

Protein and Starch Properties of Some Tetraploid Wheats

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ABSTRACT

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The tetraploid relatives (subspecies) of commercial durum wheat (*Triticum turgidum* L. subsp. *turgidum* conv. *durum* (Desf.) MacKey) offer a source of economically useful genes for the genetic improvement of durum cultivars. Thirty-two accessions, representing five different subspecies: var. *durum* (13 accessions), *polonicum* (7), *persicum* (3), *turanicum* (5), and *turgidum* (4) were grown at Tamworth, Australia, in 1997 and 1999. These accessions were compared with three durum cultivars: Wollaroi and Kamilaroi (in both years) and Yallaroi (in 1998 only). In this study, the glutenin subunit composition and molecular

weight distribution, together with starch properties of these accessions, were studied. A much wider range in both the glutenin subunit composition and the starch RVA paste viscosities and gelatinization profiles were found in the accessions compared with the cultivated durum wheats. Most of the accessions had lower gluten strength and the presence of poor quality LMW alleles, and low proportions of unextractable polymeric protein could explain this. For starch, RVA peak viscosity correlated strongly with cooking loss of pasta, the only significant correlation between starch properties and measured aspects of pasta quality.

Both the quantity and composition of the durum endosperm protein affect pasta quality (Matsuo et al 1984; Autran et al 1986; D'Egidio et al 1990). However, the effect of starch, the major component of the endosperm, has not been clearly resolved. Genetic manipulation of durum wheat to improve pasta-making quality or to produce novel foods (for example, durum wheats with waxy and high resistant starch types, enrichment of essential amino acids) through conventional breeding would benefit from knowledge of the relationships between protein and starch composition with functional properties. An important limitation to the improvement of the durum quality of commercial cultivars is the limited genetic variation in gluten protein composition in breeding populations (Liu and Shepherd 1996). The same may be true for starch characteristics once their technological importance is established. Much greater variation in protein composition occurs in other tetraploid wheats (Ciaffi et al 1992; Liu and Shepherd 1996) and this would have contributed to the large variation in technological quality reported on a limited set of tetraploid wheats (Sissons and Hare 2002). Information about variation in the starch properties of tetraploid wheats is, however, limited (Watanabe et al 1998; Stoddard 1999).

The aim of this study was to characterize in detail the diversity in protein composition and starch properties of a selection of tetraploid subspecies whose technological quality has been reported previously (Sissons and Hare 2002). Relationships between technological quality and protein composition and starch properties are discussed.

MATERIALS AND METHODS

Plant Material

A total of 32 accessions of tetraploid wheats, representing five different subspecies of *Triticum turgidum*: var. *durum* (13 accessions), *polonicum* (7 accessions), *persicum* (3 accessions), *turanicum* (5 accessions), and *turgidum* (4 accessions), and the durum cultivars Wollaroi and Kamilaroi (in both years) and Yallaroi (in 1999 only) were grown at Tamworth, Australia, in 1997 and 1999. The lines studied are described in more detail by Sissons and Hare (2002).

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Thermal Measurements

Differential scanning calorimetry (DSC) studies were performed using a Pyris 1 instrument (Perkin Elmer, Norwalk, CT). Native starch slurry samples (1:2 starch-to-water ratio, sealed in stainless steel pans) were heated from 20 to 130°C at a heating rate of 10°C/min. The results were processed using Pyris software v. 1.7.

Rapid Visco Analyser (RVA)

Starch viscosity was analyzed using a Rapid Visco Analyser (RVA) (Newport Scientific Pty. Ltd., Warriewood, Australia). Pasting viscosities of 3.5 g of semolina in 25.0 mL of 12 mM silver nitrate solution or 3.0 g of purified starch in 25.0 mL of water were analyzed using an RVA profile as described by Batey and Curtin (2000). In brief, the sample was held at 50°C for 2 min, heated to 95°C over 6 min, held at 95°C for 4 min, cooled to 50°C over 4 min, and held at this temperature for a further 4 min. The parameters measured were peak viscosity (PV, the maximum hot paste viscosity), holding strength (the trough at the minimum hot paste viscosity), and final viscosity (FV, the viscosity at the end of the test after cooling to 50°C and holding at this temperature). In addition, breakdown (peak viscosity minus holding strength) and setback (final viscosity minus holding strength) were calculated. All values were expressed in RVA units (RVU).

Amylose Determination

Amylose was determined using the SE-HPLC method of Batey and Curtin (1996).

