An Economical and Healthy Food Ingredient – New Applications for Barley

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Introduction:

The primary uses for barley in Australia are animal feed (58%) and malt and beer production (27%) (ABARE, 2006). In Australia, barley for food use ranks a distant third (<1%) with current estimates of up to 10,000 tonnes/pa of hulless barley produced for niche food markets, mainly supplying closed-loop contracts for small, local millers of barley flour and pearled barley. Organic barley is commonly grown for this purpose. As dietary fibre is identified as being an important component of the human diet, the potential of barley as a food grain is beginning to be recognised. The Food and Drug Administration's final ruling on May 22, 2006 supports this by adding barley as an additional source of β-glucan.

The University of Adelaide Barley Program has been developing hulless barley breeding since 1989 and the major objective is to develop hulless types for the following end uses:

1. Feed types for monogastric animals (normal starch), 2. Food industry (waxy and high amylose), 3. Various industrial and commercial uses (e.g. low and high amylose), and 4. Malting and brewing industries.

Barley contains two types of starch, amylose and amylopectin. Three main groups of barley types have been identified with respect to their amylose content: Low or 'waxy' amylose (0 – 10%), Normal amylose (~25%) or High amylose (>35%). The waxy gene, *wax* (formerly called *glx* or *wx*) is located on chromosome 7HS. The waxy starch phenotype is conferred by various mutations at this locus that either preclude transcription or encode an inactive form of the starch synthase enzyme. The high amylose gene, *amo1* is located on chromosome 1H. The genes controlling both these starch mutants are recessive in their expressions.

The endosperm of waxy barley has a mealy white appearance. This bright white colour is a desirable feature for pearled barley and products derived from milling. In addition, the changes that waxy starch undergoes when it is heated with water are responsible for the unique character of many of our foods. Some examples are the viscosity and mouth-feel of gravies, and texture of some confectionaries, pie fillings and, to a lesser extent, baked products.

Compared to other cereals, barley contains high levels of β-glucan, which are important contributors to dietary fibre. The waxy gene tends to be associated with elevated levels of (1→3, 1→4)-β-D-glucan. High β-glucan content in food has been shown to have numerous health benefits including lowering blood cholesterol and reducing colon cancer. High fibre content in foods also gives the consumer a feeling of "full-ness" aiding in satiety (Hyunsook *et al*., 2006), and as a consequence, may be useful in weight control. Overseas, β-glucan has been extracted from waxy barley for uses in biodegradable films and wound preparations for burns.

The hulless waxy line WI3693 was developed from a three-way cross between Azhul, a waxy hulless cultivar (University of Arizona, US) and two CCN resistant, covered feed types, Barque and Keel (South Australian cultivars). Older waxy cultivars tend to be low yielding, susceptible to disease and have poor straw strength, however WI3693 is a medium height plant that is lodging resistant, has bright white grain and straw at maturity, has a similar plant maturity to Torrens but later than Schooner and also has resistances to cereal cyst nematode (CCN) and spot form of net blotch (sfnb). In 2005, 7 tonnes of grain was produced and will be made available by the University of Adelaide for food processing, yield and agronomic performance. WI3693 is not yet available for commercial production.

Equally, high amylose hulless barley also has the potential to make an important contribution to improving human health, particularly the prevention and management of diabetes, coronary heart disease, obesity, colon and rectal cancers, constipation and diverticular disease. In Australia the benefits of foods enriched with non-starch polysaccharides (NSP) and resistant starch (RS) to the general population have been highlighted over the past 20 years and are slowly gaining consumer acceptance (Topping *et al*., 2003). Initially, these foods were enriched with high amylose maize starch; however, the scope can be broadened to develop additional products and whole grain ingredients.

Currently, the barley program is assessing three high amylose genotypes (ANBX98002-11, ANBX98002-58 and ANBX98002-126) developed from a two-way cross between SES15-30, a high amylose covered 2-row genotype (Scotland) and a CCN resistant, hulless, 2-row feed type (South Australia). All three lines are showing improvements in grain yield upon comparison to SES15-30. These genotypes have a short to medium plant height that is lodging resistant. All genotypes are later maturing than Torrens and Schooner. There is no conclusive data for CCN and leaf disease resistances. In 2005, a total of 70kg of grain was produced and all grain will be used for seed production in 2006. These genotypes are not yet available for commercial production.

