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Blending: consequences for wheat breeding

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1 Summary

An experiment was conducted to examine the effects that different alleles in a Kukri-Janz Doubled Haploid (KJDH) population had on dough quality both separately and when different flour samples were blended. During this experiment, it was discovered that the population was also segregating at the *Glu-B1* locus. The pure flour results (from 2 years of data) showed that there are interactions between all alleles at the 4 loci in the KJDH sample set to produce the final dough quality. The *Glu-B1* and *Glu-D1* alleles had important effects on dough mixing characteristics, strength and extensibility, but these were often modified by interactions with the *Glu-A3* and *Glu-B3* alleles. Overall, the *Glu-B1al* allele tended to produce flours with higher extensibility and more uniform dough characteristics than the *Glu-B1u* allele, and the *Glu-D1d* allele produced flours with higher mixing times, higher stability and stronger dough than the *Glu-D1a* allele.

When these alleles were blended together, some non-linear effects were seen. The extent of the non-linearity depended both on the background allele combinations, and the alleles being blended. For example, extensibility per unit of flour protein (E/P) was increased over the predicted amount by the addition of 25% *Glu-B1al* flour in a *Glu-D1a* background, but decreased in a *Glu-D1d* background. These complex interactions show that it is necessary to know both the alleles present in the flour and how they interact to make accurate predictions of rheological behaviour and hence end-product quality.

2 Introduction

Wheat quality, the suitability of a particular wheat for a certain end-use, is determined by combination of environmental and genetic factors. Some of the genetic factors that are highly important in dough quality are the proteins coded by the glutenin genes on the long and short arm of chromosome 1. At each glutenin gene there are a number of different possible alleles. Due to the large number of different alleles, many different dough characteristics can result, which are used for a wide range of end-products.

When varieties with different alleles are blended to produce flour for a specific purpose, the dough properties are not always those that would be predicted by a linear relationship between the original flours. Synergistic effects can occur which unexpectedly increase or decrease dough properties from those which would be envisaged. These non-linear effects need to be known so that blending can be carried out and the resulting flour properties known. Bekes and Wrigley (1999) in the QWCRC Project Report #35 showed that blending different *Glu-D1* and *Glu-B1* alleles could result in non-linear effect for a number of dough quality parameters.

The current experiments used a Kukri-Janz Doubled Haploid (KJDH) population to investigate:

1) the effects of the alleles present in the population at the *Glu-B1*, *Glu-D1*, *Glu-A3*, and *Glu-B3* loci on dough properties as measured by the farinograph and extensograph, and
2) the effects of blending various alleles on the dough properties of the flour produced.

3 Materials and Methods

3.1 Experiment Design

Initially, the experiment was designed to test the interactions between the glutenin alleles present in a Kukri-Janz Doubled Haploid population. Kukri and Janz were thought to differ at 3 loci (see Table 1).

Table 1: Differing alleles of Janz and Kukri (2000).

	Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
Janz	a	b	a	b	b	b
Kukri	a	b	d	d	h	b

In 1999, a doubled haploid population of approximately 200 lines (double plots, replicated twice) was sown at the University of Adelaide's Roseworthy Agricultural Campus, South Australia (34° 32' S, 138° 42' E). The allele combinations were checked using the SDS-PAGE method of Singh *et al* (1991) (with minor modifications), and 70 lines, containing all 8 allele combinations known (although not in equal numbers), were selected for quality testing.
These lines were Buhler milled, and the flour tested for dough rheology and baking quality by the SARDI Grain Quality Laboratory staff (now Australian Grain Technologies). For the rheology and baking methods, see Measurements.

The same 200 lines were also sown at Roseworthy in 2000 (also double plots, replicated twice). Initially, a statistically balanced subset (8 allele combinations, 3 lines of each, replicated twice) of the 70 lines from 1999 were selected and quality tested using the 35g Mixograph, 50g Farinograph and 50g Extensograph. However, it was discovered that Kukri contains the *Glu-B1al* allele, and Janz the *Glu-B1u* allele, (Table 2) instead of both of them being the *Glu-B1b* allele as previously thought. The samples selected were tested for the *Glu-B1al/u* alleles using the RP-HPLC method of Vawser and Cornish (2004), which distinguishes between the elution times of the 8 and 8* sub-units (Figure 1).

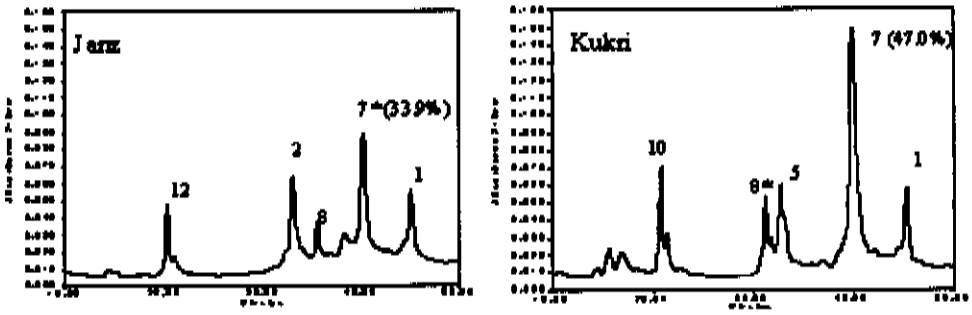

Figure 1: RP-HPLC traces for Janz and Kukri. Note the different positions of the 8 and 8* sub-units.

It was found that the set was missing several allele combinations. New samples in the 200 line set were found to fill the gaps and create a new statistically balanced design (16 allele combinations, 2 lines of each, replicated twice), and the entire set was re-tested using the Farinograph and Extensograph. There was not enough flour remaining to re-test the Mixograph results.

Table 2: Actual alleles of Janz and Kukri (2001).

	Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
Janz	a	u	a	b	b	b
Kukri	a	al	d	d	h	b

With the information gained from the testing of the pure lines, a number of allele combinations were selected for testing as blends. As only small quantities of flour remained, only limited testing could be carried out. The blends tested are detailed below.

Table 3: Testing the *Glu-B1al/u* alleles with a *Glu-D1a*, *Glu-A3b* and *Glu-B3h* background.

	J-014 (*Glu-B1al*)	
J-040 (*Glu-B1u*)	Rep 1	Rep 2
Rep 1	25% *al*, 75% *u* 50% *al*, 50% *u* 75% *al*, 25% *u*	
Rep 2		25% *al*, 75% *u* 50% *al*, 50% *u* 75% *al*, 25% *u*

Table 4: Testing the *Glu-B1al/u* alleles with a *Glu-D1d*, *Glu-A3b* and *Glu-B3h* background.

	J-130 (*Glu-B1al*)	
J-083 (*Glu-B1u*)	Rep 1	Rep 2
Rep 1	25% *al*, 75% *u* 50% *al*, 50% *u* 75% *al*, 25% *u*	
Rep 2		25% *al*, 75% *u* 50% *al*, 50% *u* 75% *al*, 25% *u*

Table 5: Testing the *Glu-D1a/d* alleles with *Glu-B1u*, *Glu-A3b* and *Glu-B3h* background.

	J-040 (*Glu-D1a*)	
J-083 (*Glu-D1d*)	Rep 1	Rep 2
Rep 1	25% *a*, 75% *d* 50% *a*, 50% *d* 75% *a*, 25% *d*	
Rep 2		25% *a*, 75% *d* 50% *a*, 50% *d* 75% *a*, 25% *d*

Table 6: Testing the *Glu-D1a/d* alleles with *Glu-B1al*, *Glu-A3b* and *Glu-B3h* background.

	J-014 (*Glu-D1a*)	
J-130 (*Glu-D1du*)	Rep 1	Rep 2
Rep 1	25% *a*, 75% *d* 50% *a*, 50% *d* 75% *a*, 25% *d*	
Rep 2		25% *a*, 75% *d* 50% *a*, 50% *d* 75% *a*, 25% *d*

3.2 Measurements

Flour moisture and protein were measured using NIR spectroscopy.

3.2.1 Farinograph

In the first year of the experiment, the farinograph method used was that of the SARDI Grain Quality Laboratory in 2000. Fifty g of flour (adjusted for a moisture content of 13.5%) was used for each test. The sample was dry mixed for 1 minute and sufficient water added using a burette to centre the dough on the 500 BU line at maximum dough development. Each sample was run for 10 minutes after the peak dough development to obtain a stability reading.

Measurements of the farinographs are shown below (Figure 2). There were differences in the methods used to calculate certain measurements, which must be

taken into account. In the first year, farinograph measurements were Water Absorption (WA), Dough Development Time (DDT), Stability and 5-minute Breakdown. Water Absorption was calculated from the amount of water added to the mix, corrected for the moisture percentage of the flour to give total water content as a percentage of flour weight. Dough Development Time was as seen in Figure 2. Stability was measured as the time from Peak Dough Development (PDD) to the Departure Time (DT), however, if the trace did not reach the DT before 10 minutes, stability was only recorded as ">10 minutes". The 5-minute Breakdown measurement was the drop measured in the centre of the trace from peak dough development to 5 minutes after PDD.

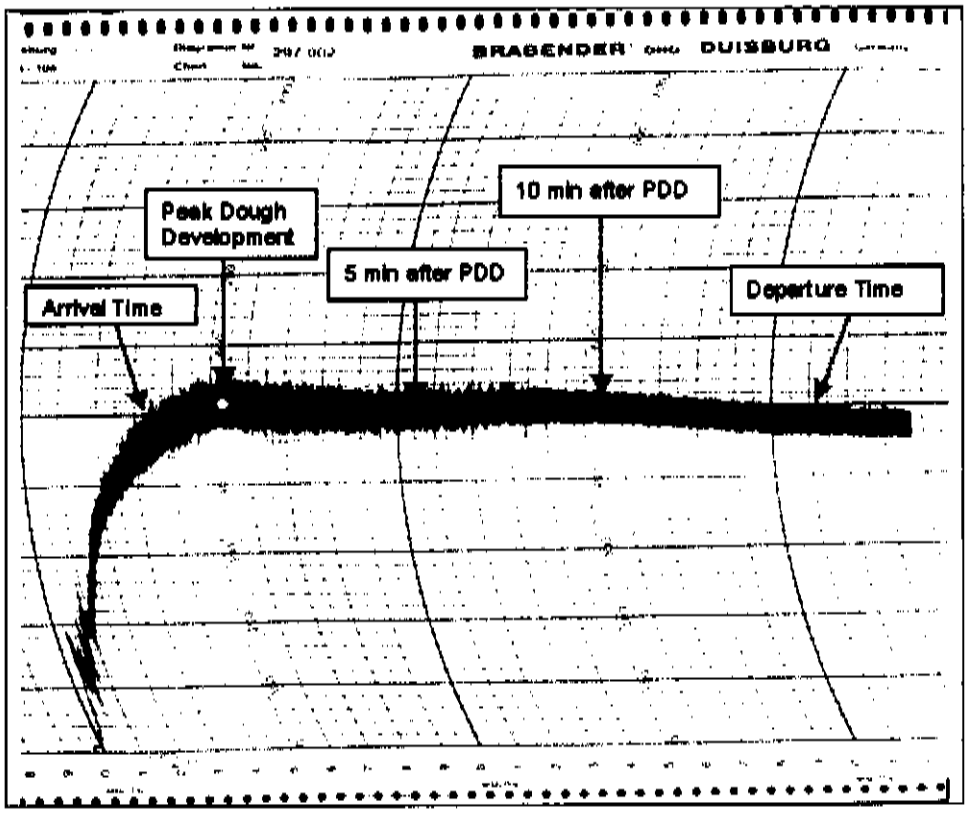

Figure 2: Measurements taken from the farinograph.

In the second year, the farinograph method used was RACI Method 06-02. WA was measured in the same way as in year 1. Dough development time could not be determined accurately for many samples (see Figure 3), and was not used in the analysis, hence neither was 5-minute Breakdown. Instead, Arrival Time (AT) and Departure Time (DT) were measured, and the difference between them used for the Stability measurement. Each sample was mixed until the trace showed a distinct change in form to a smooth narrow trace, and dropped below the 500 BU line. This was necessary as it was found that some samples could drop below the 500 BU line but then gain strength again.

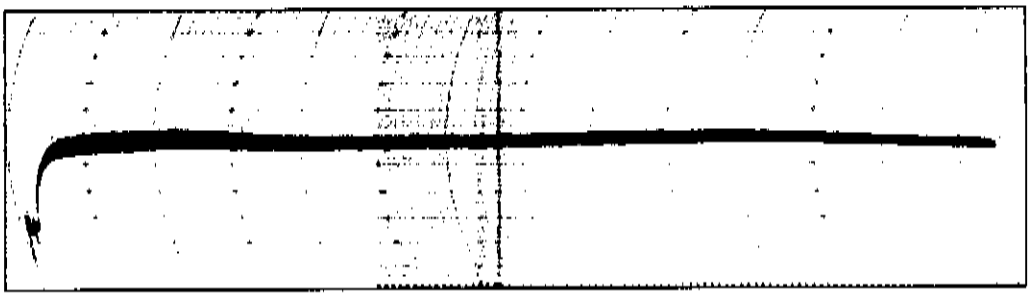

Figure 3: Why Peak Dough Development was difficult to measure.

Each sample was tested in duplicate in both years. When blended tests (second year) were being performed, appropriate amounts of each sample were measured out separately and dry mixed together for 1 minute before adding water to obtain a homogenous sample.

3.2.2 Extensograph

Extensographs were carried out using the 50g Extensograph method used by the SARDI Grain Quality Laboratory. A farinograph was used to mix the dough. Fifty grams of flour (adjusted to 13.5 % moisture content) was used for each test. One gram of sodium chloride was weighed out and dissolved in a sufficient amount of water to centre the dough trace on the 500 BU line after 5 minutes, and the solution was added to the flour after 1 minute of dry mixing. After the dough was mixed for 5 minutes, it was removed from the farinograph bowl, weighed and scaled off to 75g, moulded, rolled, and placed in a cradle in an incubation cabinet at 30°C for 45 minutes. After this time had elapsed, the dough piece was stretched on the extensograph, with 75g of lead weights added to the cradle.
Samples were measured in duplicate for maximum resistance (Rmax) and extensibility. Blends were mixed together as in the farinograph.

3.2.3 Mixograph

Mixograph tests were only conducted in the second year of the experiment, using a selection of flours from the 70 lines tested in the first year. This selection was statistically designed to be balanced with the allele information available at the time, but was subsequently found to be incomplete, and not enough flour remained for re-testing. The flours were randomly tested in duplicate on the mixograph using AACC Method 54-40 at a water absorption of 63 %, using 35 g flour (adjusted for moisture content of 14%) and 22 ml de-ionised water, a cabinet temperature of 21 °C and the spring set at 10. All mixographs were run for 10 minutes.
The graphs were examined and readings taken for Peak height (cm), Mix Time (s), 5 min height, Bandwidth at Peak Height, Bandwidth at 5 min, Ascending Angle (degrees) and Descending Angle.

3.3 Statistical Analysis

Statistical analysis was carried out using the packages Genstat 5 and Genstat 7. Each year's results were analysed separately since the quality testing measurements and

experimental designs were not the same in each year. After the discovery of the *Glu-B1al/u* alleles, the results from the first year and the mixograph results were analysed using the residual maximum likelihood (REML) method as the numbers of lines in each allele category were uneven, and some allele combinations were missing entirely.

