

New Insights Into the Role of Gluten on Durum Pasta Quality Using Reconstitution Method

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ABSTRACT

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The effects of varying the gluten composition at constant protein, protein content at constant composition, and glutenin-to-gliadin (glu/gli) ratio on durum semolina rheological properties and the quality of the spaghetti derived from these doughs was investigated using the reconstitution method. Reconstituted flours were built up from a common durum starch and water-soluble fraction but with varying gluten types from a range of wheats at both 12 and 9% total protein. A 10-g mixograph and microextensigraph properties were affected by the source of the gluten, which was related to glutenin composition and polymeric molecular weight distribution. Cooked pasta firmness was highly correlated to mixograph development time

(MDDT). Furthermore, varying the protein content (9–20%) showed an increase in mixograph peak resistance (PR) with no effect on extensigraph Rmax. Pasta firmness increased and stickiness decreased with increasing protein content. In another experiment, the glutenin and gliadin fractions isolated from durum wheat were added to the respective base semolina to investigate the effect of varying the glu/gli ratio by 1.3–1.6 fold. Increasing the ratio increased MDDT but had no effect on PR and resistance breakdown. Variable effects were obtained for spaghetti firmness. The information obtained should prove useful to durum breeders by providing further evidence for the importance of protein to pasta quality.

Durum wheat (*Triticum turgidum* L. var. *durum*) is the preferred raw material for the production of pasta, and cooking quality is one of the most important criteria in assessing the suitability of durum semolina for this purpose. Cooking quality depends on two main parameters: viscoelastic behavior (particularly firmness after cooking) and surface condition (extent of disintegration) which determines stickiness and degree of smoothness of the cooked product. These two aspects of pasta quality are relatively independent of each other (D'Egidio et al 1990). Dry pasta must appeal to the consumer at the point of purchase and the cooked pasta should be of al dente quality and must meet consumer criteria for good yellow color retention, smooth surface, firmness, and resilience; it must tolerate moderate overcooking, have minimal cooking loss, and offer a pleasing flavor.

Early studies have shown a consistent relationship between protein and gluten content of durum wheat and pasta cooking quality (Sheu et al 1967; Matsuo et al 1972; Dexter and Matsuo 1977). Evidence obtained using a statistical approach where variation in cooking quality is partitioned according to variance contribution (Matsuo et al 1982; Autran et al 1986; D'Egidio et al 1990) shows that protein content is the primary factor influencing pasta quality and that gluten strength is an important secondary factor, especially in pasta dried at high temperatures (D'Egidio et al 1990).

Other approaches have utilized a control or base flour and added a component such as glutenin or even a glutenin subunit (Bekes et al 1994; Uthayakumaran et al 1999; Edwards et al 2003). This is satisfactory when there is a major effect of the component and significant differences between the effects of materials being compared. Alternatively, separation of flour into components followed by recombination to form reconstituted flour allows the substitution of one component at a time, while the remaining components are identical, which is perhaps more sensitive. Sheu et al (1967) were the first to apply this technique to pasta production and showed that interchange of the gluten fractions resulted in the largest quality differences. In most reconstitution studies, the com-

ponent being changed has usually been protein (Delcour et al 2000; Grassberger et al 2003; Uthayakumaran and Lukow 2003), although there have been a number of reports of reconstitution being used to study the effects of lipids (Matsuo et al 1986) and starch (Delcour et al 2000; Gianibelli et al 2005). It is important that the fractionation and reconstitution retain the original functional properties of the unfractionated flour for this approach. A procedure for small-scale reconstitution of durum semolina components was established using durum wheat semolina (Sissons et al 2002).

The purpose of this study was to use a combination of reconstitution and addition studies to determine the influence of varying both the gluten composition and content and the effect of variation in the glutenin-to-gliadin (glu/gli) ratio on both rheology and pasta quality using small-scale techniques.

MATERIALS AND METHODS

Isolation of Components and Reconstitution of Semolinas

Influence of gluten source at 12 and 9% constant protein. A bulk sample of semolina (courtesy of Allied Mills, NSW Australia) was used to isolate the starch, soluble material, and tailings. Gluten was isolated from selected hexaploid and tetraploid wheats and these were combined with the starch, solubles, and tailing fractions keeping the same protein and moisture content as described previously (Sissons et al 2002). Protein content was adjusted to either 12 or 9% and moisture to 12%. Control samples used were the original semolina from which the fractions were derived and a reconstituted semolina (ReSem) where all the components were recombined in their separated proportions. Sources of gluten included the common Australian wheats Sunbrook, Hartog (prime hard wheats), Triller (soft wheat), and Cadoux (Udon noodle wheat), a Canadian extra strong wheat Glenlea, a waxy hexaploid triple Wx-null wheat (courtesy of Value Added Wheat CRC), two durum cultivars grown in Australia (Yallaroi and Kamilaroi), and three accessions of tetraploid wheats described elsewhere (Sissons and Hare 2002) AUS 3549 *Triticum persicum*, AUS 22342A and AUS 3917, both *T. polonicum*. There was not enough gluten from Cadoux and Glenlea to perform reconstitutions at the 9% protein level.

Effect of protein content variation. To determine the effect of increasing gluten content in a reconstituted dough system with a constant background, the amount of gluten was varied to give a protein range of 9–20% in the reconstituted semolina using gluten separated from the bulk semolina. In this experiment, a different bulk semolina (to that used previously) was the source of the starch, water soluble, and tailing fractions.

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Effect of variation in glu/gli ratio. Glutenin or gliadin prepared from three different sources (cv. Kamilaroi, cv. Yallaroi, and accession AUS3917) were used in this experiment. Glutenin-rich and gliadin-rich fractions were added back to the control semolina to vary the glu/gli ratio, with the protein content being adjusted to 120% of that of the control semolina.

Chemical Analyses of Fractions and Semolinas

Moisture was assayed by oven-drying a sample for 1 hr at 130°C according to Approved Method 44-16 (AACC International 2000). Protein content ($N \times 5.7$) was determined according to Approved Method 46-30 (AACC International 2000) with duplicate measurements per sample using EDTA as a standard.