Size-Exclusion HPLC and Electrophoretic Analyses of Proteins

SE-HPLC was performed to assess the proportions of the main classes of storage proteins typically fractionated into three peaks as glutenins (peak A), gliadins (peak B), and albumins+globulins (peak C). Proteins were extracted with buffer containing 0.5% SDS and 0.05M sodium phosphate, pH 6.9, into two fractions: a SDS-soluble fraction (soluble), and an insoluble residue, which after sonication in buffer, solubilizes the large polymeric glutenins (Gupta et al 1993) and is referred to as "after sonication". Fractions were injected onto a BIOSEP-SEC4000 column (Phenomenex, Torrance, CA) to run for 10 min at 2 mL/min in a 1:1 mixture of deionized water (containing 0.1% trifluoroacetic acid) and acetonitrile (containing 0.1% trifluoroacetic acid) using an HPLC system (Waters Corporation, Milford, MA). The system comprised two model 510 pumps, model 717 WISP automatic sampler, model 490 UV-visible detector at 214 nm, and Millennium chromatography manager software v. 3.05.01 for integration of profiles.

Calculation of the polymeric-to-monomeric ratio (P/M), glutenin-to-gliadin ratio (Glu/Gli), and % unextractable polymeric protein (%UPP) was from areas under the peaks. P/M = (soluble

peak A + peak A after sonication) / (soluble peaks B+C + peaks B+C after sonication); Glu/Gli = (soluble peak A + peak A after sonication) / (soluble peak B + peak B after sonication); %UPP = [(peak A after sonication) / (soluble peak A + peak A after sonication)] × 100.

For analysis of the glutenin subunit composition, SDS-PAGE of glutenins extracted from several seeds was performed. Glutenins were extracted from whole meal (Sissons et al 1998) and the proteins separated by SDS-PAGE (Ames et al 1998). Nomenclature used to classify alleles at the *Glu-A3*, *Glu-B3*, and *Glu-B2* loci followed the system proposed by Nieto-Taladriz et al (1997). Gliadin proteins were extracted from whole meal (100 mg of ground seed mixed with 400 µL of 1M urea with constant shaking for 1 hr followed by centrifugation in a microfuge and addition of 1 µL of 10% methyl violet as a dye marker). Fractionation was by electrophoresis under acidic (0.125% aluminum lactate buffer, pH 3.1) conditions that fractionates them on the basis of charge and size. Conditions for electrophoresis were 8.3% T and 2.9% C on gels (19 × 16 cm) containing 0.024% ascorbic acid, 0.0002% ferrous sulfate heptahydrate, and 0.25% aluminum lactate. Gel pH was adjusted (pH 3.1) with lactic acid and polymerized with hydrogen peroxide and prerun for 1 hr at 45 mA constant current at 7°C. Samples were loaded (15 µL) and the gel run at 45 mA constant current until dye reached the bottom of the gel. Proteins

were fixed and stained (0.5% Coomassie Brilliant Blue R250, 12% trichloroacetic acid) overnight then destained (12% trichloroacetic acid, 10% ethanol).

Analytical Tests

For protein estimation, a sample of cleaned grain was milled on a falling number mill (Perten, Reno, NV) and protein measured on the whole meal by NIR (Infralyzer 450, Bran+Luebbe, Sydney, Australia) calibrated against Kjeldahl nitrogen (Approved Method 39-10, AACC 2000).

Quality Tests

Objective measurements of quality were performed as described by Sissons and Hare (2002).

Statistical Analysis

S-Plus 2000 software (MathSoft Inc., Data Analysis Products Division, Seattle, WA) was used for analysis of variance, multiple comparisons, and calculation of correlation coefficients. Least significant difference (LSD) was calculated for each parameter using year as a replicate and this was used to test for significance ($P < 0.05$) between accessions. The data for both years was pooled to determine simple correlations where homogeneity was found in the correlations.

TABLE I
SE-HPLC Data on Endosperm Proteins Extracted from Tetraploid Wheat Accessions and Commercial Cultivars

