al., 1980). 2 mls of extract were added to 5 mls of 1% Pfansteil starch and mixed for 15

secs before being measured for turbidity in a nephelometer (Swanston, 1982). The loss of turbidity after 2 mins was indicative of starch breakdown and was quantified by comparison to results observed with standard concentrations of α-amylase.

The test readily distinguished between cultivars, with eight samples of cvs Golden Promise, Triumph and Koru giving respective mean values of 23.4, 21.6 and 19.9 enzyme units. Standard errors were very low and differences between cultivars were shown to be highly significant by means of a 't'-test (Table 7). When the technique was applied to barley trials, correlations between α-amylase activity and HWE varied between trials, due largely to the wide range in α-amylase activities present in poor malting types. Results from a typical trial are presented in Table 8, where samples are graded on the basis of their HWE results. Among good malting genotypes, with HWE over 300 L°/Kg, the range of α-amylase activities is restricted and no genotypes with low levels of the enzyme are present. Among moderate and poor malting types, α-amylase values range more widely. It appears therefore that high levels of α-amylase will not confer good malting quality where modification has proceeded slowly, but a certain threshold level of the enzyme is necessary to permit adequate starch breakdown during mashing.

TABLE 7. Results of a 't'-test for significance of differences between mean α-amylase values of three cultivars.

	Triumph	Golden Promise
Golden Promise	5.657**	
Koru	5.226**	10.999***

**$0.001<p<0.01$ ***$p<0.001$

TABLE 8. α-amylase activities of entries in a spring barley trial

Range of Hot Water Extract Values	High	Medium	Low
	>300 L°/Kg	280-300 L°/Kg	<280 L°/Kg
Range of α-amylase Activities (enzyme units).	13.5 - 25.9	17.1 - 27.4	22.1 - 26.5

Reduction in malting sample size.

Providing more rapid methods of malt assessment is of limited use unless malt production can also be increased. Recent developments have either been in automation (Haselmore et al., 1985) (Gothard & Smith, 1986) or in scaling down of the process to make it applicable to earlier generations. In experiments at SCRI, a variation of the decro-malting system (Aastrup, 1983) was employed with steeping and germination carried out in partially water filled plant propagators at 17°C. Sample sizes tested were 10 g and 7 g, the latter being the smallest that would yield a sample for testing with the comparamill. When malt milling energies obtained from these sample sizes were compared to those observed on malts produced by the standard method employed at SCRI, on 60 g samples (Taylor & Swanston, 1987) a similar ranking of cultivars was observed (Table 9).

TABLE 9. Effect of different sample size on malt milling energy results for three cultivars.

Seed Quantity Malted	Malt Milling Energy (joules) Triumph		Golden Promise		Koru	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
7 g	204.6	8.79	270.4	32.80	434.8	19.42
10 g	191.6	4.48	244.3	17.47	387.7	12.14
60 g	195.3	4.70	229.5	8.68	314.4	12.56

However, the 7 g samples had modified less extensively and showed a much greater variability between replicates. Analysis of 10 g malts gave results for cvs. Triumph and Golden Promise similar to those observed with 60 g malts, although the latter did show an increase in variability. The feed cultivar, Koru was less well modified by the 10 g method, but showed the same level of variability as with 60 g.

A limited amount of further analyses have been performed on 10 g malts and initial hot water extract determinations have suggested that the technique would be suitable as a rapid screening system within a barley breeding programme. More extensive testing with a wider range of genotypes will be pursued. Ranking of genotypes for HWE on the basis of malt milling energies of 10 g malts has proved feasible, so the next stage is to ascertain whether HWE values can be accurately measured.

Rapid screening for aspects of quality

Malting is essentially germination under strictly controlled conditions of temperature and humidity. In this way, embryo development is restricted but modification of the endosperm is maximised. Germination in petri-dishes does not produce such a control and considerable rootlet and plumule development is visible after 5 days. However, Taylor and Swanston (1987) indicated that it was possible to observe differences in enzyme activity between cultivars and that these differences were related to malting performance.

This study was extended to investigate two further tests. The malt α-amylase test, described earlier in this report, was also applied to germinated grain and initial results indicated that it gave ready and repeatable discrimination between cultivars. Screening of germinated grain should therefore identify lines likely to produce inadequate levels of the enzyme during malting.

The malt modification test, developed at the Carlsberg Laboratory

(Aastrup & Erdal, 1980) uses a fluorescence method, based on the binding of calcafluor to the β-glucan component of the endosperm cell wall. As cell walls are broken down, as the first stage of endosperm modification, the level of fluorescence is decreased. This is visualised by examining 50 longitudinally bisected grains under ultra-violet light. At SCRI, the test was considered time consuming in relation to other procedures and measurement is subjective and therefore much more prone to experimental error. However it was possible to screen and rank the petri-dish germinated grain of 18 cultivars (Table 10) and rankings are compared with those observed for half-grain mashing and malt HWE as reported by Taylor and Swanston (1987). In addition, a close correlation was observed between results for % modification and half grain mashing with the correlation coefficient, $r = 0.787$ ($p<0.001$).