This paper describes the progress made for some products made from waxy hulless barley (WI3693) and agronomic improvements for high amylose barley and their preliminary quality attributes. This paper will highlight the improvements we have made to animal feed cultivars (WI3930) with respect to yield potential and agronomic characteristics.

Methods:

Barley samples:

All barley samples were obtained from the University of Adelaide Barley Program. The sample of Euro Oats was obtained from the Oat Breeding Program, SARDI, Waite Campus. Hulless barleys can be susceptible to embryo damage during harvesting. Consequently, gentle harvesting techniques were adopted, resulting in 10 – 50% of the husk remaining attached to the grains. These samples were de-hulled before processing as described below.

β-glucan analysis:

Total mixed linkage β-glucan was measured using the McCleary method, enzyme Assay kit, Megazyme, Australia.

Pearled barley:

Five barley genotypes (WI3693 – waxy, hulless; Morrell, Namoi and Torrens – normal, hulless; and Schooner – normal, covered) were grown in three replicated trials at four South Australian sites (Brinkworth, Clinton, Yeelanna and Weetulta) during 2004. Barley samples (40 grams) were de-hulled using a laboratory scale oat de-huller (except for covered barley). The de-hulled sample was pearled in a Satake grain testing, mill model TM-05, until 20% (hulless types) or 35% (Schooner) of kernel was removed (approximately 2-7 minutes depending on sample). The sound kernels measurement was calculated as a percentage of whole pearled grain above a 2.0mm screen. Whiteness (L-value) of pearled samples were measured with a Minolta colorimeter using the CIE L*a*b* colour score.

Flaked/Rolled barley:

500 grams of WI3693, Morrell (both grown at Charlick SA, 2003) and the oat cultivar, Euro were rolled at BRI (Sydney, NSW). Prior to rolling, the samples were de-hulled (described above) and conditioned to a moisture content of 25%, approximately 12 hours before processing. Once conditioned, the grain was steamed at atmospheric pressure for 35mins. The steamed grain was then immediately passed through smooth flaking rolls on a Vario roller mill. The material was passed through the rolls for a single pass and another sample was passed through the rolls for two passes to produce a thinner flake. The gap on the mill was set to approximately 0.01mm. The flakes were then dried at 125°C for 15minutes and cooled at room temperature before packing. Screenings were measured on 50 grams of each flaked sample sieved (3 times) over 1.7mm, 2mm and 3.86mm Endecott sieves. The product above the sieve was weighed and recorded as a percentage of the whole sieved sample. Whiteness (L-value) was measured as described above.

Milling and Baking:

Samples of WI3693 and Morrell were de-hulled prior to milling in a laboratory scale Buhler mill, AGT laboratories, SA. Samples were not conditioned prior to milling. Moisture content was approximately 10%. Flour products were reconstituted to produce whole grain flour. The total mixed linked β-glucan of Morrell flour was 4.1%, and WI3693 flour was 6.7%. Three types of pita breads were made at Regency TAFE, SA containing either 50% WI3693 flour: 50% bakers flour, 50% Morrell flour: 50% bakers flour and 100% bakers flour.

High Amylose Barley:

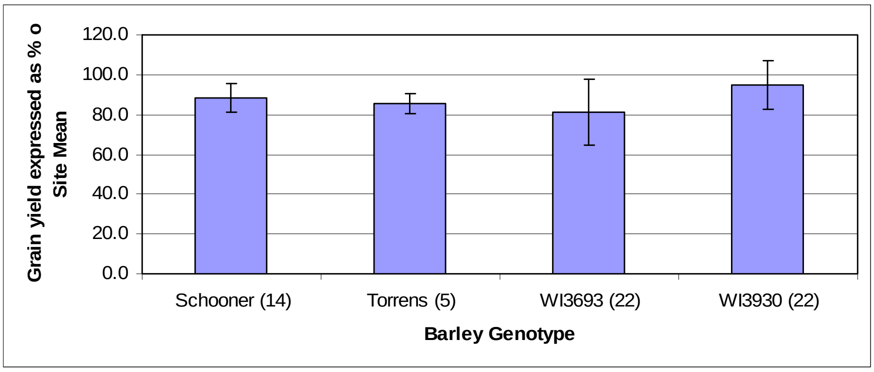
Three high amylose barley genotypes were grown at Charlick Experimental Research Station over summer 2005. Amylose/Amylopectin Ratios and total mixed linkage β-glucan was measured using the McCleary method, enzyme Assay kits, Megazyme, Australia.

Results:

Agronomy:

A large number of introduced hulless lines are poorly adapted to many agro-ecological regions in Australia. Therefore, our biggest challenge has been to develop hulless lines that are more comparable to Australian locally adapted cultivars. In year 1992, Namoi was released as an Australian hulless cultivar, but suffered from poor yields. As a result, Torrens was released in 2001 with a strong focus on market development for hulless barley. Torrens averages 4% higher yields than Namoi, with higher yield potential in most agro-ecological regions of South Australia. However, its average yield potential compared to Schooner is 92%. The University barley program has now also targeted the development of hulless barley adapted to higher rainfall areas. In 2005, WI3930 yielded up to 8% and 10% higher than Schooner and Torrens respectively (Figure 1). Pure seed production of the hulless feed barley line WI3930 commenced in 2005, with potential release in 2007. WI3930 has improved grain yield and feed quality compared to Torrens. In 2005, WI3693 yielded 7% and 4% lower than Schooner and Torrens respectively. In 2005, 7 tonnes of grain was produced and will be made available by the University of Adelaide for food processing, yield and agronomic performance. WI3693 is not yet available for commercial production.

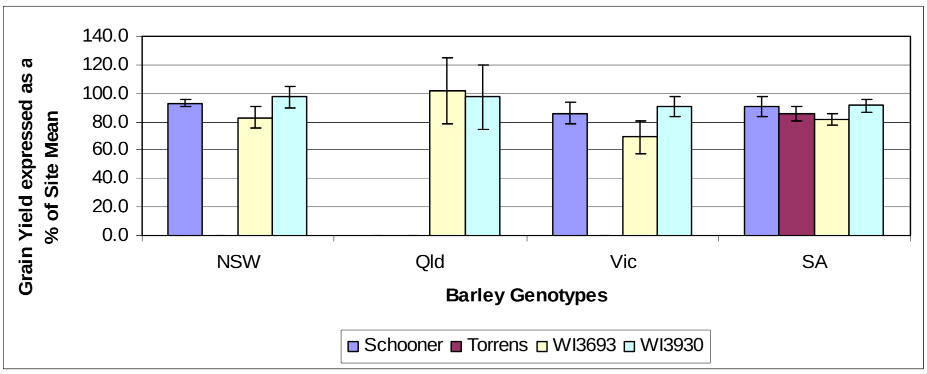
Figure 1: Summary of the improved yield potential of WI3693 and WI3930 compared to Schooner Torrens in National Variety Trials. Numbers of trials testing the barley genotypes are indicated in the brackets.



Yield Stability and Adaptation:

WI3693 and WI3930 are better adapted to the agro-ecological zones that have medium to high annual rainfall (particularly NSW and Qld) as shown in Figure 2. In addition, WI3930 averaged 3% and 6% higher yields than Schooner and Torrens in all districts, with best performances in Meandarra and Dalby, Queensland; Hamilton and Charlton, Victoria. Their superior leaf diseases resistances may explain these higher yields. Lower grain yields for WI3930 and WI3693 were observed in the Murray Mallee districts (SA and Victoria), where the lighter, less fertile soils may be contributing factors.

Figure 2: Grain yield performances for WI3693 and WI3930 compared to Schooner and Torrens expressed as a percentage of Site mean for the different agro-ecological zones of Australia. These results are from the 2005 National Variety Trials planted at 22 locations around Australia.