Isolating Glutenin and Gliadin and Characterizing Fractions

The method of Fu and Kovacs 1999 was used but with extensive modification to separate the monomeric from polymeric protein in gluten on a preparative scale. Two 40-g extractions of gluten were used to prepare glutenin and gliadin for this study and the dried products obtained were mixed before use. Each batch involved extraction of 40 g of gluten with 800 mL of extractant (0.3M sodium iodide in water containing 7.5% 1-propanol) for 1 hr, followed by centrifugation ($10,000 \times g$) for 30 min. The pellet was re-extracted with the same buffer 3× using an homogenizer (Ultraturrax, IKA Works (Asia), Kuala Lumpur, Malaysia) to disperse the gluten pellet. The combined supernatants containing the gliadin-rich fraction were dialyzed against 0.1 mM acetic acid (20 L) for three days (to remove the sodium iodide), changing the medium each day. The pellet (glutenin-rich fraction) was dialyzed in the

same way. This procedure retains the protein functionality (Skerritt et al 1996). After dialysis, fractions were freeze-dried, ground in a mortar, and passed through a sieve (1 mm pore size). Fractions were characterized for protein content and for purity by SDS-PAGE (Sissons and Batey 2003). Glu/gli ratio was determined by size-exclusion high-performance liquid chromatography (Table I) as described elsewhere (Sissons and Batey 2003) with the only modification being the need to dilute the samples 3–5 fold to avoid glutenin “sticking” to the column and causing excessive column backpressure. Duplicate injections were used for each sample and data presented is the mean. The LMW-GS alleles were classified according to the nomenclature of Nieto-Taladriz et al (1997).

Quality Tests

Analysis on a 2-g mixograph (TMCO, Lincoln, NE) was performed by adding distilled water to semolina and correcting for the moisture and protein content of the sample so as to obtain a constant dough mass of 3.5 g. Software (Rath et al 1994) was used to obtain mixograph dough development time (MDDT), peak dough resistance (PR), and resistance breakdown (RBD). Triplicate analyses were performed on each sample. For the study on the effect of glu/gli ratio, a 10-g mixograph and the Mixsmart software (TMCO) were used to obtain MDDT, peak resistance, width at peak, and width of peak after 9 min mixing (W9). Duplicate analyses were performed. Dough extension tests were performed as described previously (Sissons et al 2002) with quadruplicate analyses.

For pastamaking, dough samples were mixed in a 50-g farinograph bowl and processed into spaghetti using a small-scale extruder and dried using conditions described previously (Sissons

TABLE I
Characterization of Glutenin and Gliadin Fractions Isolated from Durum Wheat Semolina

| Sample | Protein Fraction | % Yield ^a | Protein Content (%) | Purity by SDS-PAGE | Glu/Gli Ratio |
|-----------|------------------|----------------------|---------------------|----------------------|---------------|
| Kamilaroi | Glutenin | 83 | 62 | Gliadin contaminants | 1.13 |
| | Gliadin | 25 | 82 | Trace glutenin | 0.14 |
| Yallaroi | Glutenin | 83 | 62 | Gliadin contaminants | 1.02 |
| | Gliadin | 25 | 80 | Trace glutenin | 0.16 |
| AUS 3917 | Glutenin | 74 | 60 | Gliadin contaminants | 1.27 |
| | Gliadin | 30 | 81 | Some glutenin | 0.23 |

^a From 400 g of semolina.

TABLE II
Effect of Gluten Substitution in Reconstituted Semolina on Dough Rheology and Pasta Quality at 12% Constant Protein^a

| Sample ^b | %Protein ^c | Pasta Quality | | | | | | | | | | | | |
|---------------------|-----------------------|---------------|---------|---------|--------------|----------|-----------|--------|--------|--------------|------|------------|--------|--------------|
| | | Mixograph | | | Extensigraph | | Firmness | | | | | Stickiness | | |
| | | MDDT (sec) | PR (AU) | RBD (%) | Ext (cm) | Rmax (N) | MCT (min) | CL (%) | PH (g) | Area (g·sec) | Res | WA (%) | PH (g) | Area (g·sec) |
| Semolina | 11.8 | 476 | 346 | 5 | 12.20 | 0.27 | 12.0 | 6.7 | 389 | 195 | 0.70 | 168 | 26.1 | 6.7 |
| ReSem | 12.1 | 467 | 328 | 4 | 9.80 | 0.34 | 13.0 | 6.6 | 388 | 192 | 0.68 | 168 | 27.8 | 7.1 |
| Sunbrook | 12.5 | 337 | 414 | 10 | 14.43 | 0.30 | 12.0 | 6.4 | 348 | 171 | 0.67 | 172 | 27.1 | 7.7 |
| Triller | 12.1 | 235 | 356 | 9 | 13.23 | 0.21 | 12.0 | 6.4 | 332 | 157 | 0.66 | 174 | 27.1 | 6.7 |
| Hartog | 12.1 | 515 | 406 | 8 | 13.35 | 0.22 | 11.5 | 6.1 | 379 | 193 | 0.73 | 163 | 27.1 | 6.9 |
| Cadoux | 11.9 | 459 | 390 | 8 | 13.50 | 0.18 | 11.0 | 6.6 | 365 | 187 | 0.74 | 168 | 32.2 | 7.8 |
| Glenlea | 12.0 | 838 | 434 | 3 | 11.65 | 0.39 | 12.5 | 6.7 | 431 | 236 | 0.75 | 164 | 31.8 | 8.5 |
| Waxy | 11.9 | 222 | 334 | 6 | 12.70 | 0.13 | 11.5 | 6.1 | 277 | 134 | 0.72 | 169 | 33.6 | 9.0 |
| AUS3549 | 11.9 | 300 | 214 | 4 | 8.73 | 0.08 | 11.0 | 6.5 | 324 | 148 | 0.65 | 165 | 28.7 | 7.4 |
| AUS22342 | 11.9 | 410 | 310 | 2 | 10.60 | 0.19 | 12.0 | 6.4 | 363 | 179 | 0.71 | 168 | 27.7 | 7.1 |
| AUS3917 | 11.5 | 215 | 241 | 5 | 10.08 | 0.10 | 11.5 | 6.3 | 305 | 145 | 0.68 | 169 | 29.8 | 7.3 |
| Yallaroi | 12.1 | 597 | 326 | 3 | 9.50 | 0.43 | 13.0 | 6.3 | 389 | 190 | 0.66 | 164 | 28.4 | 7.1 |
| Kamilaroi | 12.1 | 412 | 328 | 3 | 10.35 | 0.34 | 13.0 | 6.5 | 379 | 178 | 0.64 | 163 | 27.8 | 6.5 |
| S/Sol/T | 3.2 | 330 | 286 | -1 | nd | nd | 5.5 | 6.2 | 137 | 44 | 0.57 | 194 | 26.8 | 9.1 |
| LSD ^d | 0.1 | 30 | 12 | 3 | 1.27 | 0.03 | | 0.9 | 28 | 16 | 0.04 | 15 | 4.1 | 1.1 |