The results from the second year, when the testing had been statistically designed, were analysed using a 4-way ANOVA.

4 Results

4.1 Pure Lines

4.1.1 1999 Farinographs

The farinograph data from 1999 is not as useful as that of 2000 due to the way in which measurements were taken. The running of the samples for only 10 minutes after the peak dough development has problems for the stability values, which are underestimated for a number of samples. Also, some samples, as has already been noted, may not reach their true peak development for some time after the first part of the trace has levelled off. Thus the Dough Development Times may also be inaccurate for a large number of samples. Further, the *Glu* allele combination *Glu-B1u, Glu-D1a, Glu-A3b* and *Glu-B3h* is missing from the data set. Nevertheless, some conclusions can still be drawn from this data.

Figure 4 shows the mean water absorption values for the 1999 flour data. Analysis showed that there was a significant main effect of the *Glu-A3* allele on water absorption, with flours with the *Glu-A3b* allele having a higher mean water absorption (63.6%) than those with the *Glu-A3d* allele (62.3%). This difference is clearer for those samples which also had a *Glu-B1al* allele, but there was no interaction.

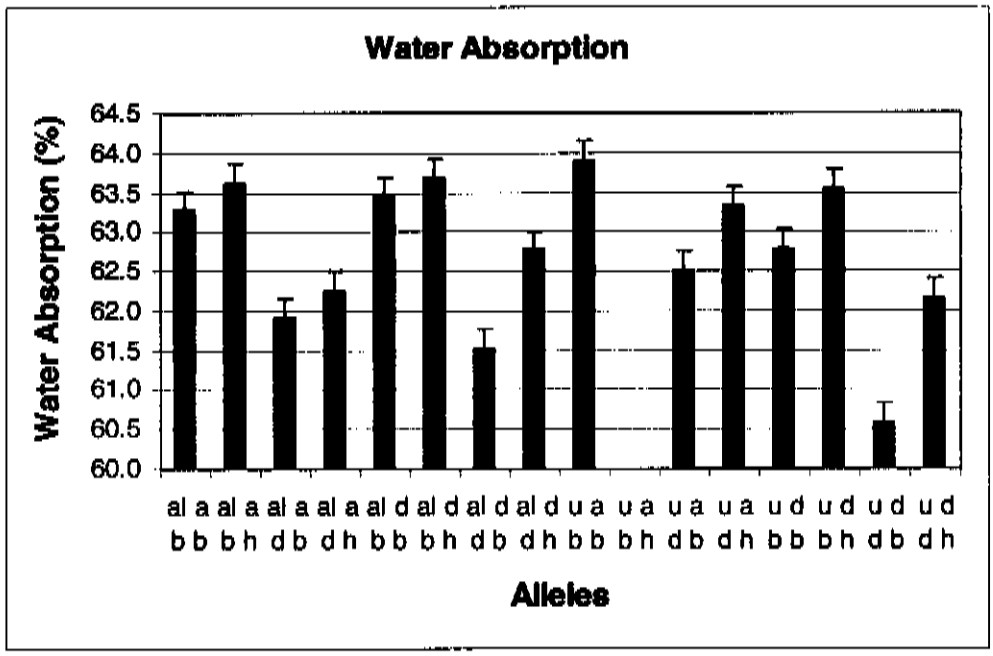

Figure 4: Water absorption for 1999 data.

Water absorption data should be treated with caution as there may be another factor present due to possible segregation of *Pina* and *Pinb* alleles in this population (Cane *et al*, 2004).

Dough Development Time showed a significant interaction of the *Glu-B1* and *Glu-B3* alleles, as well as a significant main effect of the *Glu-D1* allele. Development Time was higher for flours containing the *Glu-D1d* allele (mean 7.8 min) than for flours

containing the *Glu-D1a* allele (mean 6.4 min, lsd 0.64). The *Glu-B1* and *Glu-B3* interaction is shown in Table 7.

Table 7: Means of DDT for *Glu-B1* and *Glu-B3* interaction, lsd 0.93. Letters indicate statistical similarities or differences.

	Glu-B1b	*Glu-B3h*
Glu-B1al	8.062^{A}	7.125^{B}
Glu-B1u	6.372^{C}	6.910^{B}

Means of the entire data set are shown in Figure 5. DDT cannot be compared to the 2000 results as DDT was not measured in 2000.

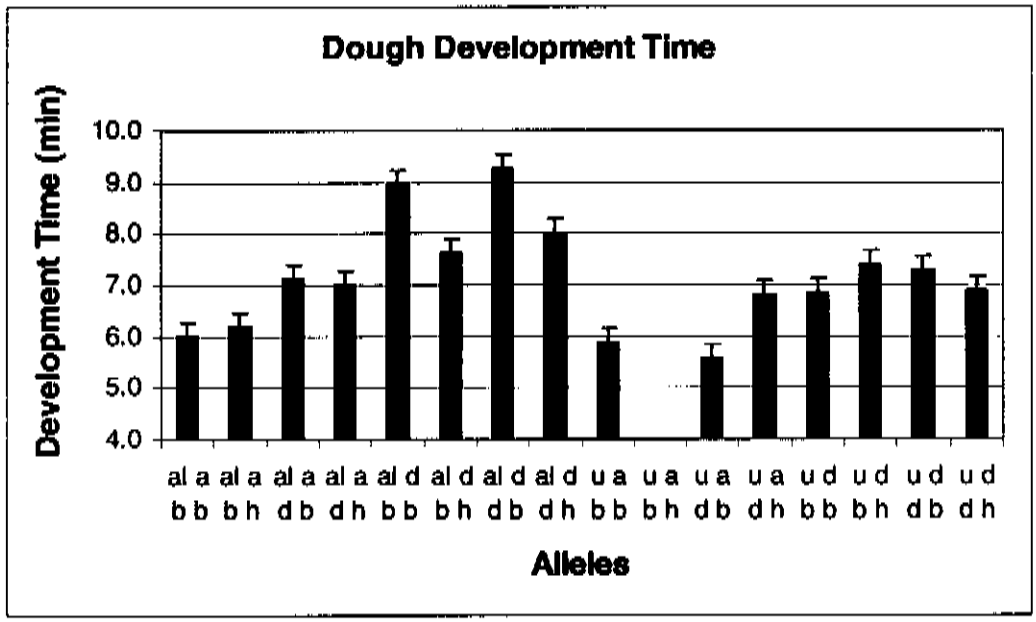

Figure 5: Dough Development time, 1999 data.

The stability data showed a strong interaction of the *Glu-B1* and *Glu-D1* alleles. The doughs with *Glu-B1al + Glu-D1a, Glu-B1al + Glu-D1d* and *Glu-B1u + Glu-D1d* had mean stability values of 9.06, 9.8 and 9.3 minutes respectively. The dough with alleles *Glu-B1u + Glu-D1a* had a stability of 6.6 minutes, much lower than the others (lsd 0.97). This can be seen in Figure 6, as the group of lines with the *Glu-B1u + Glu-D1a* alleles has a clearly lower stability than the rest.

Note that the stability data from 1999 were not measured in the same way as in 2000, as stability was only measured to a maximum of 10 minutes in 1999, starting from PDD. When the means from the 2 years were correlated to check for a relationship, none was found (r^2 = 0.057). Although the *Glu-B1* and *Glu-D1* alleles played an important part in both years, the results from 2000 are much more accurate.

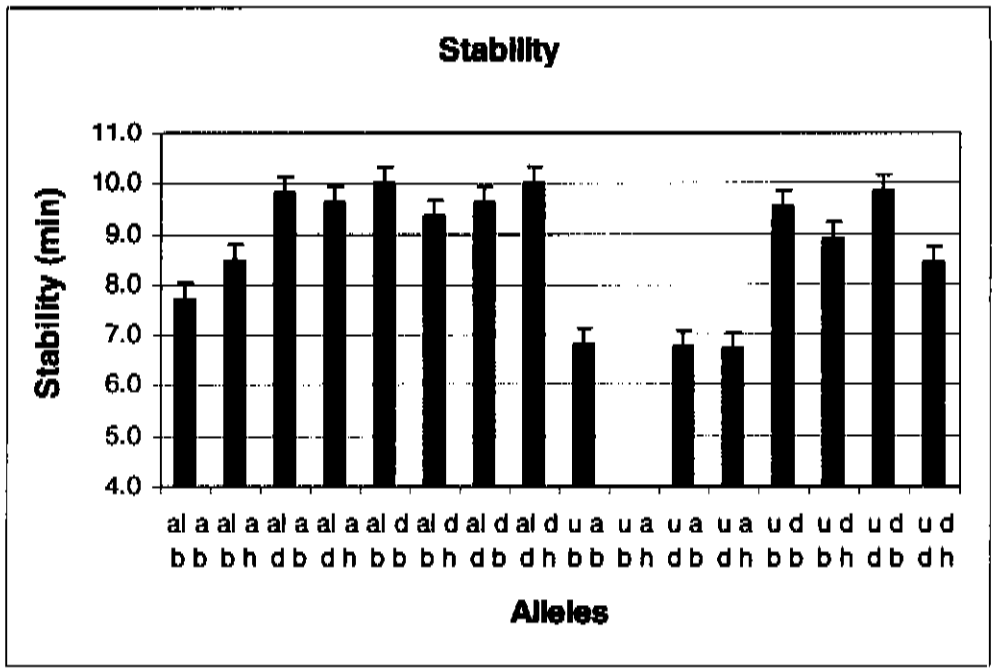

Figure 6: Stability measurements in 1999.

Breakdown had two separate main effects of the *Glu-B1* and *Glu-D1* alleles. Flours containing the *Glu-B1al* allele had lower breakdown (21.1 BU) than those with the *Glu-B1u* allele (28.4 BU, lsd 4.4). Flours with the *Glu-D1d* allele had lower breakdown (19.8 BU) than those with the *Glu-D1a* allele (29.6 BU lsd 4.3). This grouping can be seen in Figure 7.

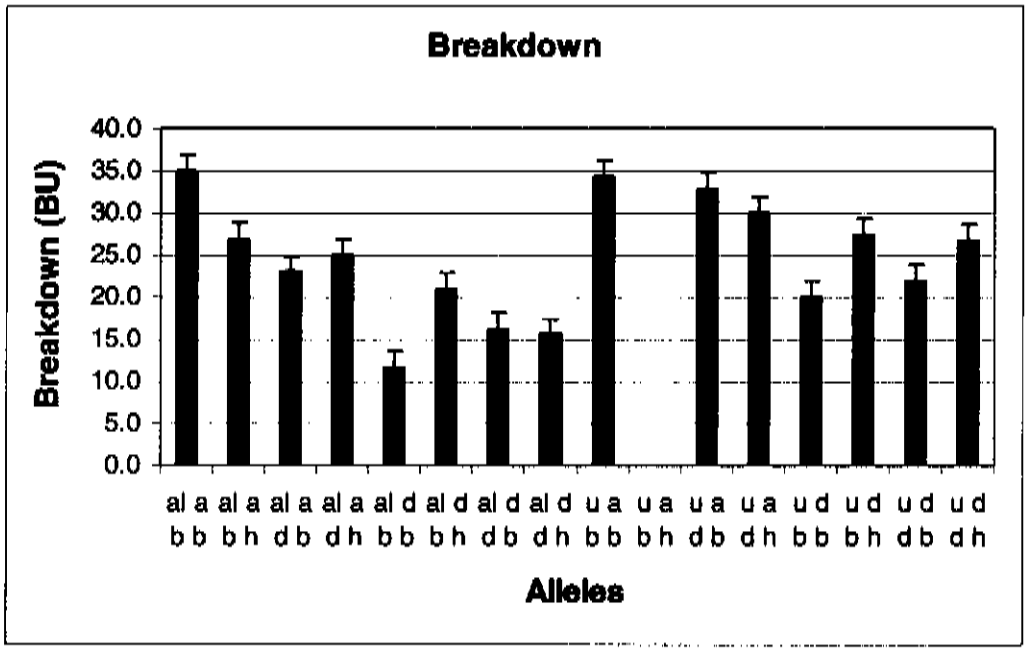

Figure 7: Five-minute Breakdown for flours in 1999.

4.1.2 1999 Extensograph

Extensograph measurements were done in the same way in both 1999 and 2000, and are much more comparable. However, it should be noted that due to the very long mixing times of some of the samples, and the standardised nature of the test with a 5 minute mixing time, some of the samples may have performed well below their potential due to undermixing.

Dough strength (Figure 8) was significantly affected by the *Glu-B1*, *Glu-D1* and *Glu-B3* alleles in 1999. There were no interactions. Doughs with *Glu-B1al* were stronger (446 BU) than those with *Glu-B1u* (354 BU, lsd 35.7). The doughs with *Glu-D1d* alleles were stronger (483 BU) than those with *Glu-D1a* (317 BU, lsd 34.9). Also, doughs with *Glu-B3b* alleles were stronger (435 BU) than those with *Glu-B3h* (365 BU, lsd 36.0). Rankings between alleles at a given locus are similar to those reported by Eagles *et al* (in press).

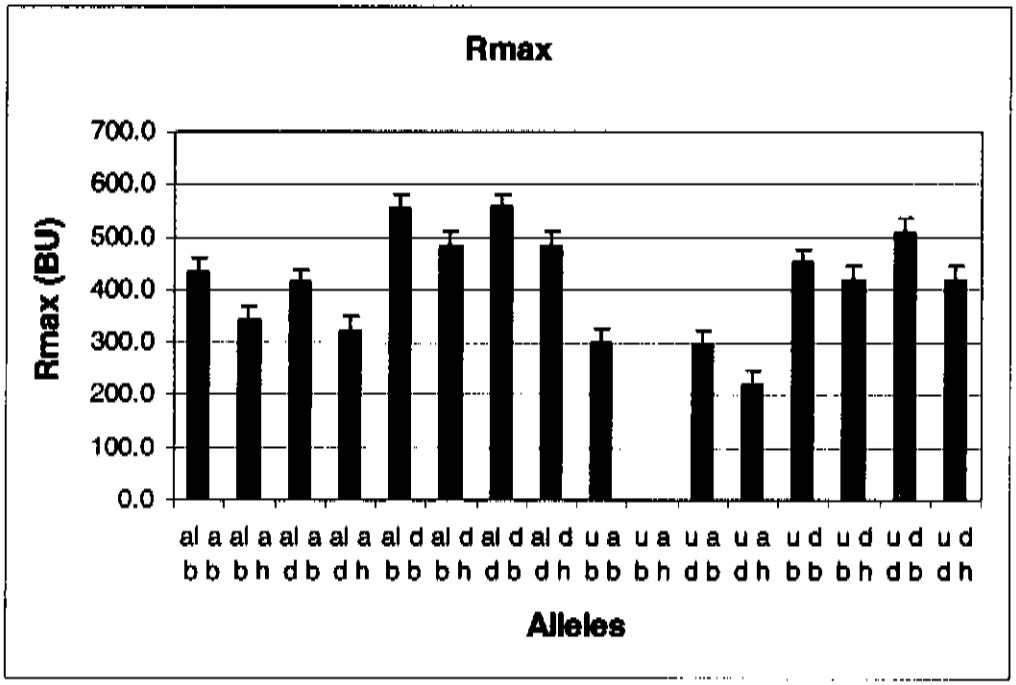

Figure 8: Dough strength (1999).