Lodging and head loss:

A large number of introduced hulless lines from Canada and Mexico have the propensity to be very tall resulting in severe lodging, stem break and head loss, all culminating in up to 50% reduction in grain yields.

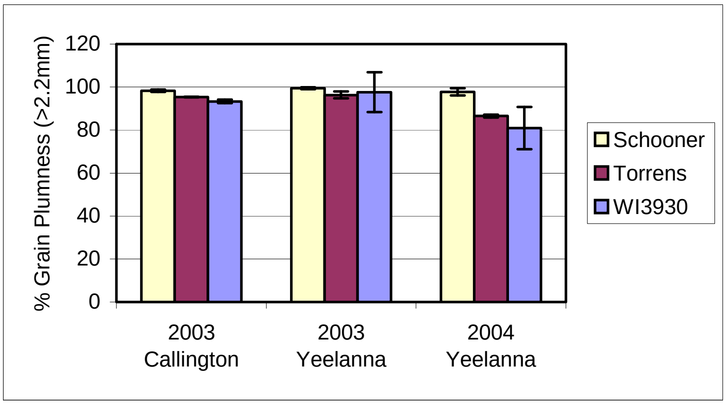
To reduce the severity of these negative traits, the University of Adelaide Barley Program has been actively seeking and using barley germplasm that is semi-dwarf in stature, resulting in the development of WI3930. WI3930 has a semi-dwarf habit and is lodging, stem break and head loss resistant. Whereas, Torrens has a medium plant height and is susceptible to head loss but has better resistance to lodging and stem break compared to Schooner (data not shown).

Grain Plumpness and 1000 Kernel Weights:
A major objective of the University of Adelaide Barley Program is to enhance the profitability of hulless barley for the feed industry. This is achieved by developing hulless types with improved and stable grain size and increased 1000 kernel weights.

1. Grain Plumpness (>2.2mm (%))

Torrens, WI3930 and Schooner were grown in three replicated trials at Yeelanna, Eyre Peninsula, South Australia during 2003 and 2004, and Callington, Upper Murray Mallee, South Australia during 2003. 100 gram samples were cleaned and screened over a 2.2 mm screen using Sample Cleaner Model SLN 3, Rationel Kornservice, Denmark. The results indicate that both Torrens and WI3930 have smaller grain than Schooner (Figure 3). However, the 2003 season was more favourable for plumper grain than 2004, when drier conditions were experienced during grain filling (September – October period). This trend is reflected in the 17% lower grain plumpness of WI3930 compared to Schooner in Yeelanna 2004. This can be explained by WI3930 having a later flowering time than Schooner, making WI3930 unable to avoid flowering and grain filling during the drier Spring months. Similarly, Torrens had an 11% decrease regardless of comparable maturities with Schooner. This trend was also reflected in 2003 Callington, with Torrens having 2% and WI3930 having 5% lower grain plumpness than Schooner. This could be explained by the lighter, less fertile soils observed at Callington. Such an environment would favour the maturity and adaptation of Schooner and Torrens, rather than WI3930.

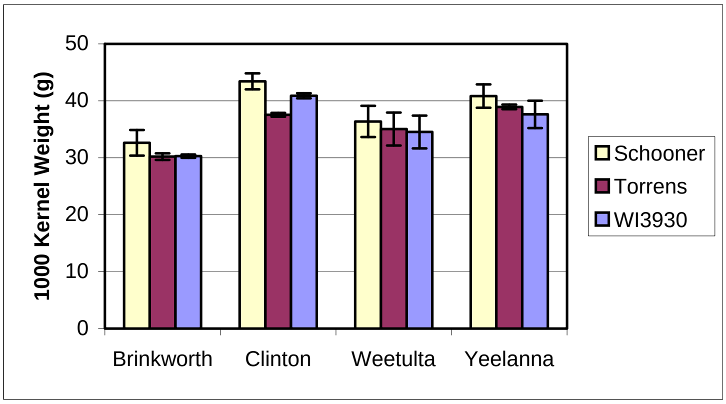
Figure 3: Grain plumpness performance for Schooner, Torrens and WI3930 grown at three locations in South Australia during 2003 and 2004