Cultivar/Accession	SE-HPLC Analysis of Whole Meal Proteins ^a							
	1997 Season				1999 Season			
	GP%	P/M	Glu/Gli	%UPP	GP%	P/M	Glu/Gli	%UPP
Wollaroi	14.0	1.27	1.69	46.78	13.3	0.78	1.09	44.07
Kamilaroi	13.7	1.21	1.59	36.01	12.6	0.77	1.12	27.23
Yallaroi					12.2	0.82	1.18	40.58
Mean	13.9	1.24	1.64	41.40	12.7	0.79	1.13	37.29
AUS285	13.1	0.94	1.27	32.42	13.7	0.65	0.89	36.21
AUS297	12.7	1.06	1.51	23.52	13.8	0.69	1.00	31.33
AUS7812A	16.5	0.87	1.11	14.03	16.6	0.56	0.78	24.74
AUS7829	18.2	0.89	1.13	33.68	14.1	0.57	0.84	34.90
AUS7842	17.5	0.92	1.17	30.75	15.0	0.52	0.77	39.61
AUS7922	15.4	0.93	1.55	17.13	15.3	0.53	0.76	21.90
AUS16041	15.6	0.90	1.14	25.43	15.2	0.52	0.70	33.17
AUS17045		0.82	1.06	27.46	15.6	0.51	0.72	24.24
AUS17294	13.9	1.09	1.43	37.04	13.3	0.77	1.10	39.08
AUS20677A	16.1	1.06	1.33	40.22	15.2	0.50	0.72	30.08
AUS20677B	15.9	1.02	1.29	39.27	15.2	0.58	0.81	30.03
AUS22300	15.0	1.13	1.49	35.08	14.0	0.52	0.74	33.24
AUS22303A	14.7	1.05	1.38	29.75				
AUS3549A	13.4	0.88	1.22	12.49	14.7	0.48	0.68	
AUS3830	16.5	1.02	1.39	29.38	16.0	0.60	0.87	33.42
AUS3838	15.6	0.89	1.19	14.64	15.2	0.46	0.66	
AUS3824	15.5	0.99	1.33	22.38	15.8	0.62	0.90	21.48
AUS3917	16.8	0.91	1.17	15.21	15.8	0.49	0.69	16.33
AUS4049	18.2	0.90	1.16	12.87	18.3	0.39	0.56	
AUS5533	15.3	0.98	1.26	25.70	15.5	0.61	0.86	21.46
AUS16133	15.4	0.97	1.26	23.66	15.6	0.61	0.87	22.35
AUS22342A	15.9	1.31	1.71	47.88	15.8	0.73	1.03	42.63
AUS22342B	16.3	1.13	1.43	46.38	15.3	0.68	0.95	33.63
AUS3603	13.0	0.83	1.10	18.79	13.5	0.42	0.60	
AUS15914	15.5	0.96	1.22	31.39				
AUS18721A	14.2	1.20	1.57	39.74	13.6	0.69	0.99	30.64
AUS18721B	14.2	1.22	1.58	41.47	13.5	0.76	1.09	29.82
AUS7810	13.9	0.87	1.16	27.11				
AUS7812B	17.0	0.86	1.13	14.19	16.4	0.55	0.78	22.90
AUS13539	14.4	0.84	1.13	23.87	13.9	0.55	0.78	38.12
AUS15198	14.2	0.82	1.08	23.83	14.8	0.52	0.73	38.19
AUS17210	13.7				13.6			
Mean	15.3	0.98	1.29	27.64	14.98	0.57	0.82	30.40
Min	12.7	0.82	1.06	12.49	13.30	0.39	0.56	16.33
Max	18.2	1.31	1.71	47.88	18.30	0.77	1.10	42.63
LSD	1.5	0.13	0.17	4.41	1.5	0.08	0.12	1.86

^a GP%, grain protein percentage; P/M polymeric/monomeric ratio; Glu/Gli, glutenin/gliadin ratio; %UPP, percentage of unextractable polymeric protein.

RESULTS AND DISCUSSION

Protein Quality

The quality of pasta depends to a large extent on the variations in the amount, structure, and type of gluten proteins. Large and significant variation of protein content (13–18%) was detected among the accessions, relative to the cultivar checks (Table I). The cultivar checks produced protein contents typical for this growing location (>12%). Many of the accessions produced grain protein percentages in both years that were significantly higher than the mean of the checks. In only two cases (AUS3838 and AUS3917) were the grain of low 1,000 grain weight, suggesting poor grain filling, low starch content, and consequently elevated protein percentage. Higher grain protein content was associated with longer dough mixing time and greater stability and with firmer pasta (Sissons and Hare 2002). Many of the accessions with higher grain protein than the cultivar checks belonged to the *durum*, *persicum*, and *polonicum* subspecies. Dough functional properties (gluten strength) are influenced by a combination of factors including the glutenin subunit composition (Liu and Shepherd 1996). Nearly all the accessions were lower in gluten strength than the cultivar checks (Sissons and Hare 2002). Information about the protein composition may help to explain these results. The cultivar checks were quite similar in protein composition all with γ -gliadin 45 and low molecular weight glutenin (LMW-GS) caa allele pattern but differing only in the HMW-GS. In contrast, there was a greater diversity in the glutenin composition of the accessions (Fig. 1 and Table II). Most of the samples had γ -45 but five of the accessions had γ -42, known to be associated with lower dough strength in

durum wheat (du Cros et al 1982). Thirteen different HMW-GS patterns were found with the majority null at *Glu-A1* but with two other variants found expressing subunits 1, 2*, or 2**. There were eight different *Glu-B1* subunits found; accession AUS285 had 2+12, indicating hexaploid wheat contamination (after testing individual seeds). This type of variation is similar to that reported in genotypes from a Turkish population (Turchetta et al 1995).