Extensibility was significantly affected by a *Glu-B1* and *Glu-A3* interaction. Flours with the *Glu-A3b* allele were similar in extensibility but those with the *Glu-A3d* allele were different. The interaction results are summarized in Table 8.

Table 8: Interactions present in Extensibility data, 1999 (lsd 0.83).

	Glu-A3b	*Glu-A3d*
Glu-B1al	24.9^{B}	25.9^{A}
Glu-B1u	24.6^{BC}	23.8^{C}

Overall, flours with the *Glu-B1al* allele were clearly more extensible on average than those with the *Glu-B1u* allele (see Figure 9).

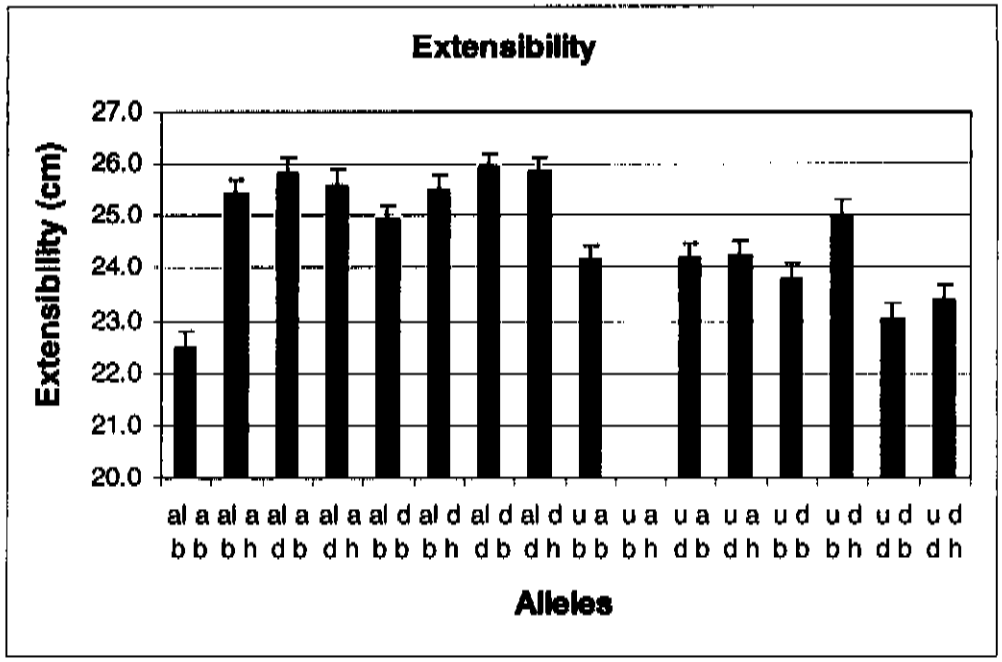

Figure 9: Extensibility data (1999).

When the extensibility was measured per unit protein, the *Glu-A3* allele effect was removed and the only significant effect was caused by the *Glu-B1* allele type. Flours with the *Glu-B1al* allele had more extensibility per unit of protein (2.0 cm per percentage point) than those with the *Glu-B1u* allele (1.86 cm per unit protein, lsd 0.07.) This difference is visible in the full data set (Figure 10).

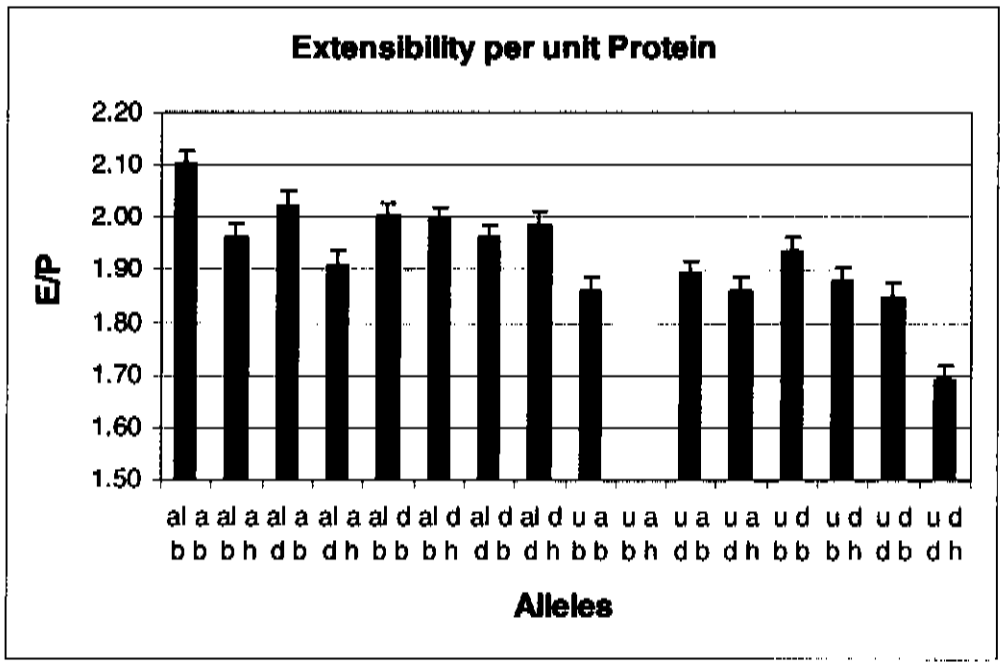

Figure 10: Extensibility per unit Protein (1999)

4.1.3 1999 Correlations

The correlations between the various farinograph and extensograph measurements were examined, with most measurements showing low correlations. The highest correlations were found between stability and breakdown (-0.84), flour protein and E/P (-0.78), stability and Rmax (0.77) and development time and breakdown (-0.76).

Table 9: Correlations between the measurements taken in 1999.

	PSI	FP	WA	DDT	Stab	BD	Rmax	Ext	E/P
PSI	1								
Flour Protein	0.12	1							
Water Absorption	-0.31	0.49	1						
Development Time	0.00	0.38	0.11	1					
Stability	-0.12	0.17	0.005	0.73	1				
Breakdown	0.08	-0.13	0.01	-0.76	-0.84	1			
Rmax	-0.02	0.06	-0.22	0.66	0.77	-0.72	1		
Extensibility	0.15	0.45	0.22	0.37	0.22	-0.22	0.093	1	
E/P	-0.04	-0.78	-0.37	-0.16	-0.03	-0.01	0.005	0.200	1

PSI – Particle Size Index; FP – Flour Protein; WA – Water Absorption; DDT – Dough Development Time; Stab – Stability; BD – Breakdown; Rmax – Maximum Dough Resistance; Ext – Extensibility; E/P – Extensibility per unit Flour Protein.

4.1.4 2000 Farinograph

In year 2 of the experiments, there was a 4-way interaction of the *Glu-B1*, *Glu-D1*, *Glu-A3* and *Glu-B3* alleles affecting the farinograph WA (Figure 11). This complex interaction, together with the small differences between various samples, makes it difficult to recognise trends in the data.

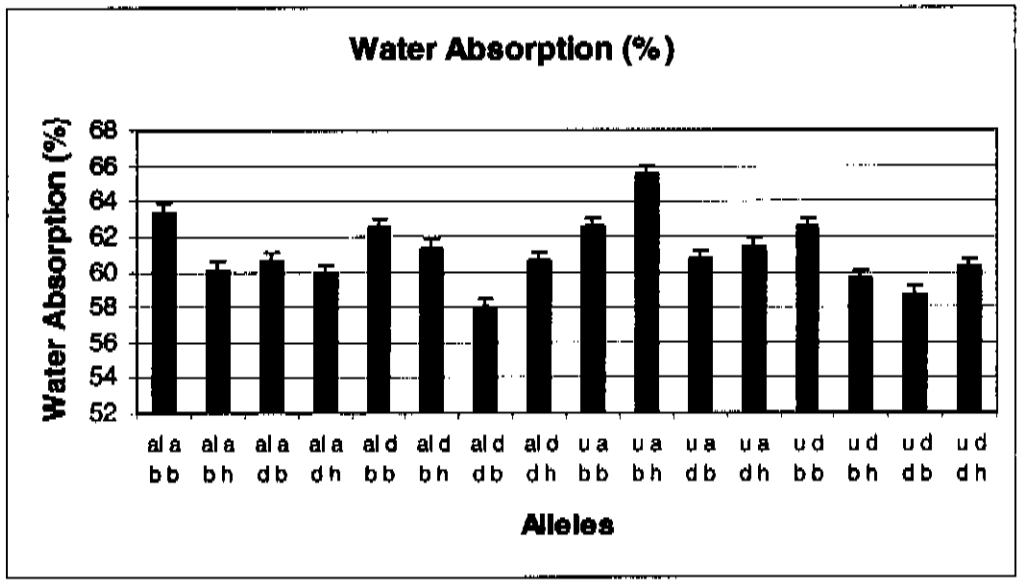

Figure 11: Water absorption of pure Kukri-Janz Doubled Haploid lines, 2000 harvest.

The dough stability measurements (Figure 12) showed a large amount of variation, again with a 4-way interaction ($P = 0.022$). Lines containing the *Glu-B1al* allele had higher and more consistent stability measurements than those with the *Glu-B1u* allele.

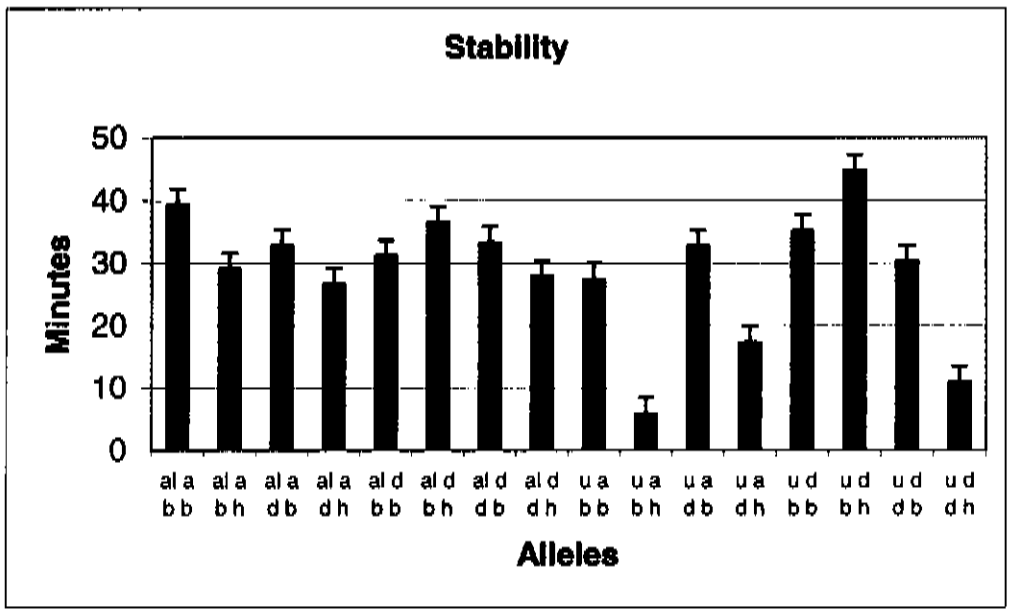

Figure 12: Stability times of pure flours, 2000.

The Arrival Times (Figure 13) showed clearly higher times for a group with the combination of *Glu-B1al* and *Glu-D1d* alleles. Flours with the alleles *Glu-B1u*, *Glu-D1d* and *Glu-B3d* also showed high arrival times, although not as high as those in the first group. There was no 4-way interaction, but there were 3-way interactions between *Glu-B1*, *Glu-D1* and *Glu-A3*, between *Glu-B1*, *Glu-A3* and *Glu-B3* and between *Glu-D1*, *Glu-A3* and *Glu-B3*.

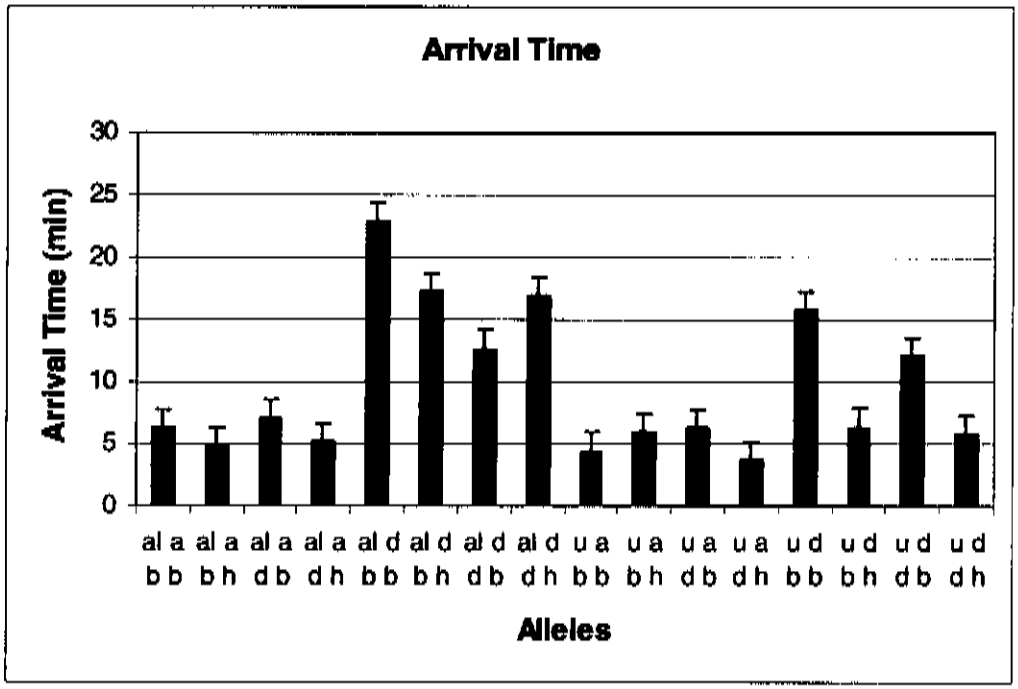

Figure 13: Arrival times of pure flours, 2000.

Most samples had departure times over 30 minutes, with some as high as 50 minutes. Those allele combinations with high arrival times also tended to have the highest departure times. There was a 4-way interaction of all the alleles.