2. 1000 Kernel Weights (g)

Torrens, WI3930 and Schooner were grown in three replicated trials at four locations across South Australia (Brinkworth, Clinton, Weetulta and Yeelanna) during 2004. Samples were cleaned and screened over 2.2mm screen and 1000 grains were counted using a Contador Pfeuffer Seed Counter and weighed. The results indicate that both Torrens and WI3930 have lower grain weights for all three sites when compared to Schooner (Figure 4). This difference can be explained by the presence of an attached husk in the Schooner samples.

Figure 4: 1000 Kernel Weights for Schooner, Torrens and WI3930 grown at four locations in South Australia during 2004.



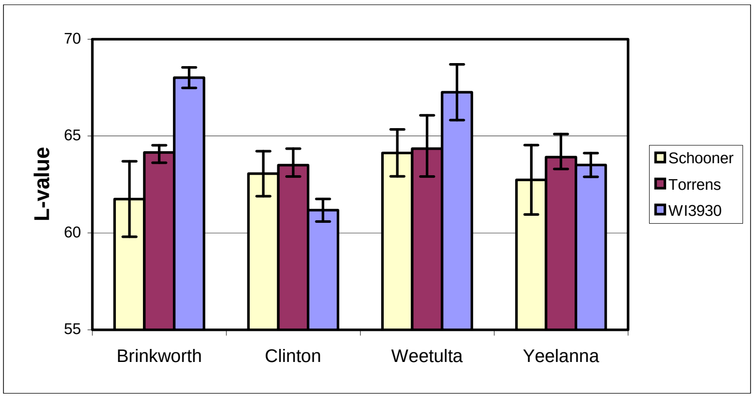
Kernel Colour:

Kernel colour has no effect on food value but the product must be aesthetically pleasing to the grower and the purchaser of the product for it to be successfully marketed. We have observed genetic variation in kernel colour with earlier crosses Galleon/CIMMYT 42002 and Galleon/Richard and the worst crosses having Chebec as a parent, for example, Chebec/SB85216 and Nudinka/Chebec (data not shown).

More recently, we have been using germplasm from Canada (for example, TR118) and Japan (for example, Nasu nijo) and the progeny are being assessed in early generations trials in 2005, with the first yield trials to be assessed in 2007.

Whiteness (L-value) of grain samples were measured with a Minolta colorimeter using the CIE L*a*b colour score. The results indicate the best locations for producing the whitest grain are Brinkworth and Weetulta, with Yeelanna producing the lowest L-values (Figure 5). It is difficult to compare whiteness values between covered and hulless genotypes due to the component of the grain being measured, for example, husk in covereds and aleurone in hulless. The issue of threshability in hulless genotypes further complicates this. However, colour differences may be explained through the spike morphology. The developing hulless grain can often get too large for the surrounding palea and lemma. Consequently, there can be a gap of a few millimetres between the palea and lemma and so the grain can be exposed resulting in ‘sunburn’. It is a problem that has not been observed in wheat, triticale or oats. The results demonstrate the large environmental influence on kernel colour.

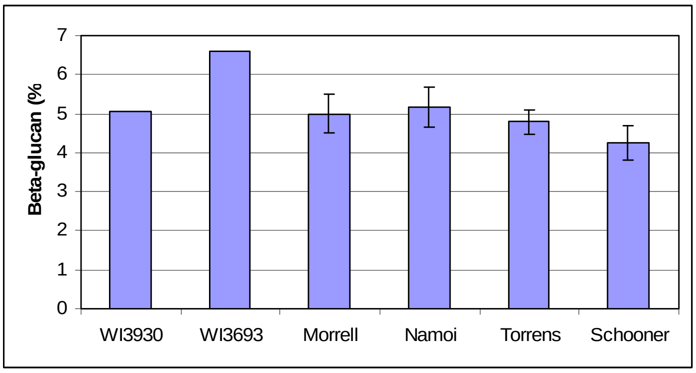
Figure 5: Colour measurement (L-value) for Schooner, Torrens and WI3930 grown at four locations in South Australia during 2004.