It was not possible to assign LMW-GS allele classification to all accessions as their patterns did not fit the scheme described by Nieto-Taladriz et al (1997). A total of 22 different LMW-GS patterns were found in this small group of 32 accessions. Such diversity in LMW-GS has also been reported previously in a much larger set of wild tetraploids (Liu and Shepherd 1996). The most common allelic groups in the accessions were caa and aaa. Several of the accessions had the *Glu-A3* b allele, encoding subunit 5 and less frequent was the *Glu-B3* h allele encoding subunits 1, 3, 14, and 18. The *Glu-B3* a allele was the most common (encoding subunits 2, 4, 15, 19). This is the case in cultivated durum where selection pressure toward good pasta-making quality occurs, typically in a narrow genetic background (Nieto-Taladriz et al 1997). There were seven accessions with subunit patterns that did not fit the allelic designation devised by Nieto-Taladriz.

Information about the size-distribution of the glutenin polymers provides a guide to the gluten strength (Southan and MacRitchie 1999). For bread wheat gluten, there is a good correlation between the polymeric-to-monomeric ratio (P/M) and gluten strength as measured by an extensigraph. The mean values for the cultivar checks for P/M and the glutenin-to-gliadin ratio (Glu/Gli) were higher than the mean of the accessions (Table I). Nearly all the accessions had weaker dough strength compared with the cultivar checks (Sissons and Hare 2002) which is in agreement with the highly significant correlation ($P < 0.01$) between P/M, Glu/Gli ratios, and dough strength tests (Table III). For example, the correlations between Glu/Gli and farinograph breakdown, $r = -0.57$; extensigraph R_{max} , $r = 0.52$. Only accessions AUS22342A, 17294, and 18712A, B had P/M and Glu/Gli ratios similar to the cultivar checks. These accessions showed correspondingly strong and stable dough properties comparable to the cultivar checks. The P/M and Glu/Gli ratios varied in absolute value between the two seasons, with lower ratios for the 1999 season. This is likely to be due to a change in the amount of glutenin and gliadin formed during grain filling due to seasonal influences, thereby affecting their ratios (Panozzo et al 2001). Research with hexaploid wheat has shown that a more useful parameter relating dough strength, in particular, extensigraph R_{max} , with glutenin size distribution is the percentage of unextractable polymeric glutenin of the total glutenin (%UPP) (Southan and MacRitchie 1999). The %UPP is highly correlated to P/M and Glu/Gli ratios and to technological quality in this set of samples (Table III). Correlations between %UPP and farinograph dough development time, $r = 0.81$; farinograph breakdown after 5 min, $r = -0.79$; R_{max} , $r = 0.61$, and mixograph peak time are all larger than the correlations with P/M and Glu/Gli. This parameter appears to be a better indicator of dough strength in these tetraploid samples. Other research with durum wheat supports this (Gianibelli et al 1995). Strong gluten cultivars like Wollaroi and Yallaroi have high %UPP, whereas the weaker Kamilaroi cultivar has a much lower amount of %UPP. Several of the accessions had even lower levels of %UPP than Kamilaroi. In general, those accessions with %UPP < 20 have weak dough properties (mean values for FDDT 2.1, FB10 174, R_{max} 155, MPT 1.9, width at peak 10.4) (Sissons and Hare 2002). Stronger durum dough is characterized by longer FDDT and MPT, large R_{max} , and low FB10 and large values for mixograph width at peak. On average by contrast, accessions with %UPP > 30 have superior dough strength close to that of the cultivated durum wheats (mean values for FDDT 2.6, FB10 104, R_{max} 235, MPT 2.8, width at peak 13.3). Accessions with %UPP of 20–30 also have poor dough strength but intermediate between the above two groups (mean values for FDDT

TABLE II
Electrophoretic Data on Endosperm Proteins Extracted
from Tetraploid Wheat Accessions and Commercial Cultivars