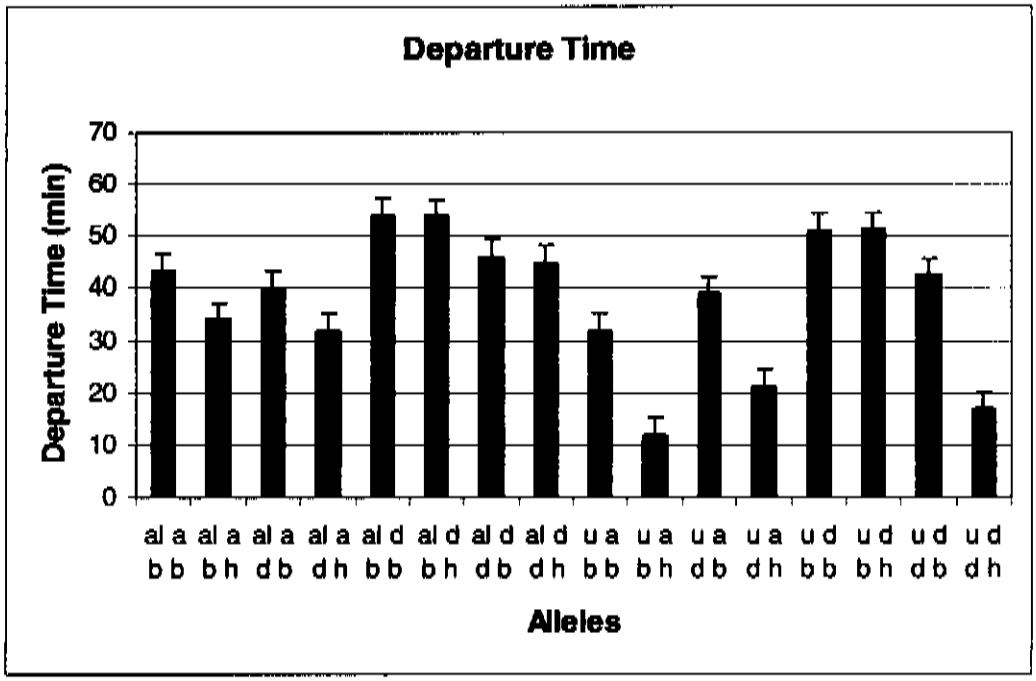

Figure 14: Departure Times of pure flours, 2000.

4.1.5 2000 Extensograph

Maximum dough strength was over 300 BU for most allele combinations (Figure 15). Flours with the *Glu-B1u* and *Glu-D1a* alleles tended to have lower dough strengths, and the lowest dough strength was found in the allele combination of *Glu-B1u, Glu-D1a, Glu-A3b* and *Glu-B3h.*

There were significant 3-way interactions between *Glu-B1, Glu-D1* and *Glu-A3,* and *Glu-D1, Glu-A3,* and *Glu-B3.* Flours with the *Glu-B1al* allele had higher dough strengths than those with the *Glu-B1u* allele, except when the *Glu-B1u* allele was paired with *Glu-D1d,* when dough strength was increased. The combination of *Glu-A3d* and *Glu-B3b* also increased dough strength above the average for the *Glu-D1a* allele combinations.

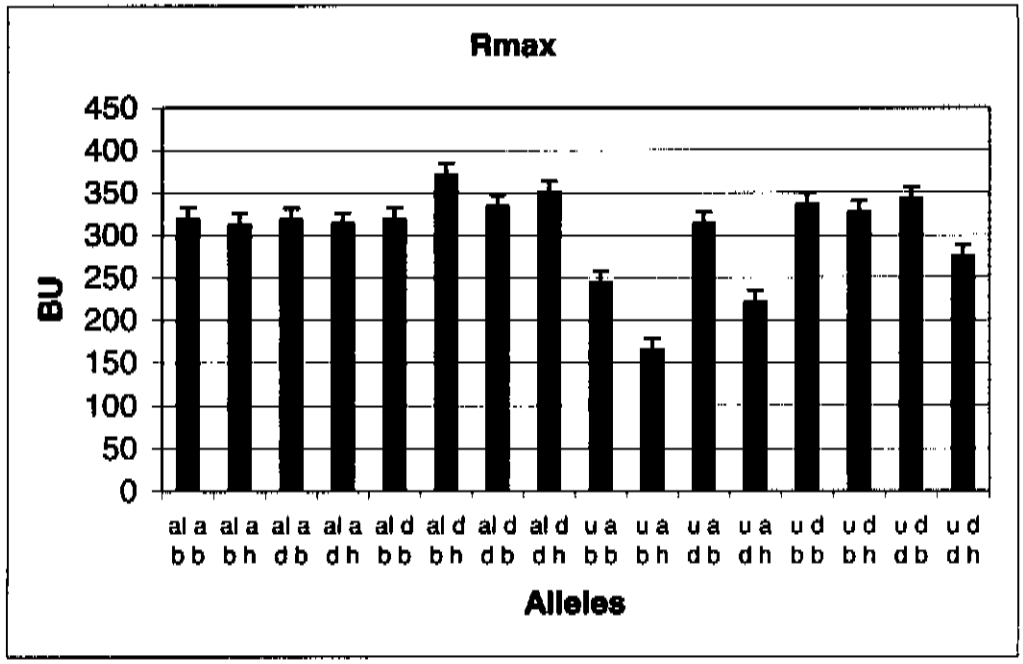

Figure 15: Maximum Dough Strength of pure flours, 2000.

Dough extensibility ranged from almost 25 cm to a low of 17 cm (Figure 16). Most doughs reached an extensibility of about 21 cm. There was a 4-way interaction of all 4 alleles, but a few broad trends can be distinguished. *Glu-B1al* alleles had higher extensibility than *Glu-B1u* alleles, and *Glu-A3d* alleles had higher extensibility than *Glu-A3b* alleles (see Table 10). However, final extensibility measurements were always influenced by all 4 alleles acting in combination.

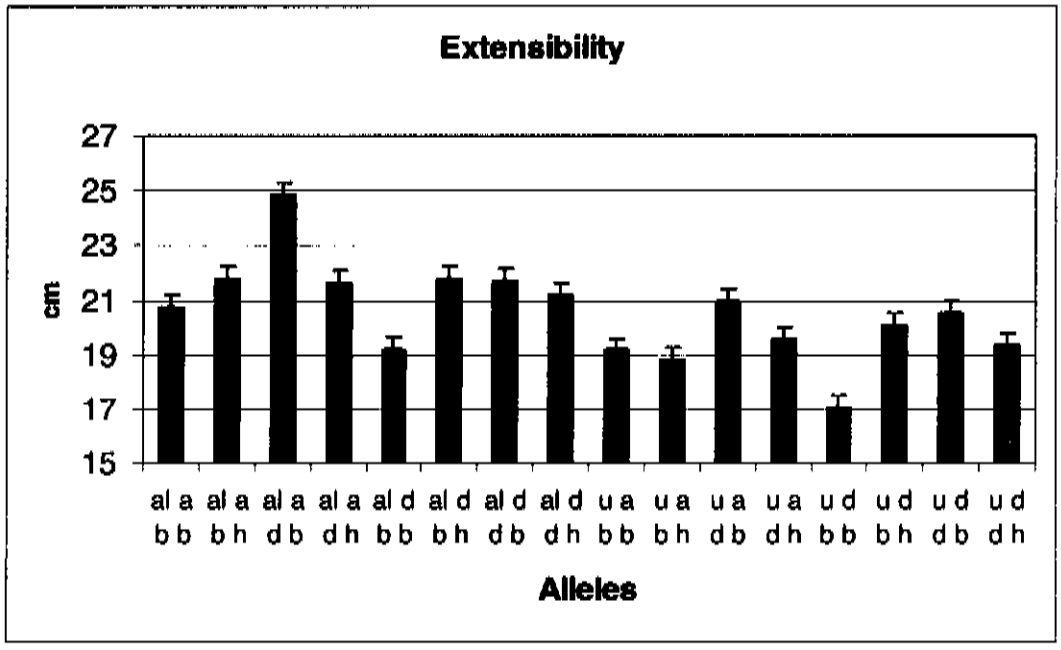

Figure 16: Dough extensibility for pure flours (2000).

Table 10: Extensibility of *Glu-B1* and *Glu-D1* groups.

	Glu-A3b	*Glu-A3d*
Glu-B1al	20.86^{B}	22.36^{A}
Glu-B1u	18.77^{D}	20.11^{C}

Extensibility per unit protein also showed a 4-way interaction of the differing alleles. Protein values ranged from 11.5% to 13.5 % with an average value of 12.3 %. The *Glu-B1al* allele still showed a tendency to have slightly higher extensibility per unit of protein than the *Glu-B1u* allele (1.734 *vs.* 1.612, lsd 0.043), but the other alleles also had a strong influence. *Glu-D1* alleles were involved in the interaction, but appear to play a lesser role in extensibility than they do for Rmax. The means for extensibility per unit protein are shown in Figure 17.

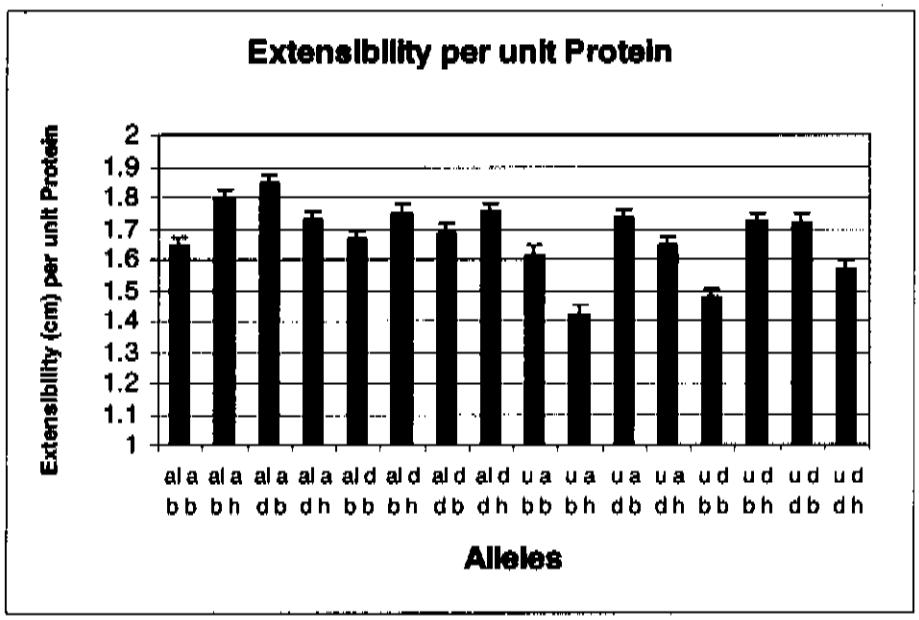

Figure 17: Extensibility per unit protein (2000).

4.1.6 2000 Mixograph

The number of allele combinations present in the mixograph test was sufficient to test for 2-way interactions between all groups, but no higher. Since the samples were mixed to a constant water absorption, there are no water absorption figures.

The most useful information from the mixograph was the mixing time results. These showed that there were significant 2-way interactions between the *Glu-A3* and *Glu-D1* alleles and the *Glu-A3* and *Glu-B3* alleles. However these were extremely complex and would probably have contributed to a 4-way interaction of all allele combinations were present, reinforcing the need to take all alleles into account when assessing dough quality of varieties.
On a more useful level, the flours with the *Glu-B1al* allele had slightly longer mean mix times than those with the *Glu-B1u* allele (203.8 s *vs.* 173.3 s, lsd 9.2). The flours with the *Glu-D1a* allele had a mean mixing time of 140.8 s compared to those with the *Glu-D1d* allele which had a mean mix time of 236.3 s. This agrees with the

farinograph results that flours with the *Glu-D1d* allele have much longer mixing requirements than those with the *Glu-D1a* allele.

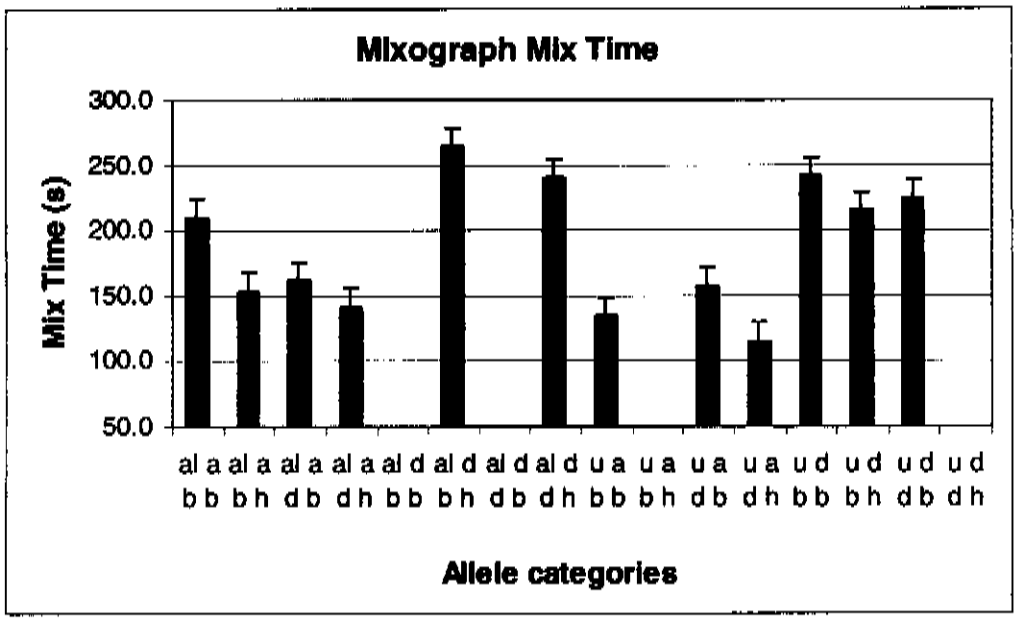

Figure 18: Mixograph Mixing time.

4.1.7 2000 Correlations

The correlations between the measurements (Table 11) were also examined in 2000. Stability was well correlated with Departure Time (0.88) but not Arrival Time (0.13). Stability also had a degree of correlation with Rmax (0.64) and with Mixograph MT (0.62). Rmax was further correlated with Departure Time (0.75) and Mixograph MT (0.85) but all other correlations were not particularly strong.

Table 11: Correlations between measurements in 2000.

	PSI	FP	WA	Stab	AT	DT	MT	Rmax	Ext	E/P
PSI	1									
Flour Protein	0.29	1								
Water Absorption	-0.30	0.17	1							
Stability	-0.17	-0.26	-0.17	1						
Arrival Time	-0.35	-0.08	0.39	0.13	1					
Departure Time	-0.30	-0.25	0.04	0.88	0.58	1				
Mixograph MT	-0.68	-0.14	0.00	0.62	0.88	0.92	1			
Rmax	-0.18	-0.15	-0.16	0.64	0.47	0.75	0.85	1		
Extensibility	0.35	0.46	-0.19	0.16	-0.02	0.12	-0.14	0.32	1	
E/P	0.42	0.49	-0.26	0.08	-0.15	-0.005	-0.11	0.22	0.95	1

PSI – Particle Size Index; FP – Flour Protein; WA – Water Absorption; Stab – Stability; AT – Arrival Time; DT – Departure Time; MT – Mixograph Mixing Time; Rmax – Maximum Dough Resistance; Ext – Extensibility; E/P – Extensibility per unit Flour Protein.

4.2 Blends

4.2.1 Glu-B1 al/u

4.2.1.1 Farinograph

The farinograph results showed a number of non-linear interactions between the two *Glu-B1* allele types, in both *Glu-D1a* and *Glu-D1d* backgrounds.
Water absorption (Figure 19) was significantly increased over the predicted amount by the addition of 25% *Glu-B1al* flour. This occurred in both *Glu-D1a* and *Glu-D1d* backgrounds. As the proportion of *Glu-B1al* flour in the mix increased, the actual water absorption became closer to the predicted amount.
There was also an interaction of the two allele types being tested. Water absorption was increased by the addition of *Glu-B1al* flour in a *Glu-D1d* background, but decreased when a *Glu-D1a* background was present.