^a MDDT, mixograph dough development time; PR, peak resistance; RBD, resistance breakdown; Ext, extensibility; Rmax, maximum resistance; MCT, pasta minimum cooking time; CL%, cooking loss percentage; Firmness, cooked pasta firmness as peak height and area of curve; Res, pasta resilience; WA, water absorption; Stickiness, cooked pasta stickiness as peak height and area of curve; nd, not determined; AU, arbitrary units

^b Semolina from commercial bulk lot A and ReSem derived from it. S/Sol/T contains starch, solubles and tailings; only one replicate due to insufficient sample.

^c Protein corrected to 12% moisture basis.

^d Least significance difference ($P < 0.05$).

et al 2002). For all three experiments, three batches of pasta were made for each sample on different days. Spaghetti samples were cooked to minimum cooking time (MCT), the time taken for the central core of the strand to disappear when crushed between two microscope slides. Samples were tested for firmness and stickiness using texture analysis (TA.XT2, Stable Micro Systems, Godalming, UK), water absorption after cooking (WABS), and cooking loss (CL%), as described previously (Sissons et al 2002). For the firmness and stickiness tests, each semolina sample was processed into pasta in triplicate. For each batch, pasta was cooked in triplicate for each sample to MCT. Mean firmness and stickiness were calculated from nine individual measurements. For WABS and CL%, duplicate measurements were made on each sample ($n = 6$).

Statistical Methods

ANOVA was performed on all data to calculate a least significance difference (LSD). Data presented in the tables are mean values. Statistical Analysis System procedures and programs (SAS Institute, Cary, NC) were used for data analysis.

RESULTS AND DISCUSSION

Influence of Gluten Source on Dough Rheology and Pasta Quality at 12% protein

The gluten from the bulk semolina used to prepare the ReSem, was substituted with gluten from other wheat sources. In this way, the effect of different gluten compositions at constant protein content on rheological properties and the quality of the spaghetti derived from such doughs could be investigated. The protein content of the ReSem was $12.0 \pm 0.2\%$, so the effect of varying protein content could be effectively eliminated. The reconstituted semolina (ReSem) and the unfractionated semolina had very similar mixograph behavior. However, the ReSem dough was less extensible than unfractionated dough and had a higher R_{max} (Table II), which agrees with the results reported previously (Sissons et al 2002). It is possible that during the fractionation procedure, where one of the first steps is to form a dough by hand, followed by washing it, that the gluten may have been partly developed, leading to an increase in strength (R_{max}) and a decrease in extensibility as observed by Delcour et al (2000) with their fractionation procedure. Nevertheless, all the reconstituted samples can be compared with the ReSem sample as the appropriate control. Interestingly, while there were no differences in the glu/gli and P/M ratios between semolina and ReSem, the latter had a much lower %UPP (Table III). Clearly there was a difference in the polymeric molecular weight distribution caused by the fractionation of the gluten. There were no differences in any of the pasta quality parameters between unfractionated semolina and ReSem as reported previously (Sissons et al 2002).

Analysis of the glutens by SDS-PAGE and SE-HPLC shows that they have a wide range in glutenin composition and molecular weight distribution of the polymeric proteins (Table III). It is expected that this variation would have an impact on gluten strength and pasta quality. Gluten strength can be estimated by the dough properties evaluated using mixograph and extension tester equipment, which provide parameters such as R_{max} , MDDT, and RBD (Table II). Strong dough has a high R_{max} , long MDDT, and is stable to overmixing (low RBD values). Kamilaroi had shorter MDDT and lower R_{max} than Yallaroi. Gluten from high-quality bread wheat such as Sunbrook and Hartog, although having reasonable R_{max} , had poor RBD compared with samples with durum gluten. Triller had low dough strength because it was bred as a soft biscuit wheat, while the waxy wheat gluten made dough with poor strength, as did AUS3549 and AUS3917. The gluten from AUS22342A had mixograph properties similar to Kamilaroi but with a lower R_{max} . Although it is not possible to ascribe differences in dough strength to particular glutenin subunit compositions due to the insufficient number of samples and multiple differences in glutenin alleles, some relationships are noted. High dough strength was associated with the presence of HMW-GS 5+10 in glutens from Glenlea, Hartog, and Sunbrook, and for durum, subunit pairs 7+8 and 7+16, except for AUS3917, due to a poor LMW-GS composition. In contrast, lower dough strength samples had the 2+12 pair (common wheats) and subunit 20 (durum). Such associations have been reported elsewhere for common wheat (Payne et al 1984) and durum (Brites and Carillo 2001; Sissons and Batey 2003) and are consistent with our results.

The size distribution of polymeric proteins estimated by the percentage of unextractable polymeric protein (%UPP) has been correlated with dough strength in bread (Gupta et al 1993) and durum wheat (Gianibelli et al 1995). The %UPP varied widely among the gluten samples analyzed in this experiment (Table III); the lowest %UPP was found in two of the tetraploid wheats. It is clear that despite some very subtle changes in protein content, many of the differences in the rheological properties observed are due to the effects of the different glutens used in the reconstitution of the semolinas. Gluten from durum wheats provides much stronger doughs than from the wild tetraploids and the hexaploid wheats (including waxy wheat), with the exception of the extra strong gluten from cv. Glenlea.