In 60-70% of the cultivars tested, rankings for % modification and half-grain mashing, which assesses breakdown of cell wall and protein matrix, were similar, but cultivars such as Golden Promise, Midas and Kym apparently showed fairly extensive cell wall breakdown without a similar level of protein degradation. Conversely, cvs Claret, Goldmarker and Tasman appear to break down the protein matrix very rapidly as soon as there is sufficient cell wall modification to permit access of the necessary enzymes. This has identified an area for future research as it could not be followed up within the time available.

On unmalted grain, a test originally developed for sorghum (Hallgren & Murty, 1983) has been applied, at the Carlsberg Laboratory, to assess the endosperm density of barley cultivars. This is suggested to relate to endosperm structure and, therefore, to malting quality. The test involves stirring 100 grains into a sodium nitrate solution with a density of 1.315 g/ml at 25°C, and counting the number which remain floating. When the test was applied to barley grown under Scottish conditions, the feed

TABLE 10. Ranking of 18 cultivars for hot water extract on malt and % modification and half-grain mashing on grain after 5 days germination.

Cultivar	Ranking		
	Hot Water Extract	%Modification	Half-Grain mashing
Aramir	4	14	11
Ark Royal	16	3	2
Claret	8	16	6
Diamont	1	2	3
Egmont	18	18	18
Europe	13	4	4
Golden Promise	6	8	14
Goldmarker	6	15	7
Julia	11	12	15
Kneifel	10	11	12
Kym	12	7	16
Lami	14	13	13
Mazurka	15	8	10
Midas	9	5	9
Minerva	17	17	17
Ortolan	5	6	8
Pegasus	2	1	1
Tasman	3	10	5

cultivar Koru could not be distinguished from the malting cultivar Triumph. Reduction of sodium nitrate concentration (Table 11) caused samples of both cultivars to predominantly sink, while 50% of a sample of the moderate malting cultivar Golden Promise remained floating. Golden Promise is

characterised by a much smaller and more globular grain than the other two cultivars and it was considered that grain size and shape might have too strong an influence to permit the test to distinguish between cultivars on grain density. Further experimentation tended to confirm this view and this test will not be pursued as a screening procedure at SCRI.

TABLE 11. Results of grain floatation test for endosperm density on three cultivars.

Cultivar	Thousand Grain Weight (g)	Density of Sodium Nitrate Solution		
		Density 1 (1.315 g/ml)	Density 2 (80% of Den. 1)	Density 3 (50% of Den. 1)
		% of Grain Floating (mean of 3 reps.)		
Triumph	44.28	96.3	51.0	4.7
Golden Promise	33.73	84.3	84.3	52.3
Koru	47.23	90.0	53.3	6.3

CONCLUSIONS

Experiments within this project have indicated that malting may be successfully carried out on 10 g samples and malts screened either by a "scaled down" hot water extract determination or by comparamill assessment of milling energy. These techniques should greatly increase throughput and enable laboratory scale malting to be applied as a screening technique at earlier generations of breeding programmes than is possible at present.

Several tests on ungerminated or petri-dish germinated grain have also been assessed as a means of predicting hot water extract. While none has been shown to correlate more closely with hot water extract than grain milling energy, certain tests on germinated grains, e.g. half-grain mashing and α-amylase determination can complement assessment of endosperm characters made on ungerminated grain. Since these tests can be applied to

very small samples of grain, the potential exists to incorporate several quality components into screening of parents and random inbred or F_3 lines, within a cross prediction strategy.

A number of factors contributing to malting quality have been identified and their relative importance appears to vary between cultivars. This is an obvious starting point for future research to understand more of the genetic control of malting quality. A number of the procedures outlined here still require more extensive testing than was possible within the timescale of the project, but initial results suggest that they have considerable potential in the assessment of experimental material.

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PU2(f) The milling energy of malted barley

The hot water extract of fermentable material, obtained during the malting and initial brewing processes, is dependent on endosperm structure and how that structure is broken down, or modified, during malting. The endosperm structures of different cultivars may be rapidly compared by measuring relative grain hardness in a milling energy test. By applying this test to malted barley, where much of the initial hardness has disappeared, it was possible to assess the extent of modification. As this is the product of both endosperm structure and enzyme activity it provided a very high correlation with hot water extract, much closer than that observed between hot water extract and grain milling energy.

Malt milling energy does not, however, assess the enzymes necessary to break down the gelatinised starch during a brewery mashing procedure. A rapid technique, to measure α-amylase activity, was therefore developed, using a nephelometer, whereby the change in light scattering properties observed, when a starch solution is broken down, may be quantified. As this method required only 600 mg of malt flour, it was applicable to the flour saved following determination of malt milling energy by the Comparamill.

The techniques described not only speed up comparison of malts obtained from different barley samples, but greatly reduce the number of full malt analyses required, enabling a great increase in the throughput of the malting quality laboratory. As between 4 and 5 times as many malts, as was previously possible, can now be assessed, work is currently being directed towards means of more rapid production of greater numbers of malts.

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J.S. SWANSTON
K. TAYLOR[1]

[1]Chemistry Department