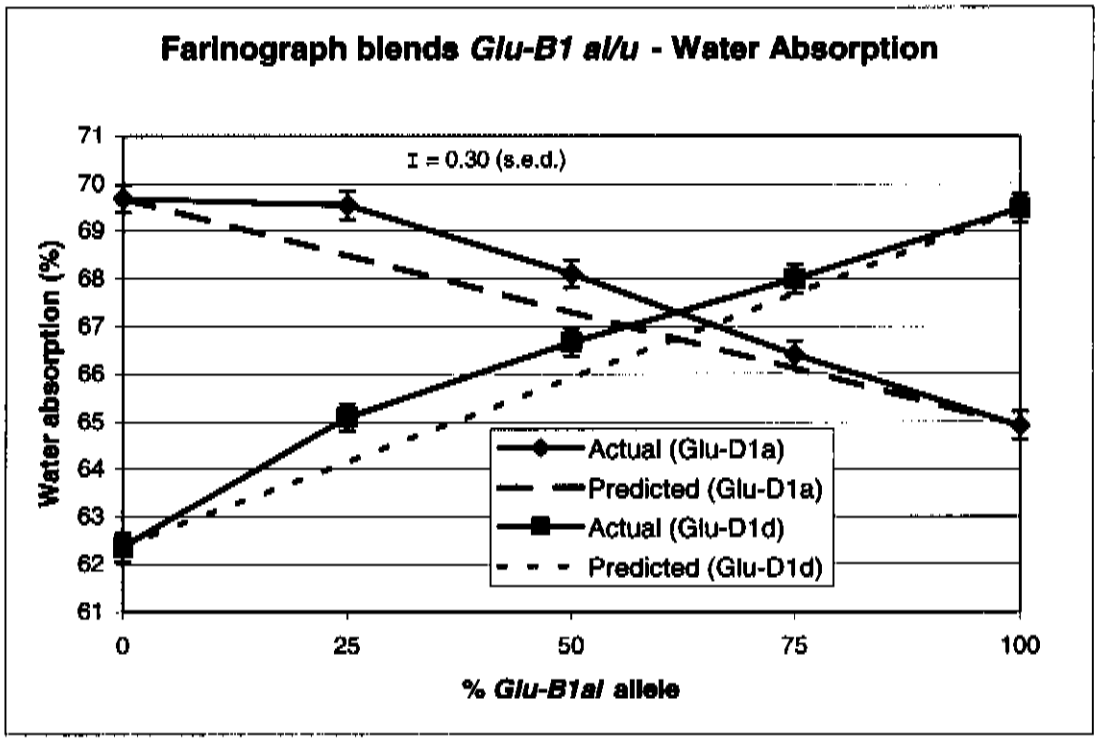

Figure 19: *Glu-B1 al/u* alleles – Water Absorption.

When stability was measured there was an interaction of the effects of the *Glu-B1* and *Glu-D1* alleles on the blended samples (Figure 20). Stability appeared to decrease with the addition of flour containing the *Glu-B1al* allele in a *Glu-D1d* background, but stability increased when *Glu-B1al* flour was added to samples with a *Glu-D1a* background. Further, there was a large degree of non-linearity present in the blends of *Glu-B1 al* and *u* alleles with a *Glu-D1d* background.

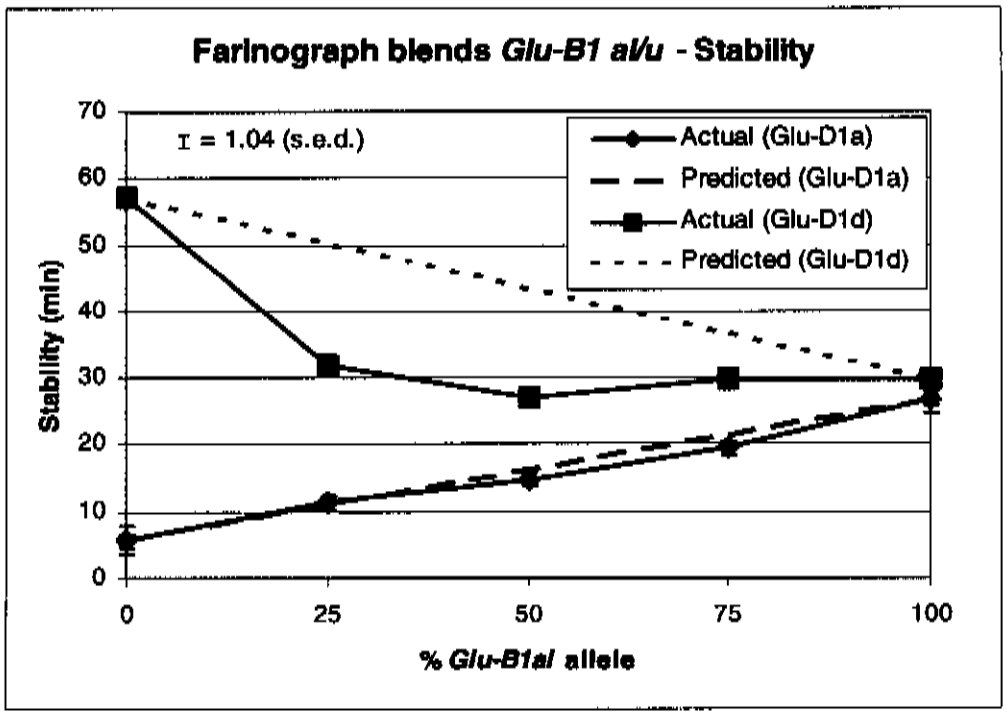

Figure 20: Stability of *Glu-B1* farinograph blends.

This large reduction in stability (50 minutes down to 32 minutes) is not as straightforward as it might seem. The stability measurement is taken from the difference between the Departure Time and the Arrival Time on the farinograph. The farinographs with mixtures of *Glu-B1al* and *u* alleles show an unusual mixing trace (Figure 21).

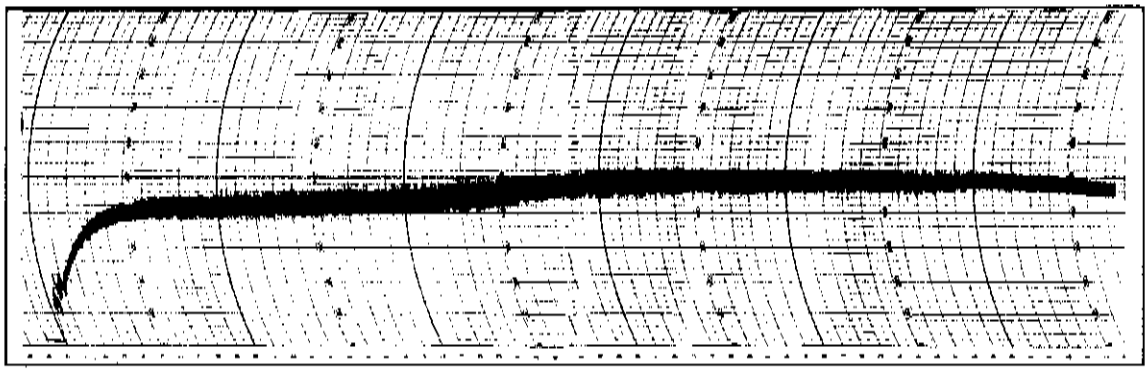

Figure 21: Example of flour sample with high Arrival Time. Blend of 75% *Glu-B1al*, *Glu-D1d*, *Glu-A3b* & *Glu-B3h* with 25% *Glu-B1u*, *Glu-D1d*, *Glu-A3b* & *Glu-B3h*.

These flour mixtures tend to plateau for approximately 20 minutes below the 500 BU line, before gaining strength and reaching it. Thus they have an unusually high Arrival Time, which can be seen in Figure 22, which causes a reduction in the Stability time.

Arrival times for those flours with *Glu-D1a* alleles were very close for both pure flours and mixtures. The flour with the *Glu-B1al* and *Glu-D1d* background had a very high Arrival Time, and the *Glu-B1al* allele caused very significant non-linearity even when mixed at a rate of only 25% in a *Glu-D1d* background (Figure 22).

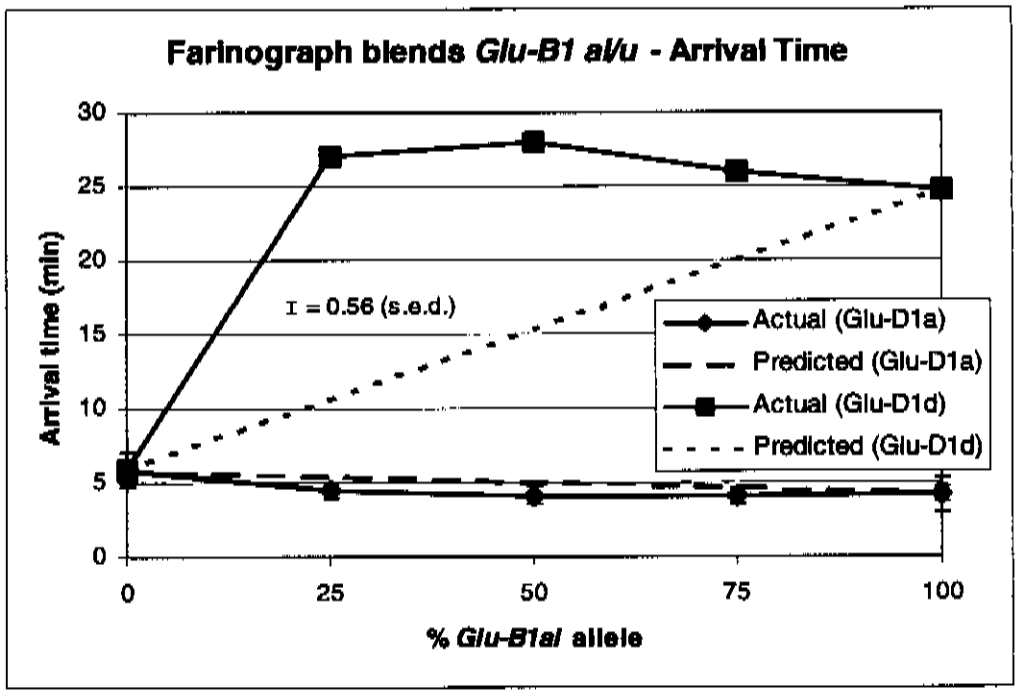

Figure 22: Arrival Times of mixed *Glu-B1* flour samples.

Departure Times showed some slight non-linearity for both *Glu-D1* backgrounds (Figure 23), as the proportion of *Glu-B1al* flour in the mix reached 50 – 75 %. There was an interaction present with the *Glu-B1* and *Glu-D1* flour types, as the addition of *Glu-B1al* flour decreased Departure Time for doughs with the *Glu-D1d* background, but increased it for doughs with a *Glu-D1a* background.

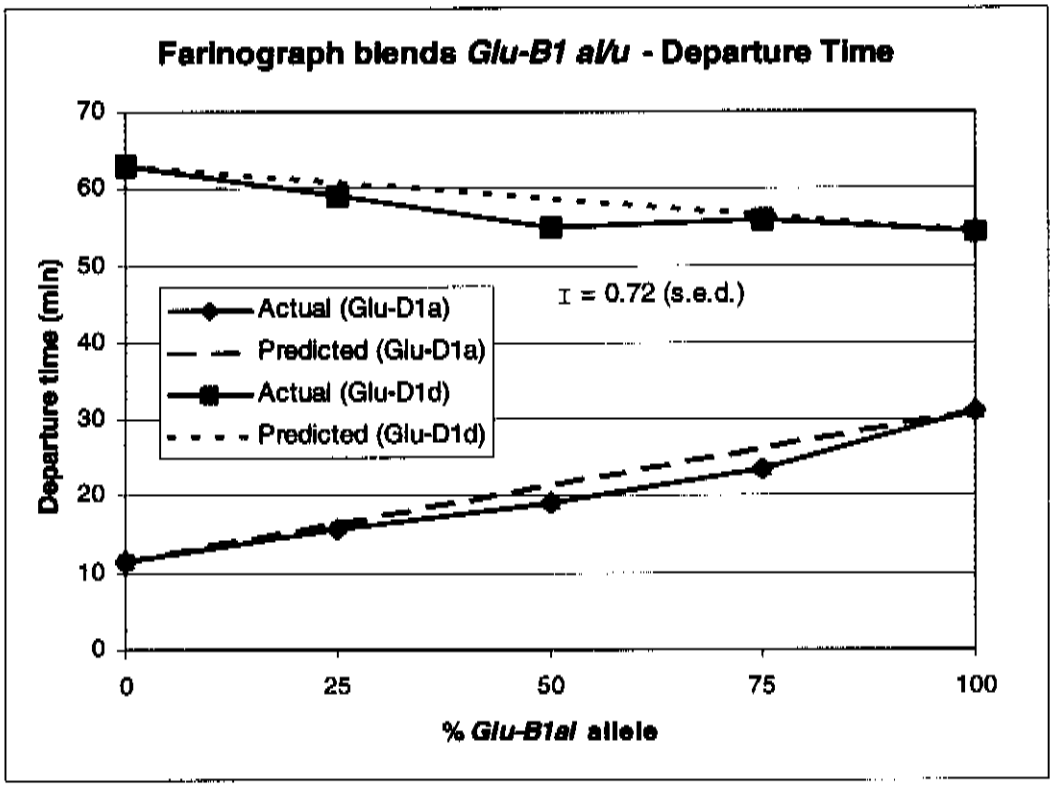

Figure 23: Departure Times for *Glu-B1* flour blends.

4.2.1.2 Extensograph

Dough strength (Rmax) was predicted well for the *Glu-B1 al* and *u* mixes by a linear equation (Figure 24). An increase in the amount of *Glu-B1al* flour in the mix resulted in an increase in the dough strength, and this was more marked for flours with a *Glu-D1a* background, but this increase was as expected from the linear equation.

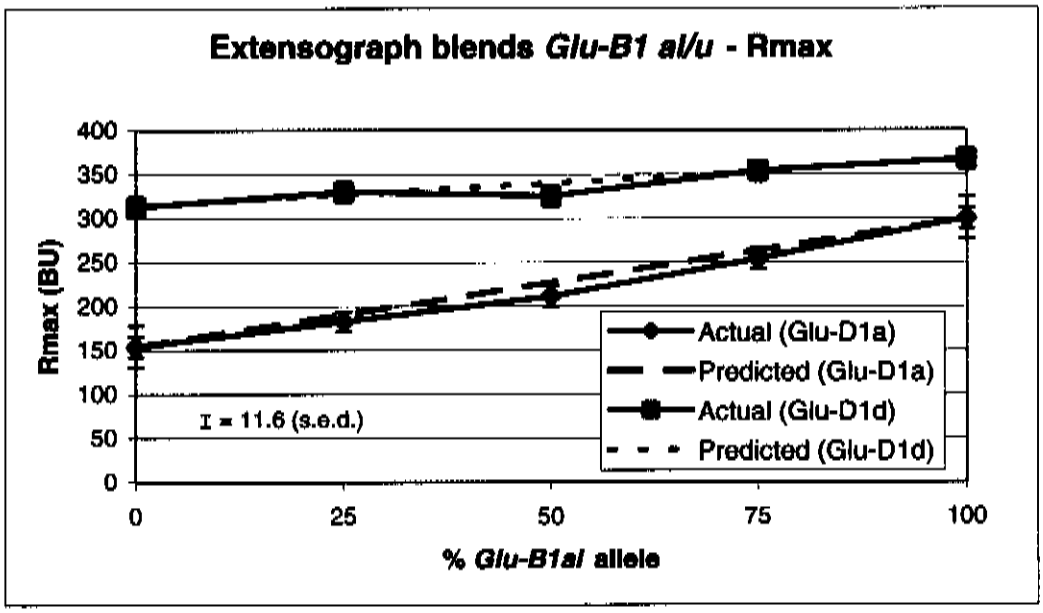

Figure 24: Rmax for *Glu-B1 al/u* extensograph blends.