Pasta minimum cooking time only varied between samples by up to 2 min, as expected because cooked pasta diameter (2.50–3.00 mm), semolina protein content, and starch type were basically unchanged and these have the largest impact on MCT. We measured the firmness and stickiness of the cooked spaghetti, which are good indicators of pasta texture. Pasta made from doughs containing gluten from Hartog, Cadoux, and AUS22342 made pasta of equivalent firmness to the durum samples (Table II). Glenlea

TABLE III
Characterization of Polymeric Molecular Weight Distribution and Glutenin Composition of Semolina and Reconstituted Samples from Gluten Substitution Study

| Sample | Poly/Mono Ratio | Glu/Gli Ratio | %UPP | HMW-GS | LMW-GS or Allele |
|-----------------------|-----------------|---------------|-------|-----------------|------------------|
| Semolina ^a | 90.24 | 1.37 | 39.01 | 7+8 | caa |
| ReSem ^a | 87.74 | 1.36 | 27.46 | 7+8 | caa |
| Sunbrook | 87.89 | 1.36 | 32.80 | 2*, 7+8, 5+10 | b/chb |
| Triller | 70.93 | 1.12 | 26.85 | 2*, 7+8, 2+12 | bjb |
| Hartog | 77.16 | 1.20 | 32.71 | 1, 17+18, 5+10 | bhb |
| Cadoux | 68.84 | 1.04 | 16.39 | 2*, 17+18, 2+12 | bba |
| Glenlea | 79.24 | 1.21 | 43.33 | 2*, 7+8, 5+10 | ggc |
| Waxy | 73.06 | 1.10 | 21.43 | 17+18, 2+12 | not determined |
| AUS3549 | 75.43 | 1.17 | 9.05 | 20 | 5, 12, 15, 19 |
| AUS22342 | 85.59 | 1.34 | 28.93 | 1, 15+16 | aab |
| AUS3917 | 69.37 | 1.04 | 12.21 | 7+8 | 5, 12, 15, 19 |
| Yallaroi | 82.98 | 1.33 | 21.09 | 7+16 | caa |
| Kamilaroi | 85.26 | 1.40 | 9.18 | 20 | caa |

^a Semolina from commercial bulk lot A and ReSem derived from it.

gluten made the firmest pasta, which is consistent with having the strongest dough but it was also slightly stickier (both peak height and area) than pasta made from the durum wheat samples, perhaps because the durum starch-Glenlea gluten matrix is less able to prevent loss of amylose during cooking than the durum samples. Waxy wheat gluten made the softest pasta, which was also the stickiest and Cadoux gluten increased the stickiness. Sunbrook, Triller, AUS3549, and AUS3917 made softer pasta than the durum glutes but had the same stickiness.

Resilience is defined as the ratio of the second to first peak in the compression test and is an indication of the recovery of the pasta after compression. Pasta made from the durum samples (Yallaroi, Kamilaroi, ReSem, semolina, and the three tetraploids) had lower resilience than pasta made from samples with gluten from Cadoux, waxy, Glenlea, and Hartog (Table II). This may be because these samples have higher gluten extensibility, although Sunbrook was the most extensible dough but had a pasta resilience comparable to that of the durum samples. There were no differences between samples in their water absorption and cooking loss. Starch has a major effect on CL% (Gianibelli et al 2005) and because this was the same in all the samples, it is not surprising that this parameter did not change.

Interestingly, a reconstituted sample consisting of only starch, water solubles, and tailings (S/Sol/T) was made into pasta. This reconstituted sample had the expected very low protein content due to a low gluten content. There was sufficient gluten coming from the tailings fraction (data not shown) in the sample to produce a mixogram showing a very weak dough (Table II). Due to the low protein content, the pasta has a short cooking time, very low firmness, and resilience. The pasta made from this sample was stickier (area) than the ReSem sample, although CL% was equivalent. This clearly shows that despite the very low gluten content, cook-

ing loss was not affected. Other work has shown that the amylose content of the starch influences cooking loss (Gianibelli et al 2005).

MDDT was strongly correlated to pasta firmness (peak area), ($r^2 = 0.90$), while Rmax showed a weaker correlation with pasta firmness ($r^2 = 0.55$). Given these correlations it could be said that, in material with a similar protein content, MDDT would be a good predictor of pasta firmness.

Influence of Gluten Source on Dough Rheology and Pasta Quality at 9% Protein

Premiums are paid for high protein durum wheat in Australia and most other parts of the world. Protein >12% is regarded as providing a good base for sound quality, while low protein durum wheat (<10%) is less desirable because it is generally thought to produce inferior pasta. We decided to investigate the influence of gluten substitution at $\approx 9\%$ protein to see whether gluten composition had a greater influence on quality at lower protein levels. The protein content in the reconstituted samples was relatively constant ($9.16 \pm 0.13\%$). Significant differences were found in mixograph curves between the samples but with fewer differences compared with gluten substitution at 12% protein, especially for RBD (Table IV). At 12% protein level, Sunbrook, Triller, Hartog, and waxy gluten had higher RBD than at 9%. In contrast, Yallaroi and Kamilaroi samples showed the opposite trend. Mixograph PR was lower for all samples at 9% compared with 12% protein, which is expected because protein has a large impact on PR (Shogren et al 1969). Based on MDDT, there were differences in the rankings at 9% versus 12% protein; for example, waxy had the lowest MDDT at 12% protein but not at 9%. Also, Hartog and Sunbrook had longer MDDT at 9% than at 12% protein level. The Rmax of the samples was similar at the two protein levels, with the exception of Sunbrook, suggesting this parameter is less affected by protein

TABLE IV
Effect of Gluten Substitution in Reconstituted Semolina on Dough Rheology and Pasta Quality at 9% Constant Protein^a