Cultivar/ Accession	Electrophoretic Analysis of Whole Meal Proteins			
	Gliadin	HMW-GS	LMW-GS	Allele ^a
Wollaroi	45	7+8	2,4,6,10,12,15,19	caa
Kamilaroi	45	20	2,4,6,10,12,15,19	caa
Yallaroi	45	7+16	2,4,6,10,12,15,19	caa
AUS285	43.5	1,2+12,18	5,7,8,12,15,19	
AUS297	43.5	2*,7+16	1,3,6,14,18	ahb
AUS7812A	45	2*,7+8	2,4,14,15,19	hcb
AUS7829	47	20,8	2,4,6,10,12,15,17,19	cda
AUS7842	45	20,8	2,4,15,19	hab
AUS7922	45	13+16	2,4,11,12,15,16	ega
AUS16041	47	7+8	2,4,14,15,18,19	
AUS17045	45	6+8	2,4,12,15,19	haa
AUS17294	45	6+8	2,4,6,10,12,15,19	caa
AUS20677A	45	6+8	2,4,6,10,12,15,19	caa
AUS20677B	45	6+8	2,4,6,10,12,15,19	caa
AUS22300	45	2*,7+8	5,8,9,16,18	
AUS22303A	45	2**,6+8	1,3,12,14,18	eha
AUS3549A	47	20	3,5,16,18	
AUS3830	45	7+8	5,12,15,19,20	
AUS3838	42	7+8	2,4,6,15,19	aab
AUS3824	45	20	2,4,6,15,19	aaa
AUS3917	43.5, 47	7+8	5,12,15,19	
AUS4049	43.5, 47	15+16	2,4,11,15,16	egb
AUS5533	45	20	2,4,6,15,19	aab
AUS16133	45	20	2,4,6,11,15,19	dab
AUS22342A	45	20	2,4,6,15,19	aab
AUS22342B	45	1,15+16	2,4,6,15,19	aab
AUS3603	45	2*,17+18	2,4,6,14,15,19	acb
AUS15914	45	2*,17+18	2,4,6,15,19	aab
AUS18721A	45	7+8	2,4,6,10,12,15,19	caa
AUS18721B	nd	7+8	2,4,6,10,12,15,19	caa
AUS7810	42	7+16	3,7,8,13,14,18	
AUS7812B	45	2*,7+8	2,4,11,14,15,19	ecb
AUS13539	42	7+16	5,7,8,12,14,18	bia
AUS15198	42	7+16	5,7,8,12,14,18	bia
AUS17210	42	7+16	5,7,8,12,14,18	bia

^a LMW-GS allele designation using nomenclature of Nieto-Taladriz et al (1997).

2.1, FB10 126, R_{max} 207, MPT 2.6, width at peak 13.1). The %UPP value is therefore quite a useful indicator of dough strength but the absolute value for a particular genotype is influenced by environment.

SE-HPLC data and glutenin patterns are related because gluten strength is dependent on the type of subunits and the molecular weight distribution of the polymers (Southan and MacRitchie 1999). Accessions AUS 7812A, B, 7922, 3549A, 3838, 3917, 4049, and 3603 all had %UPP < 20 (in 1997), poor dough strength (Sissons and Hare 2002), and LMW-GS allele patterns that were not either caa or aaa. These two alleles are associated with strong gluten strength in durum wheats (Ames et al 1998).

Starch Paste Viscosity (RVA)

In both years, the paste viscosities of semolina (measured in the presence of silver nitrate to inhibit α -amylase action) and isolated starch had ranges greater than the values observed for the cultivar checks (Tables IV and V). Values for semolina were higher in the second growing year, although values for the starch were lower. The contribution of protein to the viscosity may well explain this. Greater variability in the viscosity profiles was also observed in

the accessions. For example, some accessions had low PV but high FV in contrast to others with high PV and FV. The paste viscosity of the isolated starch was higher than for the semolina in the 1997 samples but the opposite was true in 1999. Properties such as a high PV and breakdown, low setback, and FV are related to good textural quality in white salted noodles (Konik et al 1992). In this type of noodle, softness is considered a desirable attribute, while a firmer texture is required for pasta. The relationship of starch paste viscosity to pasta quality is not known. A survey of the variation in a diverse set of tetraploid wheat species provides an insight into the possible range in paste viscosities. This information, together with knowledge obtained about the technological properties of the pasta (Sissons and Hare 2002), may help to provide information on the relationships. Different environmental conditions during grain filling will alter the starch composition and fine structure (Zeng et al 1997), which would affect RVA profiles. Therefore, it is important when making comparisons of different samples that they be grown under the same field conditions in a correctly designed experiment. Interactions between starch and protein, as well as a contribution from the protein itself, affect pasting viscosity (Morris et al 1997; Batey 2000) and changes in both components due to