Dough extensibility showed more variation than other measurements (Figure 25). A significant non-linear effect occurred with 25% *Glu-B1al* flour in the *Glu-D1a* background, but not with any other blends.
Extensibility was slightly higher for the *Glu-B1al* flours than for the *Glu-B1u* flours.

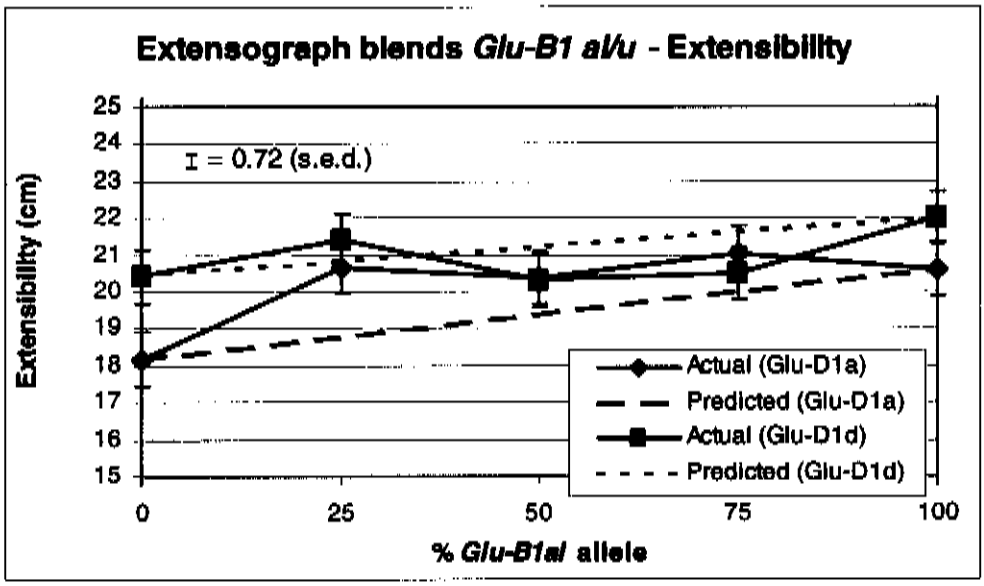

Figure 25: Extensibility of *Glu-B1 al/u* mixes.

Extensibility per unit protein showed a clearer non-linear effect (Figure 26). The addition of 25 – 50 % of flour containing the *Glu-B1al* allele to flour with the *Glu-B1u* allele in a *Glu-D1a* background caused a significant decrease in the amount of extensibility per unit protein, much lower than that expected from a straight line prediction. However, the addition of 25% *Glu-B1al* flour to flour with the *Glu-B1u* allele in a *Glu-D1d* background caused a significant increase in the amount of extensibility per unit protein over that predicted by a straight line.
Extensibility per unit protein did not significantly differ between the *Glu-B1al* and *u* flours with a *Glu-D1d* background, but extensibility was increased by the addition of flour containing the *Glu-B1al* allele when the *Glu-D1a* background was used.

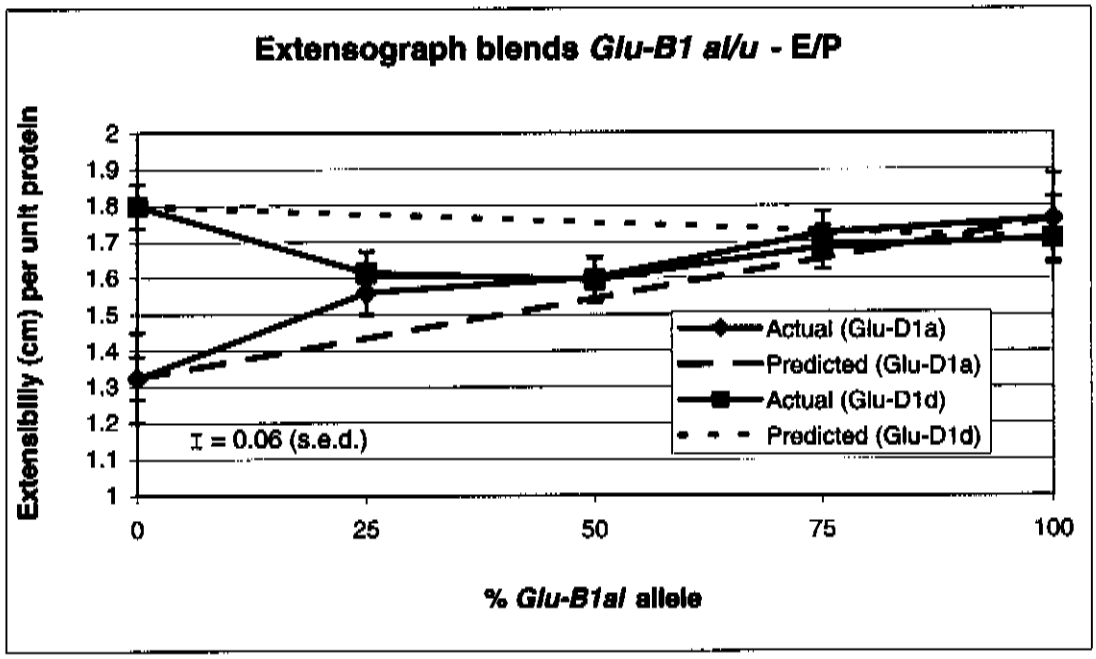

Figure 26: Extensibility per unit protein for *Glu-B1 al/u* blends.

4.2.2 *Glu-D1 a/d*

4.2.2.1 Farinograph

Water absorption could be predicted using a linear equation for a mixture of *Glu-D1 a* and *d* alleles only with a *Glu-B1al* background (Figure 27). The *Glu-B1u* background showed an increase in water absorption beyond what was predicted by a straight line equation with 50 – 75% *Glu-D1d* flour present.
There was a significant interaction of the *Glu-B1* and *Glu-D1* alleles, as the *Glu-D1* alleles had opposite effects when mixed in different *Glu-B1* backgrounds.

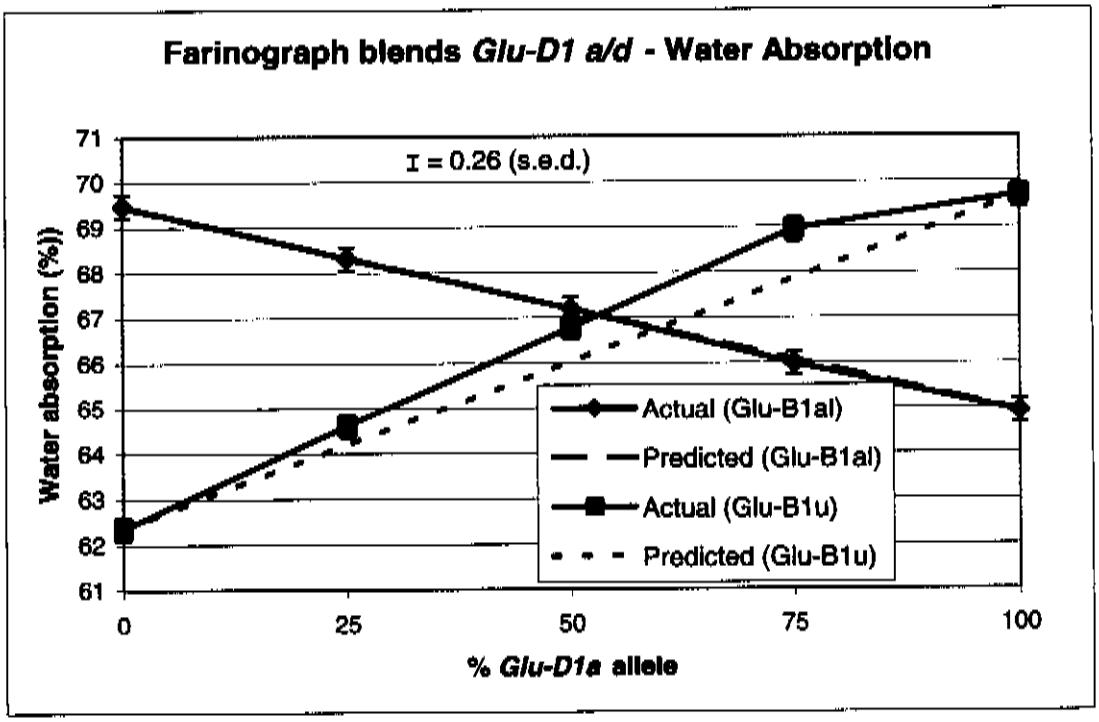

Figure 27: Water absorption for *Glu-D1 a/d* blends.

Stability showed some non-linearity in the *Glu-D1* blends (Figure 28). With a *Glu-B1al* background, stability was reduced slightly below the prediction with a 50/50 *Glu-D1 a/d* mix. With a *Glu-B1u* background however, stability was increased with between 50 and 75% *Glu-D1a* in the blend. Overall, blends with a *Glu-B1al* background showed no significant changes in stability, as the *Glu-B1al* allele seems to confer a high dough stability regardless of the other allele combinations. However, stability was greatly decreased by the increase in *Glu-D1a* flour when mixed with a *Glu-B1u* background.

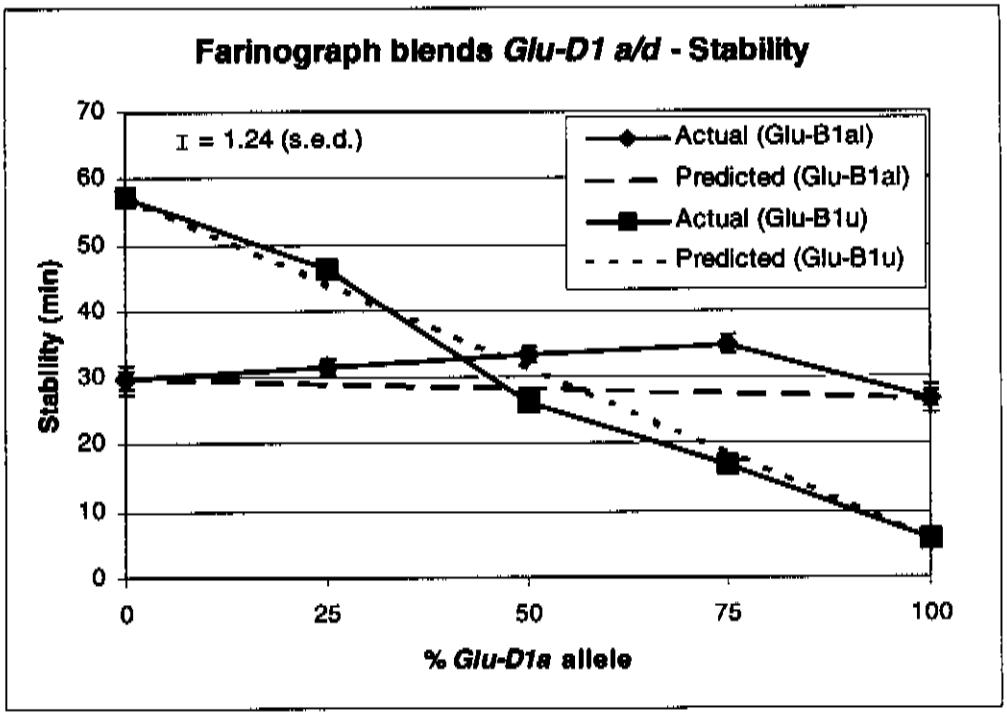

Figure 28: Stability of *Glu-D1 a/d* blends.

The Arrival Times (Figure 29) of the *Glu-D1a/d* blends show a definite non-linear trend for the flour with a *Glu-B1al* background. Arrival times were decreased below the predicted amount by the addition of *Glu-D1a* flour.

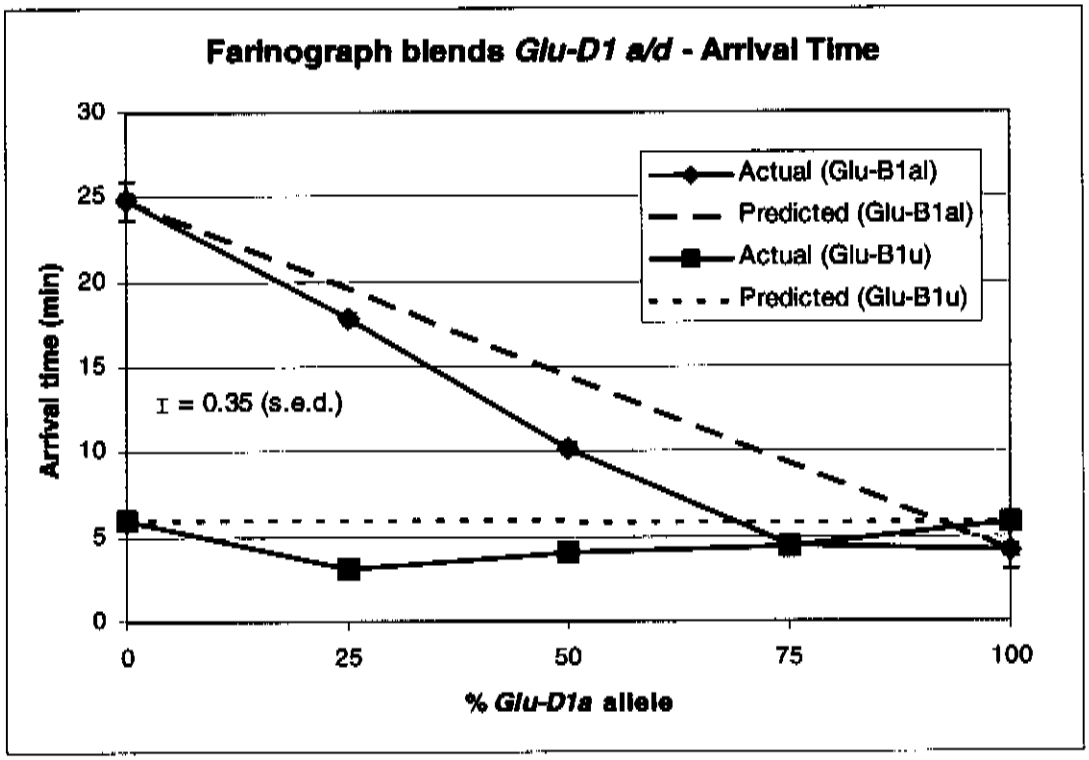

Figure 29: Arrival Times of *Glu-D1 a/d* blends.