| Sample | % Protein | Mixograph | | | Extensigraph | | Pasta Quality | | | | | | |
|-----------|-----------|------------|---------|---------|--------------|----------|---------------|--------|-----------------|-----------------------|------------|----------|-------------------------|
| | | MDDT (sec) | PR (AU) | RBD (%) | Ext (cm) | Rmax (N) | MCT (min) | CL (%) | Firmness PH (g) | Firmness Area (g-sec) | Resilience | WABS (%) | Stickiness Area (g-sec) |
| ReSem | 9.1 | 401 | 221 | 2 | 11.50 | 0.44 | 9.0 | 6.2 | 363 | 171 | 0.71 | 156 | 7.6 |
| Sunbrook | 9.2 | 513 | 258 | 4 | 11.00 | 0.16 | 9.0 | 6.7 | 319 | 152 | 0.74 | 169 | 8.1 |
| Triller | 9.1 | 282 | 234 | 3 | 13.45 | 0.19 | 9.5 | 6.9 | 274 | 129 | 0.71 | 180 | 10.9 |
| Hartog | 9.5 | 631 | 260 | 3 | nd | nd | 9.5 | 6.3 | 324 | 160 | 0.72 | 166 | 6.7 |
| Waxy | 9.1 | 346 | 226 | 2 | 12.50 | 0.10 | 8.5 | 6.3 | 286 | 134 | 0.73 | 154 | 9.0 |
| AUS3549 | 9.2 | 135 | 201 | 5 | 8.30 | 0.14 | 9.0 | 6.6 | 321 | 153 | 0.70 | 152 | 8.4 |
| AUS22342 | 9.1 | 426 | 215 | 2 | 12.20 | 0.20 | 9.5 | 8.2 | 214 | 98 | 0.73 | 208 | 9.4 |
| AUS3917 | 9.1 | 178 | 196 | 8 | 9.20 | 0.12 | 9.5 | 6.7 | 298 | 147 | 0.74 | 168 | 9.4 |
| Yallaroi | 9.1 | 393 | 230 | 6 | 10.00 | 0.45 | 10.5 | 6.6 | 316 | 155 | 0.72 | 170 | 7.0 |
| Kamilaroi | 9.1 | 257 | 234 | 8 | 9.70 | 0.40 | 10.0 | 7.0 | 352 | 167 | 0.70 | 160 | 7.4 |
| LSD | 0.2 | 116 | 18 | 3 | 0.96 | 0.04 | | 0.6 | 27 | 15 | 0.05 | 14 | 1.8 |

^a Terms as described in Table II.

TABLE V
Effect of Protein Content Variation on Dough Rheology and Pasta Quality in a Common Gluten Background

| Sample ^a | Protein % | Dough Properties | | | Pasta Quality | | | |
|---------------------|-----------|------------------|----------|----------|---------------|--------|------------|--------|
| | | RBD (%) | Ext (cm) | Rmax (N) | MCT (min) | CL (%) | Resilience | WA (%) |
| 0.7 × ReSem | 9.2 | 3 | 8.4 | 0.42 | 12.0 | 6.1 | 0.68 | 184 |
| 0.8 × ReSem | 10.9 | 2 | 9.8 | 0.38 | 12.0 | 5.9 | 0.67 | 169 |
| 0.9 × ReSem | 12.2 | 2 | 8.5 | 0.47 | 11.5 | 5.8 | 0.72 | 162 |
| ReSem | 13.4 | 3 | 8.9 | 0.52 | 13.5 | 5.9 | 0.73 | 177 |
| 1.1 × ReSem | 14.7 | 5 | 9.7 | 0.42 | 13.0 | 6.0 | 0.72 | 164 |
| 1.2 × ReSem | 16.1 | 5 | 7.0 | 0.49 | 12.5 | 5.8 | 0.76 | 165 |
| 1.3 × ReSem | 17.4 | 3 | 7.5 | 0.44 | 13.0 | 5.7 | 0.79 | 155 |
| 1.4 × ReSem | 19.2 | 5 | 6.9 | 0.53 | 13.5 | 5.8 | 0.79 | 154 |
| 1.5 × ReSem | 20.4 | 3 | nd | nd | 13.5 | 5.8 | 0.78 | 155 |
| Semolina | 12.2 | 5 | 12.6 | 0.29 | 12.0 | 6.0 | 0.72 | 156 |
| LSD | 0.2 | 3 | 2.1 | 0.08 | | 0.3 | 0.04 | 8 |

^a ReSem sample derived from a commercial bulk lot B × reduction or augmentation of gluten to achieve 0.7–1.5-fold change in ReSem protein content. Terms as described in Table II.

quantity. There were also some differences in Ext at the two protein levels. The results show that more differences were obtained between the gluten-substituted samples when this experiment was conducted at 12% protein. This feature is not unexpected because it is well known for common wheats that loaf volume differences between different genotypes narrows as flour protein falls to <10% (Hoseney 1994).

A comparison of the results (Tables IV vs. II), where the total protein of the samples differed by $\approx 3\%$, showed pasta MCT was reduced by ≈ 2.5 min. Presumably, the reduced amount of gluten in the starch-gluten matrix allowed gelatinization to occur more rapidly, reducing the cooking time. The reduced protein also led to a slightly higher cooking loss compared with 12% protein, especially for AUS2342, which suggests greater leaching of amylose from the starch-gluten matrix. Most of the samples had a lower cooked pasta firmness than at 12% protein content. Some of the samples with low firmness (waxy, AUS 3549, and AUS 3917) had similar firmness at both protein levels. A possible explanation is the poor glutenin composition and low %UPP of these samples, which prevented an improvement in firmness when protein was increased. There were no differences in resilience between the samples (Table IV). Also, there were no changes in WABS between the two protein levels, except for an increase for AUS22342 that cannot be explained. Slightly higher pasta stickiness was observed at the lower protein level in these samples, especially for the Triller sample. This is consistent with results from a later study. The results from both these studies indicate that selection for long MDDT, low RBD, and high Rmax should produce a dough that is less extensible and pasta that is firm.

Effect of Protein Content Variation on Dough Rheology and Pasta Quality

Many studies have been published that show the importance of the quantity of protein in determining cooking quality, especially for high-temperature-dried pasta (Dexter and Matsuo 1977; Feillet 1984; Autran and Galterio 1989; D'Egidio et al 1990). However, most of these studies have not isolated the effect of just the total amount of protein on technological quality from variation in protein and starch composition. An advantage of the reconstitution system is that the composition of the sample can be kept constant while the proportion of gluten in the reconstituted semolina can be altered. We achieved a range in protein of 9–20% in the reconstituted samples, which spans the range found in durum wheat. Mixograph PR was highly dependent on protein content and increased ($r^2 = 0.99$) with increasing protein (Fig. 1). The MDDT increased to $\approx 12\%$ protein and then moved up and down, with a tendency to decrease. There were no significant changes in

RBD over this range in protein (Table V), which is in contrast to results from changing the gluten composition (Tables II and IV). In a study in common wheat where blends of different base flours with added gluten and starch were used to obtain a range in protein, MDDT increased with protein in a pattern similar to that found here but was highly dependent on the cultivar and protein level (Uthayakumaran et al 1999). In that study using bread flour, PR showed a consistent linear positive relationship to protein.