β-glucan analysis:

β-glucan content was measured on the pure seed production of WI3930 and WI3693 planted at Turretfield Research Station in 2005. Both hulless genotypes produced high β-glucan levels of 6.61% and 5.04% (dwt). The result for WI3693 was significantly higher than results obtained for normal, hulless and covered genotypes from 2004 (mean of 4 sites, 3 replicates) (Figure 6).

Figure 6: Beta-glucan analysis



Pearled barley:

WI3693 performed well in pearling trials as assessed by percentage of sound kernels remaining after pearling (Figure. 8). At all sites WI3693 had at least 80% sound kernels after pearling. This was higher than or equivalent to the sound kernels of both hulless and covered types. Environment plays a significant role in pearling quality; so four diverse sites and three plot replicates were analyzed. In addition, pearled barley whiteness (L-value) was measured (Figure 9). Again, WI3693 was brighter than the other genotypes analysed, with an average L-value of 75.

Figure 8: Pearling quality as expressed as % sound kernel from 2004 South Australian trials (4 sites, 3 replicates)

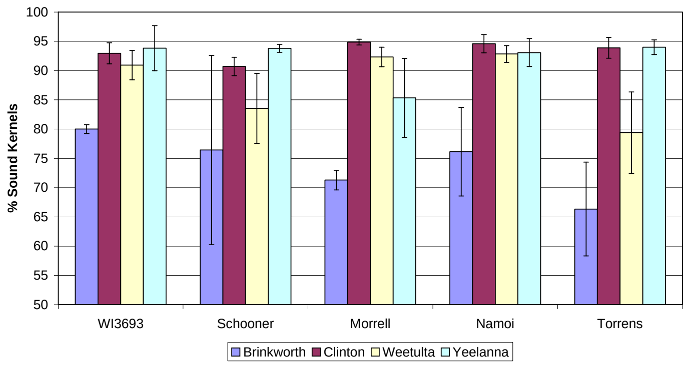
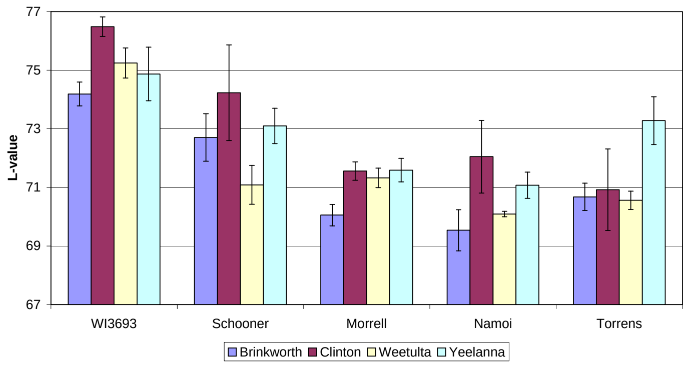


Figure 9: Colour measurement (L-value) from 2004 South Australian trials (4 sites, 3 replicates)



Flaked/Rolled barley:

Rolling of WI3693 produced a thin white flake similar in colour to Morrell (Figure 10a: WI3693 (left), Morrell (middle) and Euro Oats (right)), but marginally darker than the oat product (Figure 10a). WI3693 flakes were the only sample to be retained above all screens with greater than 80% efficiency (Figure 10b). Morrell performed satisfactory with only 60% of the "pass 2" flakes retained over the 3.86mm screen. The oat sample performed poorly with very high screenings for all sieves. The poor performance of oats was probably the result of the extreme rolling conditions compared to industry practice. However, it does demonstrate the resilience of WI3693 and Morrell during flaking.