Departure Times (Figure 30) were linear or close to for mixes with a *Glu-B1al* background, but Departure Time was decreased more than expected with 50% *Glu-D1a* in a *Glu-B1u* background. All departure times were decreased by the addition of more *Glu-D1a* flour.

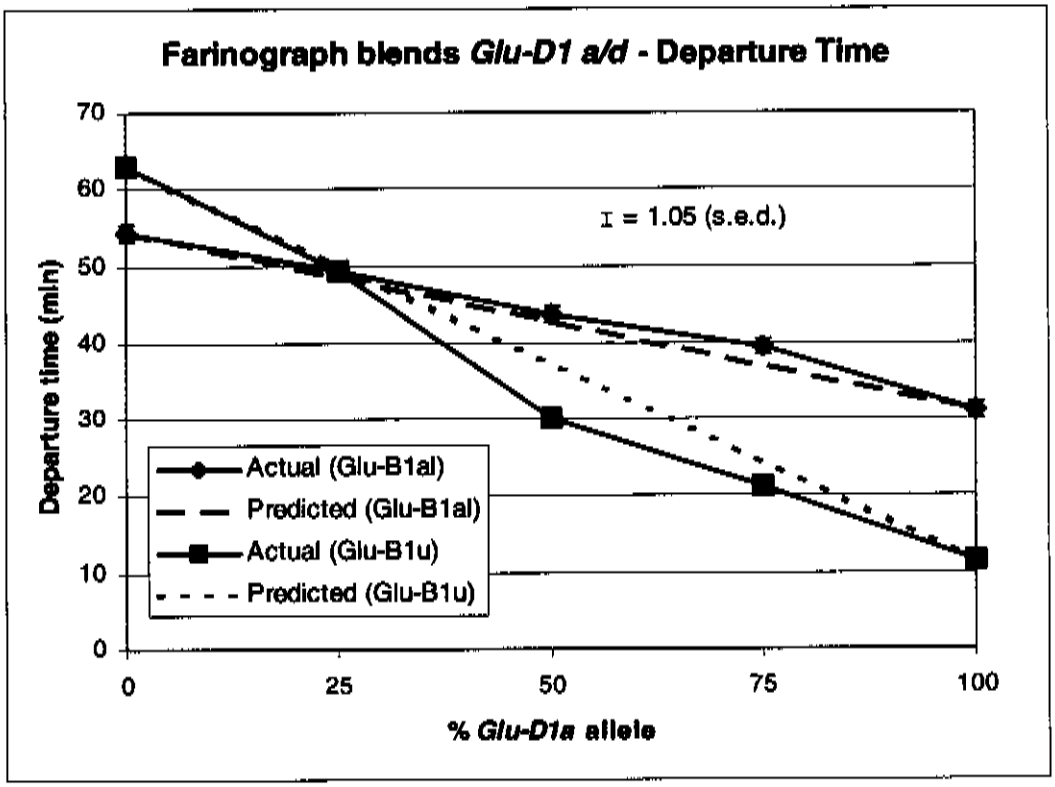

Figure 30: Departure Times of *Glu-D1 a/d* blends.

4.2.2.2 Extensograph

Dough strength was as predicted for the blends from the *Glu-B1al* background (Figure 31). Dough strength was slightly above the predicted values for those blends with a *Glu-B1u* background. Blends with higher proportions of the *Glu-D1a* allele had lower strength than those with the *Glu-D1d* allele.

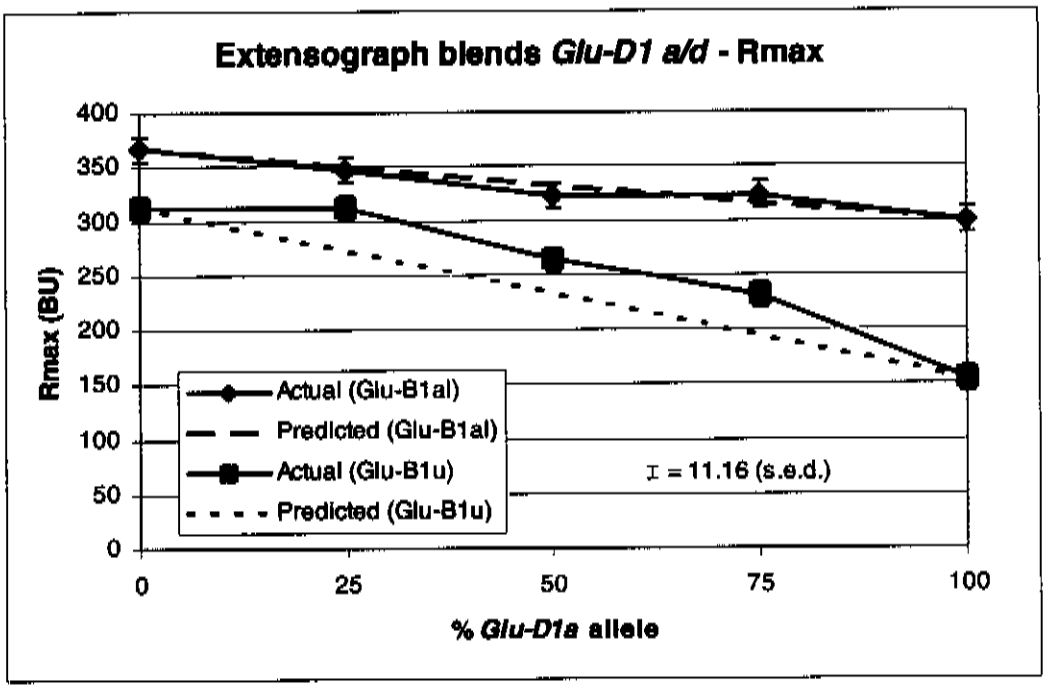

Figure 31: Dough strength of *Glu-D1 a/d* blends.

All blends of *Glu-D1 a* and *d* were close to the linear prediction for extensibility, although there was a tendency for blends with about 50% of the *Glu-D1a* allele to be slightly more extensible than predicted (Figure 32). Overall extensibility was lower in blends with higher amounts of the *Glu-D1a* allele.

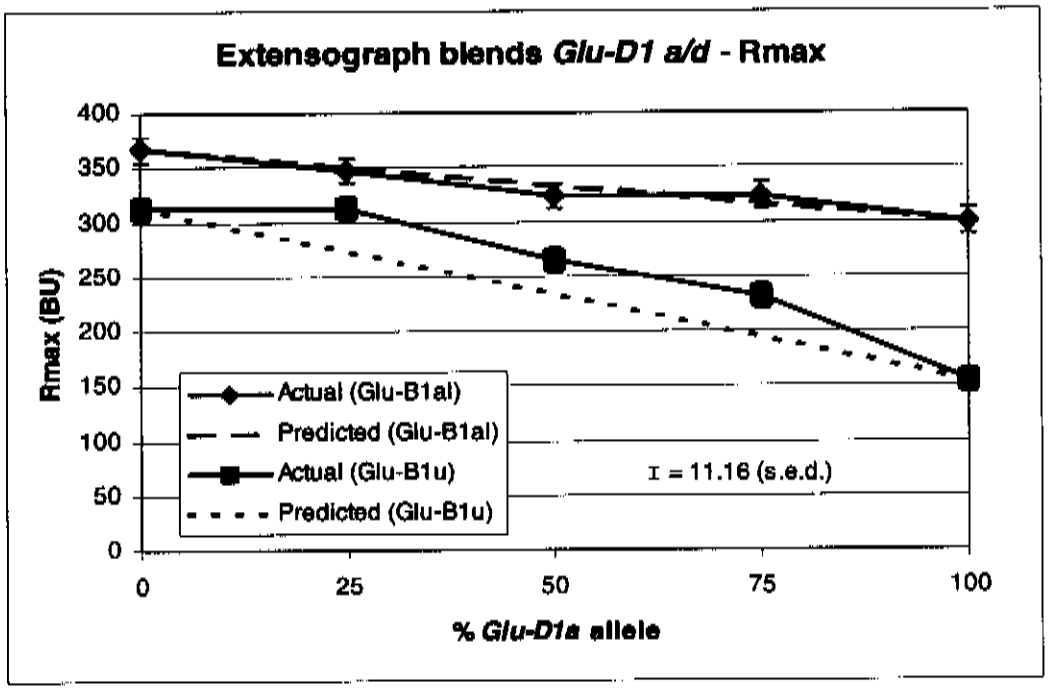

Figure 32: Extensibility of *Glu-D1 a/d* blends.

The amount of extensibility per unit protein showed some variation, but was reasonably well predicted by a linear equation for flours containing the *Glu-B1al*

allele (Figure 33). E/P was first decreased below expected by 25% *Glu-D1a* flour, then increased with 50-75% *Glu-D1a* flour. This variation is unusual and may need to be further investigated. E/P declined as the proportion of *Glu-D1a* in the blends increased for blends with a *Glu-B1u* background, while E/P remained approximately equal for blends with *Glu-B1al* backgrounds.

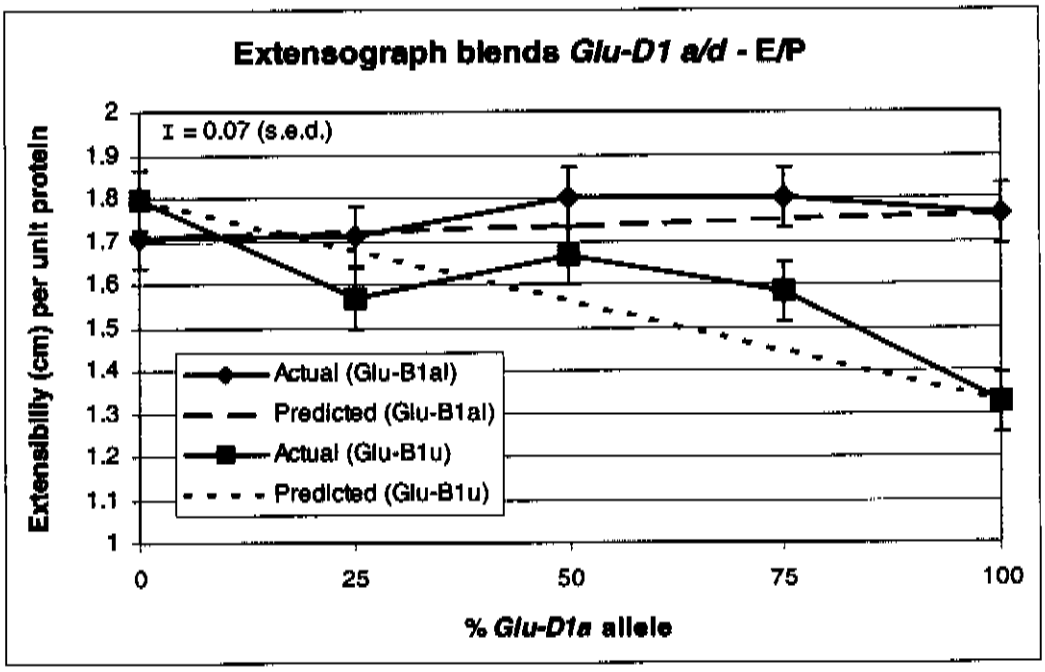

Figure 33: Extensibility per unit protein for *Glu-D1 a/d* blends.

5 Discussion

5.1 Pure Lines

Due to the differences in the methods used for the farinograph measurements in 1999 and 2000 it is difficult to compare the data over the 2 years. Therefore for the farinograph, each year is discussed separately.

The problems with the methods used for the 1999 flours make it difficult to draw useful conclusions. Peak DD times and Stability measurements are flawed since the doughs were not mixed until the true breakdown.

In 2000, it is important to note the effect that the unusually long Arrival Time had on the *al d b h* Stability measurements. Why the *Glu-B1al* and *Glu-D1d* allele combination should have such a long AT is unknown. Possibly the combination of structural differences in the 5 subunit (extra cysteine residue) compared to the 2 subunit, as well as the over-expression of the 7 subunit result in very long mixing times. If the stability had been measured from where the trace flattened out, as is usually the situation when the trace reaches the 500 line, dough stability of the *al d b h* line would have been equivalent to that of the *u d b h* and the apparent non-linear effect in the blending experiment would not have occurred. This would also eliminate the apparent non-linearity in AT. The AT of the *al d b h* line would be shortened to a level comparable with the other lines, and the addition of the *al* flour to the *u* type would probably not cause a non-linear effect.

The slow rate of energy input using a farinograph may result in an equilibrium between bonds being broken and reformed during mixing. A higher energy input machine, eg. using a DoughLab, would be expected to produce sharper, more easily defined peaks in a much reduced timeframe. This would greatly assist analysis.

Other flours with long AT were the combinations *u d b b* and *u d d b*. These traces showed similarities to the *al d b h* type, with slow AT. It seems that the *Glu-D1d* allele may be the cause of the slow AT, but this can be modified to be shorter by the presence of certain combinations of other alleles – hence the strong interactions seen in the analysis of variance. The key combinations to reduce AT when a *Glu-D1d* allele is present are the *Glu-B1 a* allele and the *Glu-B3h*. Unfortunately, in the case of the combination *u d d h*, the alleles also reduce DT, resulting in low stability. However for the combination *u d b h*, DT remained high, giving the highest stability measurement of the 16 allele combinations. The *Glu-A3* allele is interacting strongly in this case. Interestingly, the *Glu-B1al* allele does not interact with the *Glu-B3h* to bring down AT, and the AT remains high for all *Glu-B1al* + *Glu-D1d* combinations.

Extensograph methods were identical for the 1999 and 2000 flours, and some useful comparisons can be drawn.
In both 1999 and 2000 Rmax was least in the allele group with *Glu-B1u* + *Glu-D1a* alleles. Dough strength was highest in the *Glu-B1al* + *Glu-D1d* group for both years, although the effects of the *Glu-A3* and *Glu-B3* alleles were interacting with these in 2000. The *Glu-D1d* allele produced flours with much greater strength than the *Glu-D1a* allele in both years as did the *Glu-B1al* allele with the *Glu-B1u* allele. The *Glu-*

B3b allele also produced consistently higher dough strength than the *Glu-B3d* allele in both years. In 2000 the *Glu-A3* alleles had a small but significant effect, which was not seen in 1999. From all this, it can be seen that when selecting varieties for maximum dough strength, breeders should choose those containing the *Glu-B1al, Glu-D1d* and *Glu-B3b* alleles. The combination of *Glu-B1al, Glu-D1a, Glu-A3d* and *Glu-B3b* alleles possessed good dough strength and also had high extensibility. This combination also had high stability but a short arrival time, so should not have excessive mixing requirements.