Extensibility fluctuated within a range of ≈ 2.9 cm with a tendency to decline with increasing protein (Table V) but there was no significant difference in Ext between the lowest and highest protein levels. The Rmax fluctuated over the protein range, with a tendency to increase slightly with protein. This data is in contrast to the linear increases with protein over a similar range in hexaploid wheats using base bread flour enrichment with gluten (Uthayakumaran et al 1999). Our results show that increasing protein content had a negligible effect on Rmax and Ext. In contrast, changing the gluten composition had a substantial effect on these parameters (Tables II and IV). This might depend on the glutenin composition or the polymeric molecular weight distribution.

There was only a minimal variation in MCT, with a tendency to a slightly longer cooking time at the higher protein levels. Cooked pasta firmness increased from 302 to 534 g (1.8-fold increase) at a linear rate ($r^2 = 0.96$) with increasing protein (Fig. 2). In the analysis of breeder's lines, we typically see a similar range in both grain protein and pasta firmness, but the correlation between these is much lower due to the high variability of the samples (data not shown). In the reconstitution studies here, where only one factor is altered, we see that gluten quantity has a marked influence on pasta firmness. In comparison, when gluten was substituted at constant protein level with different gluten compositions, a similar but slightly smaller (1.4–1.6 fold) change in firmness was obtained (Tables II and IV). Clearly, pasta firmness is dependent on both protein content and composition. In most durum breeding populations, a narrow range in gluten protein composition exists (Liu et al 1996), so changes obtained in firmness are more likely related to the environmental impact on grain protein content. However, the relationship between gluten strength (protein composition) and pasta cooking quality is complex and somewhat controversial. Certainly under high- and ultrahigh-temperature pasta drying conditions, gluten strength has less influence on cooking quality than under low-temperature conditions (D'Egidio et al 1990; De Stefanis and Sgrulletta 1990). Gluten from a Canadian extra strong cultivar exhibit pasta cooking quality comparable to those of intermediate strength at equivalent protein (Rao et al 2001), indicating that gluten strength (gluten quality) is not as important as once thought. Sopiwnyk (1999) related the gluten strength of seven Canadian

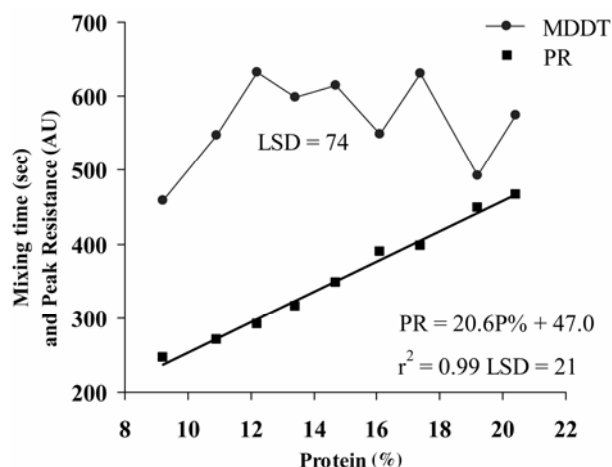


Fig. 1. Effect of changing the percentage of gluten in reconstituted sample on mixograph dough development time and peak resistance.

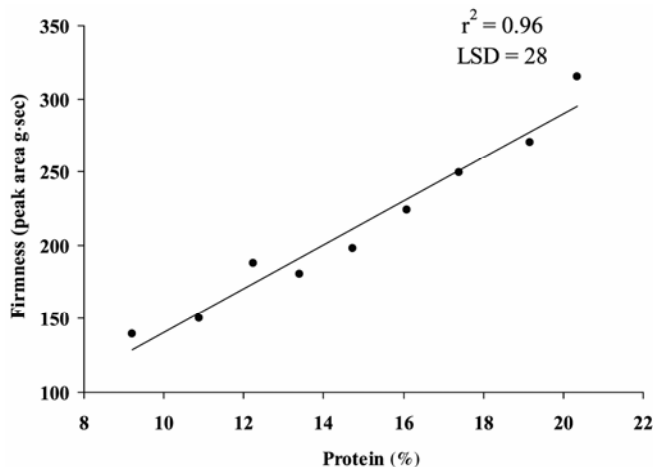


Fig. 2. Effect of changing the percentage of gluten in reconstituted sample on cooked pasta firmness.

durum wheats to cooked pasta texture and found that pasta made from samples with weaker gluten strength did not necessarily result in pasta with poorer cooking quality. Our results showed that while there was a correlation between Rmax and pasta firmness at both protein levels ($r \approx 0.55-0.57$), the relationship between pasta firmness and gluten strength is not strong.

Pasta resilience showed a small but significant increase with protein content (Table V), which suggests that the pasta becomes tougher and more resistant to deformation. Hence, in the two compression tests, the pasta largely returns to its original width with an increase in protein content. Pasta stickiness decreased at a linear rate ($r^2 = 0.96$) with increasing protein content (Fig. 3). As protein increases, starch content decreases, so there is less material to be lost during pasta cooking and exuding on the surface, resulting in a less sticky pasta. The water absorption of the pasta is the change in weight of the pasta after cooking and this decreased significantly at protein levels >13.4% (Table V). The decrease in gluten is accompanied by a greater proportion of starch, which during cooking swells and therefore contributes to the increased pasta water absorption, making the pasta softer. Cooking loss was not affected by protein content variation. A high CL% reflects a protein-starch matrix that easily allows leaching of the material from pasta during cooking, which are mainly amylose (Lintas and D'Appolonia 1973) and other carbohydrates (Bangur et al 1999). The data here shows that even at the low 9% protein level, the matrix is sufficient to prevent excessive amylose