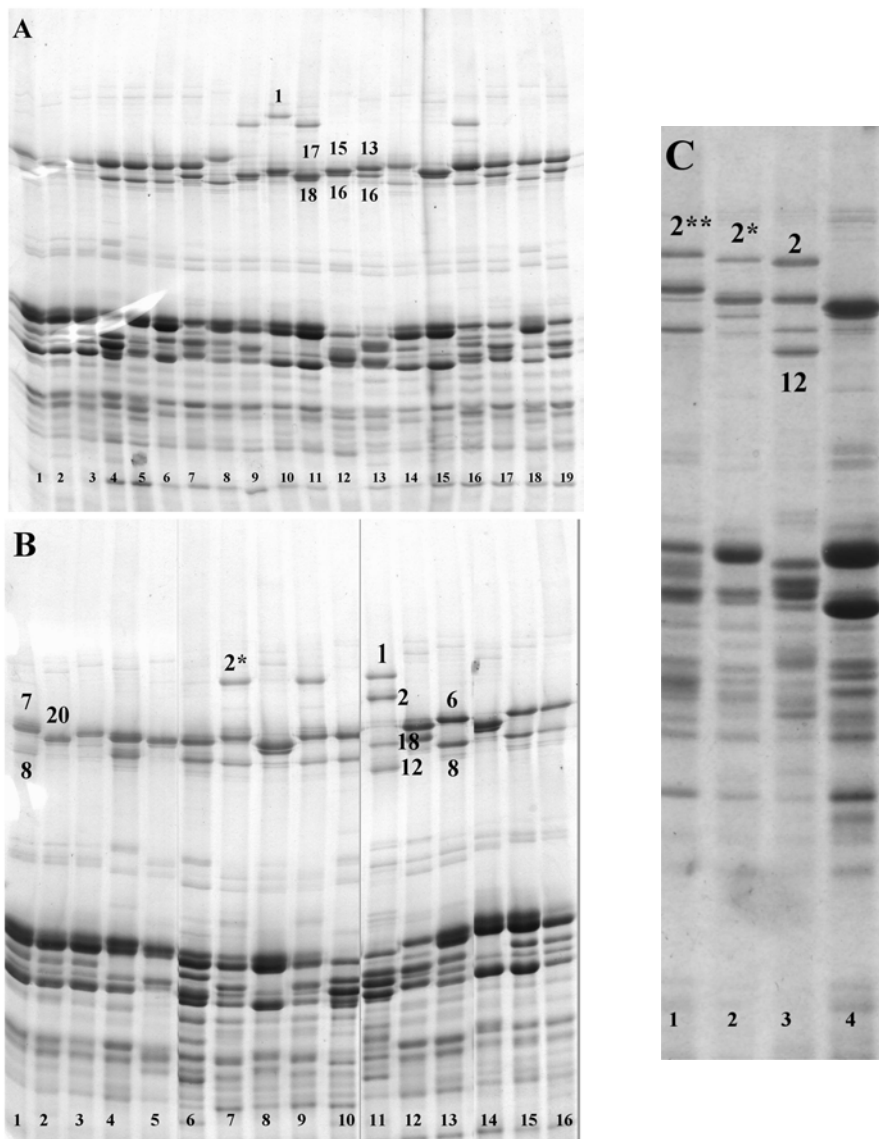


Fig. 1. SDS-PAGE of glutenins extracted from 32 accessions of tetraploid wheats compared with those of durum wheat cultivars. **A** (lanes 1–19) Wollaroi, Kamilaroi, Yallaroi, AUS 3830, 16041, 18721A, 17210, 20677B, 3603, 22342B, 15914, 4049, 7922, 18721B, 16133, 22300, 13539, 3838, and 15198. **B** (lanes 1–16) Wollaroi, Kamilaroi, Yallaroi, AUS 7842, 3549A, 7829, 7812B, 5533, 7812A, 3917, 285, 7810, 20677A, 3824, 17294, 17045. **C** (lanes 1–4) 22303A, 297, Chinese Spring, 22342A.