Figure 10a: Visual comparison of barley flakes

Figure 10b: Whiteness (L-value) of rolled barley and oats

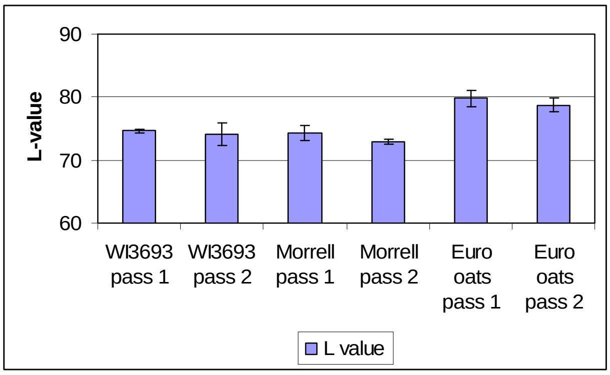
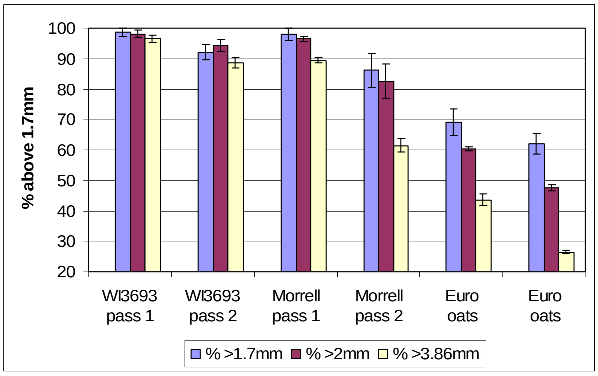


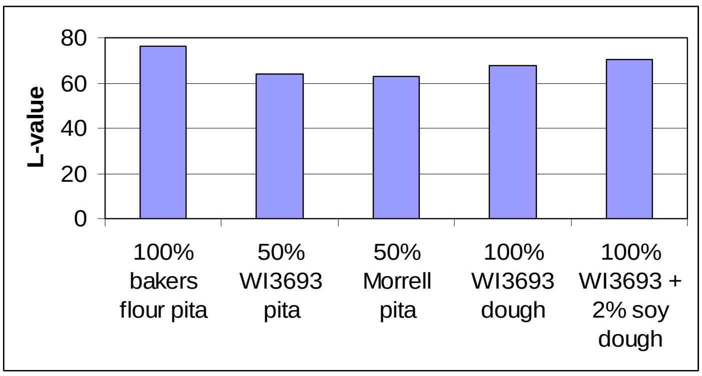
Figure 10c: % Retained above three sieves



Barley pita breads:

Both 50% barley pita breads were visually darker than those made with 100% bakers flour, with the 50% WI3693 pita having marginally better colour than the 50% Morrell pita. It also pocketed better and staled more slowly than the 50% Morrell pita. To investigate whether lipoxygenase could improve WI3693 dough colour, 2% soy flour (containing natural lipoxygenase) was added to the 100% WI3693 flour dough. Colour measurements show that dough colour can be improved by the addition of a natural lipoxygenase (Figure 11).

Figure 11: Whiteness score of pita breads and waxy barley flour dough (Minolta L-value). Colour measurements were taken on the inside of the pita pocket.



WI3693 pita bread had higher moisture, fat, ash and β-glucan content than the other two pita breads (Table 1). However, the carbohydrate and starch content were lower. Both barley pita breads had similar fibre content, which was almost twice as much as that of the bakers flour control bread, however the β-glucan component was significantly higher in the WI3693 pita. The higher moisture is favourable to industry due to lower product cost per volume. The higher fibre, β-glucan and lower carbohydrate content of the barley pita breads, provides significant health advantages.

Table 1. Carbohydrate and beta-glucan analysis of Pita breads

	50% WI3693 flour	**50% Morrell flour**	**100% Bakers flour**
Moisture %	41.6	40.5	35.6
Fat %	2.7	2.4	2.0
Protein (N x 5.7) %	8.6	8.4	8.6
Ash %	1.7	1.6	1.5
Total Dietary Fibre %	5.8	5.8	2.8
Carbohydrate (db) %	39.6	41.3	49.5
Total Starch %	37.5	39	47
Resistant starch %	ND	ND	ND
Total β-glucan %	3.94	2.76	0.43

ND=Not Detected, Detection Limit: Less than 0.5%

High Amylose Barleys:

The introgression of the high amylose trait in locally-adapted germplasm has been a high priority for the barley program. The high amylose lines we have been crossing with are derived from Scotland and they are tall resulting in severe lodging, stem break and head loss. The three lines that have been selected for promotion are a straight cross between SES15-30 (Scotland, 2-row, covered, high amylose) and WI3152 (semi-dwarf, 2-row, hulless, normal).