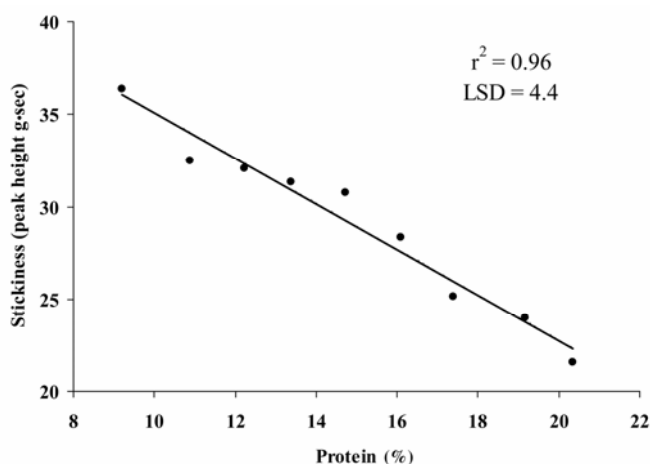


Fig. 3. Effect of changing the percentage of gluten in reconstituted sample on cooked pasta stickiness.

leaching and the pasta has acceptable CL% compared with the original semolina. Indeed, even altering the gluten composition markedly, there was no effect on CL% until protein reached 9%, where in three samples CL% increased relative to ReSem (Table IV). Even a reconstituted sample with virtually no gluten and just starch, solubles, and tailings fractions (S/Sol/T) (Table II) had the same CL% as a sample containing gluten. This indicates that starch structure and composition is much more important in cooking loss than protein content. For example, starch with reduced amylose content has decreased CL% in two studies (Sharma et al 2002; Gianibelli et al 2005), and in another study, there was no change in cooking loss reported (Grant et al 2004).

It is interesting to note that in this study at 20% semolina protein, the pasta had the greatest firmness and least stickiness compared with pastas made from different gluten types (Table II). Protein contents <10% would be undesirable because the pasta has low firmness and high stickiness. Clearly, aiming for high protein content (all other factors aside) should ensure pasta that is very firm with low stickiness. However, work by Novaro et al (1993) shows it is not necessary for durum wheat breeding programs to force the selection for grain protein content to >15–16% because there is very limited improvement in pasta sensory quality.

Variation in Glu/Gli Ratio at Constant Protein Content

Preliminary studies showed that it was not necessary to defat the semolina before the fractionation of glutenin and gliadin. Changes in the mixograph of a base semolina after adding either glutenin- or gliadin-rich fractions isolated from defatted versus nondefatted semolina produced no change in mixing properties (data not shown). The glutenin- and gliadin-rich fractions contained ≈ 61 and 81% protein, respectively. The gliadin fraction had only trace quantities of glutenin with a low glu/gli ratio (Table I). However, the glutenin had gliadin contamination and was less pure. We used a well-established protocol for examining the effect of added fractions (Uthayakumaran et al 1999; Verbruggen et al 2001; Edwards et al 2003) by addition of glutenin or gliadin fractions to a base semolina. In this study, three base semolina samples were chosen with quite different gluten strengths so that the effect of adding glutenin and gliadin could be compared. Clearly, AUS 3917 (tetraploid base flour or TBS) was the weakest sample with very short MDDT and W9 consistent with low glu/gli and %UPP values (Table VI). These relationships have been observed previously (Sissons and Batey 2003). Kamilaroi (KBS) is weaker than Yallaroi (YBS) as indicated by the mixograph results (Fig. 4A and B and Table VI). Protein content was increased to 120% of the base flour with the addition of either a glutenin- or gliadin-rich fraction or a sample containing an equal mass mixture

TABLE VI
Effect of Glutenin-to-Gliadin Content Variation at Constant Protein on Dough Rheology and Pasta Quality

| Sample ^a | SE-HPLC | | | Mixograph | | | Pasta Quality | | |
|--------------------------|---------|-----------|-------|-----------|---------|-----------|---------------|-------------------|-------------------------|
| | Glu/Gli | Poly/Mono | %UPP | PR (AU) | WP (AU) | MCT (min) | Resilience | Stickiness (PH) g | Stickiness Area (g·sec) |
| KBS | 0.86 | 0.68 | 40.59 | 68 | 24 | 10.67 | 0.67 | 24.1 | 5.3 |
| KBS+gliadin | 0.72 | 0.57 | 37.25 | 68 | 24 | 10.50 | 0.73 | 24.7 | 5.1 |
| KBS+glutenin and gliadin | 0.76 | 0.60 | 43.20 | 70 | 23 | 10.67 | 0.74 | 25.2 | 5.0 |
| KBS+glutenin | 0.98 | 0.79 | 45.48 | 60 | 24 | 11.33 | 0.70 | 22.8 | 4.9 |
| YBS | 0.78 | 0.64 | 51.75 | 64 | 19 | 10.17 | 0.70 | 22.9 | 4.4 |
| YBS+gliadin | 0.68 | 0.54 | 48.55 | 70 | 23 | 10.00 | 0.75 | 20.5 | 4.6 |
| YBS+glutenin and gliadin | 0.74 | 0.60 | 49.71 | 63 | 20 | 10.67 | 0.71 | 20.7 | 4.8 |
| YBS+glutenin | 0.91 | 0.72 | 56.52 | 64 | 20 | 10.50 | 0.72 | 18.8 | 3.6 |
| TBS | 0.48 | 0.39 | 8.73 | 50 | 12 | 9.33 | 0.63 | 22.4 | 5.5 |
| TBS+gliadin | 0.46 | 0.39 | 17.43 | 50 | 11 | 9.00 | 0.74 | 39.0 | 9.5 |
| TBS+glutenin and gliadin | 0.60 | 0.50 | 31.40 | 56 | 15 | 9.00 | 0.74 | 26.3 | 6.7 |
| TBS+glutenin | 0.75 | 0.60 | 30.73 | 55 | 13 | 8.67 | 0.72 | 25.7 | 5.5 |
| LSD | 0.08 | 0.06 | 9.89 | 6 | 5 | 0.01 | 0.06 | 4.1 | 1.0 |

^a KBS, Kamilaroi base semolina; YBS, Yallaroi base semolina; TBS, tetraploid base semolina; Glu/Gli, glutenin to gliadin ratio; Poly/Mono, polymeric to monomeric ratio; %UPP, percentage of unextractable polymeric protein. Other terms as described in Table II.