Extensibility was higher in 1999 than 2000, and the *Glu-B1al* flours had higher mean extensibility than *Glu-B1u* flours in both years. *Glu-D1* alleles had no effect in 1999, but the *Glu-D1* alleles interacted with the others in 2000. The *Glu-A3* alleles were important in both years, interacting with the *Glu-B1* in 1999 and with all the other alleles in 2000. *Glu-A3d* alleles tended to produce higher extensibility when paired with a *Glu-B1al* allele than with a *Glu-B1u* allele in both years.
Overall, for maximum extensibility the allele combination *Glu-B1al, Glu-D1a, Glu-A3d* and *Glu-B3b* showed consistently high extensibility in both 1999 and 2000. No particular combination of alleles was lowest in extensibility in both years.

Extensibility per unit protein eliminates the effect that protein content has on extensibility, showing which allele types are most effective at conferring extensibility. Again the *Glu-B1al* allele showed better extensibility per unit protein in both years. The other alleles had no effect in 1999, but all interacted in 2000. Flours with the *Glu-A3d* allele tended to have higher extensibility per unit protein than those with a *Glu-A3b* allele.

The *Glu-B1* and *Glu-A3* alleles are important influences on the extensibility of doughs, while the *Glu-D1* alleles are more important for strength. However, there may be interactions between all these alleles, so that care must be taken to select good combinations, not just good individual alleles. It is also necessary to select alleles after testing over several seasons, as environmental influences can change the quality response of some allele types.

Another factor which must be considered is the effectiveness of the various tests used in differentiating the dough quality, and how well these relate to practical outcomes. For example, the Extensograph method mixes dough for 5 minutes regardless of the actual mixing requirement to obtain maximum development. It can be seen from the 2000 Arrival Time results that some of the doughs would be seriously under-mixed at 5 minutes. This is likely to affect their Rmax and Extensibility values. A clearer idea of the effect of the *Glu-B1al* allele and its interactions could be obtained by mixing extensograph doughs to Peak Dough Development. This would be facilitated by the use of computer-linked hardware, such as the DoughLab farinograph. Measurement of energy input could also be very useful in examining dough properties.

5.2 Blends

There were a number of interesting results from the study of the blended flours. The non-linear effects seen when *Glu-D1 a* and *d* alleles are mixed were confirmed, and the effects of mixing *Glu-B1 al* and *u* alleles examined.

Note that since only single lines were used in the blending trials, not two as in the pure flour trials, different base flour values are used in the blending trial from those in the pure flour results.

Water absorption showed a synergistic effect when 25 – 50 % *al* flour was blended with *u* flour in both *Glu-D1 a* and *d* backgrounds. The slight increase in water absorption could be of importance to processors to maintain correct dough characteristics. When *Glu-D1* alleles were blended, a non-linear effect only showed with a *Glu-B1u* background. There was a slight increase in WA over the expected amount at approximately 75% *Glu-D1a* flour. This could possibly be a result of interaction by *Pin* (Puroindoline) genes, as the particular *Pin* type present in the Kukri from this experiment is not yet known.

Although Stability and AT both showed apparently large non-linear effects when *Glu-B1al* flour was added to *Glu-B1u* flour containing the *Glu-D1d* allele, the problems with this interpretation have been discussed in the Pure Lines section above. However, it can be seen from the large change in AT and Stability when a small amount of *Glu-B1al* flour is added, that a small amount of *al* flour causes a large change in the farinograph mixing behaviour of a flour blend (with a *Glu-D1d* background).

The *Glu-D1* blends were not as affected by the problems in interpretation caused by the *Glu-B1* alleles, since the *Glu-B1* backgrounds remained constant. A minor non-linear effect on stability was seen at 75% *Glu-D1a* flour (an increase over the predicted amount) when mixed with a *Glu-B1al* background. On the whole, though stability was not highly affected by non-linear effects.

Arrival Time was more strongly affected, particularly with between 50 –75 % *Glu-D1a* flour in a *Glu-B1al* background. Arrival Times were significantly decreased here. Arrival Times were also decreased in the *Glu-B1u* background, mostly at around 25% *Glu-D1a* flour, but not to the same extent as the other.
A small non-linear decrease in Departure Time was seen at 50% *Glu-D1a* flour in the *Glu-B1u* background, but DT was linear for flours with a *Glu-B1al* background.

Dough strength was well predicted by a straight line for the *Glu-B1* blends, but a non-linear effect was seen in the *Glu-D1* blends in a *Glu-B1u* background. Dough strength was higher than predicted by a straight line equation for all the blended samples. This effect was not seen with the *Glu-B1al* background. The non-linearity seems most likely to have been caused by the presence of the *Glu-D1d* allele in the blends, causing a synergistic increase in dough strength. Why this did not happen in the *al* background is unknown, but dough strength in the *al* background was higher overall anyway.

Extensibility and E/P were showed non-linear effects for both the *Glu-B1* and *Glu-D1* blends. Extensibility was increased by the addition of *Glu-B1al* flour in a *Glu-D1a* background, most particularly near the 25% region. However, extensibility was decreased in the *Glu-D1d* background, mostly at around 75% *Glu-B1al* flour. This was seen more clearly in the *Glu-B1al/u* blend E/P data, since the amount of protein present also affected extensibility.

The Extensibility changes in the *Glu-D1* blends showed increases in extensibility for both backgrounds in the 50% *Glu-D1a* samples. When this was corrected for protein content in E/P, the results were less predictable. At 25% *Glu-D1a* flour, E/P was as predicted (*Glu-B1al)* or less (*Glu-B1u*). However at 50-75% *Glu-D1a* flour, E/P was higher than predicted for both backgrounds. This increase in E/P seems to show that at low levels, *Glu-D1d* flour can increase E/P in a synergistic way, but at higher levels the effect disappears and extensibility could even be reduced.

6 Conclusions

There are several important conclusions that can be drawn from the results of these experiments.

The *Glu-B1al* allele present in Kukri has a number of useful properties. The presence of the *al* allele improves a number of dough properties, and "normalises" others, so that allele combinations containing the *Glu-B1al* allele are more uniform in characteristics than those with the *Glu-B1u* allele. Extensibility, in particular, is improved by the *Glu-B1al* allele.

The *Glu-D1d* allele showed high dough strength and long stability times for most allele combinations. The qualities of the *Glu-D1d* allele have been known and examined previously (Bekes and Wrigley, 1999) and our work agrees with these results. A potential problem may be the long Arrival Times for most flours with the *Glu-D1d* allele on the farinograph. There may be a higher energy input needed to fully develop doughs containing *Glu-D1d* alleles to properly assess their dough and baking characteristics.

It was found that all the alleles present in these flour samples interacted to give the final dough quality. Therefore it is difficult to accurately predict the quality of a particular flour unless all the alleles are known. Of course, since this experiment only has 2 year's data, there may be more interactions of genotype and environment that are yet to be examined. These will also affect predictions of dough quality.

Interactions between different allele types, such as those that occur when two different varieties are blended, can significantly affect dough quality. Our experiments have showed that non-linear effects occur when *Glu-D1a* and *d* alleles are blended, particularly for farinograph AT, Rmax and Extensibility. *Glu-B1al* and *u* blends also show non-linear effects, most clearly in Extensibility and E/P. There are apparent non-linear effects in AT and Stability, however, as has been stated earlier, this may be due to the somewhat subjective nature of farinograph readings. The interactions between *Glu-B1al* and *u* are of interest, since *al* has the subunits 7 (over-expressed 7 subunit) and 8*, while *u* has the subunits 7* and 8. It is not known whether the interaction is caused by the differences between 7 and 7* or 8 and 8*, or a combination of these.

Previous work in this area by Bekes and Wrigley (1999) showed non-linear effects when flours of differing allele composition were blended. Our results agree broadly with theirs, where similar allele blends were used, however Bekes and Wrigley seemed to find the most severe non-linear effects when samples were mixed using the Mixograph. A mixograph has a higher energy input rate than a farinograph, and our work with very long-mixing flours on the farinograph seems to indicate that measurement of energy input could be a critical factor in investigating some of these dough characteristics.

It is possible that more substantial differences may be seen by end-users of blended flours than have been found here. Industry practice is to mix doughs more towards peak dough development, or optimum energy input, while in the laboratory the doughs for extensograph testing are mixed to a standard time (5 minutes). Since some

of the doughs in this experiment had PDD times far exceeding 5 minutes, these doughs would be seriously under-developed and their extensograph scores compromised. We suggest that future work in this are should take dough mixing requirements into account, by either mixing to PDD or optimum energy input. The latter would require the use of machinery with an energy use meter fitted, such as the Newport DoughLab. Using machinery with faster energy input, such as a mixograph or a farinograph with variable mixing speeds would reduce the time taken to mix the samples for testing.

The non-linear interactions between the glutenin alleles also have implications for wheat breeding programs. In particular the use of the *Glu-B1al* allele present in Kukri and Chara needs careful consideration. This over-expressed allele has the potential to significantly increase the extensibility of flour doughs. However in order to produce wheat varieties that do not have inordinately long mixing times and over-strong dough the selection of appropriate allele combinations is critical.

Lastly, we point out that there is much scope for further work in this area. Due to time constraints, we were unable to investigate as many mixed allele combinations as we would have liked, particularly those involving LMW glutenin subunits and multiple allele combinations. It would be extremely useful also to test the *Glu-B1al* allele with other *Glu-B1* types, to see if more non-linear interactions occur.

7 References

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8 Appendices

Table A: Lines and Glutenin alleles of data set used for Farinograph and Extensograph in 1999.

VARIETY	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
J96-536-209	a	al	a	b	b	b
J96-536-069	a	u	a	b	b	b
J96-536-122	a	u	a	b	b	b
J96-536-201	a	u	a	b	b	b
J96-536-232	a	u	a	b	b	b
J96-536-244	a	u	a	b	b	b
Janz	a	u	a	b	b	b
J96-536-014	a	al	a	b	h	b
J96-536-075	a	al	a	b	h	b
J96-536-253	a	al	a	b	h	b
J96-536-256	a	al	a	b	h	b
J96-536-301	a	al	a	b	h	b
J96-536-010	a	al	a	d	b	b
J96-536-110	a	al	a	d	b	b
J96-536-187	a	al	a	d	b	b
J96-536-188	a	al	a	d	b	b
J96-536-274	a	al	a	d	b	b
J96-536-043	a	u	a	d	b	b
J96-536-095	a	u	a	d	b	b
J96-536-133	a	u	a	d	b	b
J96-536-203	a	u	a	d	b	b
J96-536-216	a	u	a	d	b	b
J96-536-259	a	u	a	d	b	b
J96-536-317	a	u	a	d	b	b
J96-536-031	a	al	a	d	h	b
J96-536-044	a	u	a	d	h	b
J96-536-057	a	u	a	d	h	b
J96-536-132	a	u	a	d	h	b
J96-536-155	a	u	a	d	h	b
J96-536-168	a	u	a	d	h	b
J96-536-067	a	al	d	b	b	b
J96-536-136	a	al	d	b	b	b
J96-536-236	a	al	d	b	b	b
J96-536-165	a	u	d	b	b	b
J96-536-200	a	u	d	b	b	b
J96-536-249	a	u	d	b	b	b
J96-536-279	a	u	d	b	b	b
J96-536-296	a	u	d	b	b	b
J96-536-328	a	u	d	b	b	b
J96-536-123	a	al	d	b	h	b
J96-536-130	a	al	d	b	h	b
J96-536-158	a	al	d	b	h	b
J96-536-239	a	al	d	b	h	b
J96-536-282	a	al	d	b	h	b

Table A continued.

VARIETY	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
J96-536-013	a	u	d	b	h	b
J96-536-048	a	u	d	b	h	b
J96-536-083	a	u	d	b	h	b
J96-536-153	a	u	d	b	h	b
J96-536-172	a	u	d	b	h	b
J96-536-025	a	al	d	d	b	b
J96-536-074	a	al	d	d	b	b
J96-536-103	a	al	d	d	b	b
J96-536-228	a	al	d	d	b	b
J96-536-085	a	u	d	d	b	b
J96-536-117	a	u	d	d	b	b
J96-536-119	a	u	d	d	b	b
J96-536-154	a	u	d	d	b	b
J96-536-252	a	u	d	d	b	b
J96-536-037	a	al	d	d	h	b
J96-536-082	a	al	d	d	h	b
J96-536-091	a	al	d	d	h	b
J96-536-100	a	al	d	d	h	b
J96-536-137	a	al	d	d	h	b
J96-536-226	a	al	d	d	h	b
Kukri	a	al	d	d	h	b
J96-536-161	a	u	d	d	h	b
J96-536-180	a	u	d	d	h	b
J96-536-309	a	u	d	d	h	b

Table B: Lines and Glutenin alleles of data set used for the Farinograph and Extensograph in 2000.

VARIETY	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
J96-536-298	a	al	a	b	b	b
J96-536-209	a	al	a	b	b	b
J96-536-244	a	u	a	b	b	b
Janz	a	u	a	b	b	b
J96-536-014	a	al	a	b	h	b
J96-536-253	a	al	a	b	h	b
J96-536-040	a	u	a	b	h	b
J96-536-054	a	u	a	b	h	b
J96-536-274	a	al	a	d	b	b
J96-536-188	a	al	a	d	b	b
J96-536-095	a	u	a	d	b	b
J96-536-203	a	u	a	d	b	b
J96-536-241	a	al	a	d	h	b
J96-536-031	a	al	a	d	h	b
J96-536-044	a	u	a	d	h	b
J96-536-132	a	u	a	d	h	b
J96-536-136	a	al	d	b	b	b
J96-536-236	a	al	d	b	b	b
J96-536-165	a	u	d	b	b	b
J96-536-279	a	u	d	b	b	b
J96-536-130	a	al	d	b	h	b
J96-536-239	a	al	d	b	h	b
J96-536-083	a	u	d	b	h	b
J96-536-172	a	u	d	b	h	b
J96-536-027	a	al	d	d	b	b
J96-536-103	a	al	d	d	b	b
J96-536-117	a	u	d	d	b	b
J96-536-252	a	u	d	d	b	b
Kukri	a	al	d	d	h	b
J96-536-082	a	al	d	d	h	b
J96-536-309	a	u	d	d	h	b
J96-536-161	a	u	d	d	h	b

Table C: Lines and Glutenin alleles of data set used for Mixograph in 2000.

VARIETY	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
J96-536-209	a	al	a	b	b	b
J96-536-201	a	u	a	b	b	b
J96-536-244	a	u	a	b	b	b
Janz	a	u	a	b	b	b
J96-536-014	a	al	a	b	h	b
J96-536-253	a	al	a	b	h	b
J96-536-301	a	al	a	b	h	b
J96-536-188	a	al	a	d	b	b
J96-536-095	a	u	a	d	b	b
J96-536-203	a	u	a	d	b	b
J96-536-031	a	al	a	d	h	b
J96-536-044	a	u	a	d	h	b
J96-536-132	a	u	a	d	h	b
J96-536-165	a	u	d	b	b	b
J96-536-279	a	u	d	b	b	b
J96-536-296	a	u	d	b	b	b
J96-536-130	a	al	d	b	h	b
J96-536-239	a	al	d	b	h	b
J96-536-172	a	u	d	b	h	b
J96-536-085	a	u	d	d	b	b
J96-536-117	a	u	d	d	b	b
J96-536-252	a	u	d	d	b	b
Kukri	a	al	d	d	h	b
J96-536-082	a	al	d	d	h	b
J96-536-100	a	al	d	d	h	b
J96-536-137	a	al	d	d	h	b