environmental conditions can explain this observation. When wheat glutes from a range of cultivars are added to a wheat starch, the viscosity is affected differently by each one (Batey et al 1998). In our samples, the interaction of the starch and gluten will be more complex, as both the starch and protein will differ between samples, and the protein content, and perhaps the proportions of the protein components, will vary between years. The environmental effect on the starch granules also cannot be overlooked. There were stronger intercorrelations between the RVA parameters for the isolated starch compared with semolina. The PV and breakdown using semolina or isolated starch were highly intercorrelated but poorly intercorrelated with FV and setback. This means that the samples did not rank the same in terms of FV or setback using semolina or isolated starch as starting material. The question of whether any of the RVA parameters are related to technological quality described previously (Sissons and Hare 2002) is important to understand the value of collecting RVA data. Apart from two weak negative correlations between grain protein and semolina FV and setback, the highest correlation (negative) was with pasta cooking loss (Table VII). Significant but small negative correlations were between cooking loss (CL) of pasta and PV (starch), $r = -0.61$ and breakdown (semolina) $r = -0.57$. Low cooking loss is a desirable feature of good quality pasta. High values for breakdown are usually correlated with high peak viscosity, which in turn is associated with the degree of swelling of the starch granule on heating. More granules with a high swelling capacity results in a higher peak viscosity. With more swelling of the granules, the tendency

is greater to leach their contents into the surrounding liquid. With cooking of pasta, this would be expected to lead to an increase in cooking loss, which is usually measured as the intensity of the blue color with iodine. The observation of a negative correlation between PV and CL contradicts this, suggesting that factors other than starch properties are affecting the cooking loss. Given the importance of protein properties in pasta quality, it is likely that it is the protein matrix that is trapping the amylose as the granules swell. In parallel, in Japanese salted noodles, a high PV may also give a softer and therefore less desirable pasta product (Batey et al 1997a). Interestingly, there was a poor correlation between PV and FV for RVA on semolina but a highly significant correlation for RVA on isolated starch (Table VI). Once again, this probably reflects the contribution of the protein. In this data set, there were no significant correlations between the RVA data and cooked pasta stickiness. This may be related to the influence of the large variation in protein content and composition in this set of samples. In another study (unpublished) we have noted a high correlation between stickiness and setback and breakdown in samples of identical protein composition and similar protein content. Other research has shown that starch does affect cooked pasta texture and there were relationships between RVA and texture (Gianibelli et al 2000a).

Amylose Content

The major components of starch are amylose and amylopectin. There were differences in amylose content for the same genotypes

TABLE III
Simple Correlations (r) Between Quality Data^a and Protein Properties of Tetraploid Wheat Accessions and Commercial Cultivars^b

	GP%	FDDT	FB10	R _{max}	MPT Yr 1	MPT Yr 2	WatP Yr 1	WatP Yr 2	P/M	Glu/Gli
P/M	-0.33	0.47	-0.61	0.56	0.75	0.17	0.73	0.29		
Glu/Gli	-0.40	0.48	-0.57	0.52	0.73	0.17	0.73	0.21	0.97	
%UPP	-0.37	0.81	-0.79	0.61	0.77	0.37		0.52	0.64	0.60

^a Sissons and Hare (2002).

^b Only significant correlations ($P < 0.05$) are shown. GP%, grain protein percentage; FDDT, farinograph dough development time; FB10, farinograph breakdown after 10 min; R_{max}, extensigraph maximum resistance; MPT, mixograph peak development time, WatP, mixograph width of curve at peak mix time; P/M, polymeric/monomeric ratio, Glu/Gli, glutenin/gliadin ratio, %UPP, percentage of unextractable polymeric protein.

TABLE IV
Rapid Visco Analyser Measurements (RVU) of Semolina (in presence of silver nitrate) from Tetraploid Wheat Accessions and Commercial Cultivars^a

Cultivar	1997 Season				1999 Season			
	PV	BD	FV	Setback	PV	BD	FV	Setback
Wollaroi	198	64	288	154	248	59	380	191
Kamilaroi	200	61	306	167	225	58	343	176
Yallaroi					275	92	373	191
Mean	199	62	297	161	249	70	366	186
Accessions								
Mean	208	81	271	144	270	104	341	175
Min	145	26	211	117	222	43	281	140
Max	257	123	319	172	318	146	379	198
LSD	8.9	7.5	7.7	5.2	13.6	9.8	14.5	8.1

^a RVU, Rapid Visco Analyser units; PV, peak viscosity; BD, breakdown; FV, final viscosity.

TABLE V
Rapid Visco Analyser Measurements (RVU) of Purified Starch from Tetraploid Wheat Accessions and Commercial Cultivars^a

Cultivar	1997 Season				1999 Season			
	PV	BD	FV	Setback	PV	BD	FV	Setback
Wollaroi	222	93	322	193	151	13	263	125
Kamilaroi	199	76	314	191	138	9	240	111
Yallaroi					183	19	306	142
Mean	211	85	318	192	157	14	270	126
Accessions								
Mean	250	108	340	197	208	31	332	153
Min	184	50	267	154	133	-3	232	107
Max	306	159	422	253	286	113	445	197
LSD	4.2	2.3	16.7	12.5	5.9	3.9	11.1	8.1