Table 2 describes the amylose content, β-glucan content, 1000 kernel weights, grain protein, germination and kernel colour (L-value).

Table 2. Preliminary grain quality analyses of high amylose lines

Genotype	**Amylose Content (%)**	**Beta-glucan (% dwt)**	**1000 kernel weight**	**Grain protein (%)**	**L-value**
ANBX98002-11	39.63	5.43	43.35	12.86	63.67
ANBX98002-58	41.11	6.88	48.00	12.71	-
ANBX9802-126	33.45	4.46	48.91	14.58	59.10
Schooner	27.66	4.25 (2004)	38.31 (2004)	-	62.92
High Amylose Glacier	40.52	-	-	-	-

Conclusion:

The University of Adelaide Barley Program has been developing hulless barley breeding since 1989 and the major objective is to develop hulless types for the following end uses:

1. Feed types for monogastric animals (normal starch), 2. Food industry (waxy and high amylose), 3. Various industrial and commercial uses (e.g. low and high amylose), and 4. Malting and brewing industries.

Our biggest challenge has been to develop hulless lines that are more comparable to Australian locally adapted cultivars. As a result, the University of Adelaide Barley Program released Torrens in 2001 with a national, co-ordinated approach as a first step to supporting similar developments observed in the Canadian pig industry. Compared to older hulless cultivars, Torrens has improved grain yield, agronomy and feed quality. The barley program has now also targeted the development of hulless barley adapted to higher rainfall areas. Pure seed production of the hulless feed barley line WI3930 commenced in 2005, with potential release in 2006. WI3930 has improved grain yield, foliar disease resistance and feed quality compared to Torrens.

The challenge with our germplasm development has been in assessing those breeder's lines that offer a wide variety of quality traits. In addition, the requirements of the respective industries are constantly changing and reviewing their objectives. However, we have shown results for two advanced genotypes that indicate potential for a diverse range of industries including food and feed. The future for the development of hulless barleys is looking very favourable.

We have described the nutritional benefits of the waxy, hulless lines WI3693. Results indicate a grain that is fibre-rich, low fat and potentially low GI. In addition, it also displays excellent physical properties in pearling, flaking and pita bread production compared to normal hulless genotypes and oats.

WI3693 whole milled flour incorporated at 50% into pita breads, produced an acceptable product that pocketed well with a fibre and β-glucan content more than 2 times and 9 times, respectively, than that of the wheat control. The high moisture content of this product is also attractive to manufacturers, who can save on ingredient costs. The high β-glucan content of waxy dough results in a higher moisture uptake. There was also an indication that the waxy product staled slower than the normal starch barley product, although staling occurred more slowly in the wheat control. The issue of staling needs to be further investigated. The addition of 2% soy flour showed an improvement in the pita bread colour.

Barley has been shown to have a very low Glycemic Index (GI) compared to other grains (Arndt *et al.*, 2006). Waxy hulless barley could offer a low cost, value-added ingredient to the health conscious food producer and consumer. Other foods and products made from WI3693 are a high priority for future research.

Finally, we also described the preliminary results obtained for the high amylose types. Initial results indicate these lines are comparable to normal starch types for grain 1000 kernel weights, grain protein, and kernel colour. They also have the added nutritional benefit of having at least a 2% higher β-glucan level than Schooner. High amylose hulless barley also has the potential to make an important contribution to improving human health, particularly the prevention and management of diabetes, coronary heart disease, obesity, colon and rectal cancers, constipation and diverticular disease. There is still a large amount of research to be done to improve the agronomy and product development of high amylose barleys and make them more competitive with high amylose maize.

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