of glutenin- and gliadin-rich fractions. A direct comparison with the base flour is not strictly correct as the slight increase in protein could affect the measured properties, especially WP and pasta firmness. Instead, emphasis was placed on the effect of increasing the glu/gli ratio where a 1.3–1.6 fold change was obtained by these additions. The addition of gliadin decreased the glu/gli ratio of the base flour while the addition of glutenin increased this ratio (Table VI). More subtle changes in the %UPP were obtained with a tendency to decrease %UPP with addition of gliadin and increase %UPP with addition of glutenin. This was not the case for TBS where even gliadin addition increased %UPP significantly. However, when the glutenin was added, a much bigger increase occurred approaching the %UPP found in KBS. Increasing the glu/gli ratio increased MDDT in all flours (Fig. 4A). For

Kamilaroi, adding glutenin made a dough with a longer MDDT than Yallaroi base flour (YBS), while for TBS, MDDT increased to a level similar to KBS but still weaker than YBS. The MDDT of TBS is considerably lower than that of YBS where presumably a much greater quantity of glutenin would need to be added to achieve the same MDDT. Both PR and WP were unaffected by the changes in the glu/gli ratios in all three base flours (Table VI) because these parameters are more affected by variation in protein content. However, width at 9 min or increased tolerance to over-mixing was improved markedly in all three base flours to varying extents when the glu/gli ratio exceeded that of the base flour (Fig. 4B). These results show that dough strength can be increased in semolinas with variable dough strength by increasing the glu/gli ratio by ≈ 1.3 –1.6 fold. These results are similar to what was found in common wheat flours where the glu/gli ratio was altered by addition of glutenin fractions to base flours (Uthayakumaran et al 1999). Edwards et al (2003) found that the addition of gliadin to a base semolina dough resulted in weaker mixograph curves while glutenin addition increased dough strength measured by mixograph and dynamic and creep compliance.

The effects of increasing the gluten strength through increases in the glu/gli ratio were not readily transferred to pasta texture. Increasing the glu/gli ratio in YBS and TBS produced a decrease in pasta firmness at the highest ratio, but for KBS there was an increase in firmness when the glu/gli ratio increased from 0.72 to 0.76, followed by no further change at 0.98 (Fig. 4C). There were no changes in resilience, MCT, and stickiness, except for the much higher stickiness of the TBS + gliadin sample, which cannot be explained (Table VI).

CONCLUSIONS

Using reconstitution methods, the relationship between gluten strength, protein content, and pasta texture has been established under carefully controlled conditions. Durum gluten substituted with gluten from extra strong Glenlea wheat produced the firmest pasta. Variation in protein content had a larger effect on pasta firmness and stickiness than gluten substitution. A protein >17% produced very firm pasta with a low stickiness. The data suggests durum breeding programs should attempt to increase grain protein once a favorable gluten composition has been achieved. Increases in the protein content at constant glu/gli ratio and increase in glu/gli ratio at constant protein increased dough properties such as mixograph PR and MDDT. Pasta firmness and resilience were affected by changes in protein content.

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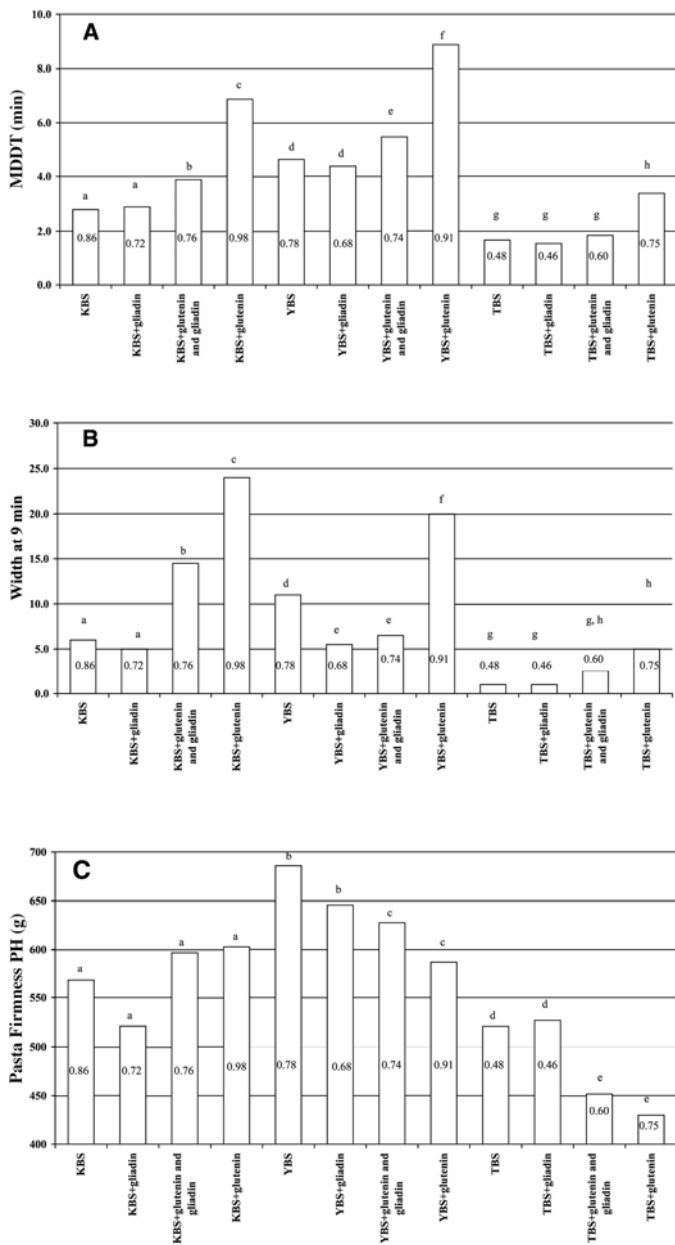


Fig. 4. Mixing and pasta firmness properties of three durum cultivars with glutenin-to-gliadin ratio altered by adding glutenin or gliadin. **A**, Mixograph dough development time (MDDT). **B**, Mixograph width at 9 min after peak (AU). **C**, Cooked pasta firmness. KBS, Kamilaroi base semolina; YBS, Yallaroi base semolina; TBS, tetraploid base semolina. Letters above the bars (for each sample group) that are the same are not significantly different ($P < 0.05$). Values for histogram rectangles are glu/gli ratios.

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