^a RVU, Rapid Visco Analyser units; PV, peak viscosity; BD, breakdown; FV, final viscosity.

between the two years with values generally lower in the 1999 season (Table VII), reflecting the influence of environment on the starch composition during grain filling (Batey et al 1997b). Some of these differences were quite large, as with AUS16041, Wollaroi and AUS20677A (1997/1999 AUS16041 22.1/15.1%; Wollaroi 22.8/15.5%; AUS20677A 21.6/14.6%). Most of the accessions had amylose contents similar to the cultivar checks. The accession AUS3830 could be a partial waxy because its amylose content was $\approx 3\%$ lower than the mean for the cultivar checks. A 5% reduction in the amylose content was found in partial waxy null-4A durum wheats (Sharma et al 2002). This result is similar to a survey of Australian durum breeding lines (Batey, unpublished results) but slightly narrower than for nonwaxy hexaploid wheat, 19–27% (Batey et al 1997b). There was a weak but significant negative correlation between semolina PV, breakdown, and amylose content (Table VI), consistent with reports for durum (Sharma et al 2002). This agrees with the observations for hexaploid wheat (Zhao et al 1998). There were no other significant correlations with other quality data.

Starch Gelatinization (DSC)

The differential scanning calorimeter measures heat flow as a function of temperature. When starch is heated in excess water (water-to-starch, 2:1), a sharp endothermic peak is obtained, the start of which corresponds to the start of birefringence loss. The area under the curve is a measure of the energy (enthalpy, ΔH) required for the transition from an ordered to a disordered state (i.e., for the crystalline area to melt). The end point of the loss of birefringence and the end of the peak correlate well (Hoseney 1986). Under the conditions used in the present report, a water-to-starch ratio of 2:1 was used in the DSC analysis. In this situation, a bi-phasic endotherm occurs. Thus, the first endotherm corresponds to the gelatinization of the starch granule and the second to the melting point of the amylose-lipid complex (Hoseney 1986). In semolina starch, a sharp, narrow, well-defined peak was observed at $\approx 60^\circ\text{C}$ and a smaller, broad peak at 103°C (data not shown), which were assigned to the gelatinization and the dissociation of the lipid-amylose complex, respectively. Transition temperatures (onset, T_o ; peak, T_p ; completion, T_c) and enthalpies for the gelatinization behavior of the starch are summarized in Table VIII. The overall temperature range for gelatinization from T_o to T_c for all samples ranged from 50 to 70°C . This variation is greater than that reported for a survey of durum wheat cultivars from three different countries (Gianibelli et al 2000b) but comparable to that reported for bread wheat, except for a narrower range in gelatinization enthalpy in this study (Bhattacharya and Corke 1996).

There was minimal variation in values for all DSC gelatinization parameters between the two years or between the cultivar checks, indicating the independence of DSC parameters with environmental effects on starch. The gelatinization temperature (GT) range for the accessions was greater than for the cultivar checks with many differing significantly from the cultivar checks for all four DSC gelatinization parameters measured. For example, four accessions had significantly lower onset values (1997 data) and 17 had significantly higher values than the mean of the cultivar checks (Table VIII). In the 1997 season only, three of the four *turanicum* accessions had the lowest T_o and T_c while the *persicum* group was at the highest end of the range (Table VIII). The four DSC parameters were all highly intercorrelated (Table VI). The onset and end temperatures were significantly correlated with semolina RVA breakdown, setback, and FV. There was a significant but small negative correlation with CL% (Table VI), indicating that higher gelatinization temperatures are associated with lower cooking loss. The end of gelatinization temperature was well correlated to RVA breakdown and FV so that starch achieving a higher final viscosity achieves a higher end gelatinization temperature. A weak but significant negative correlation between amylose content and T_c was found. The reason for the low correlation in this study is probably because of the narrow range in amylose contents found. Other research using reconstitution techniques has shown that decreasing the content of amylose in durum starch from the normal 30 to 0%, increased T_p and T_c (Gianibelli et al 2000a). Low amylose content was associated with a stickier and less firm pasta, suggesting that reduced gelatinization peak and completion temperatures may improve pasta stickiness. Because common wheat starches gelatinize at higher temperatures than durum wheat starches (Vansteelandt and Delcour 1999), this may partly explain why pasta made from common wheat

TABLE VII
Amylose Contents of Tetraploid Wheat Accessions and Commercial Cultivars

Cultivar	% Amylose	
	1997 Season	1999 Season
Wollaroi	22.8	15.5
Kamilaroi	21.7	18.8
Yallaroi		16.6
Mean	22.3	17.0
Accessions		
Mean	21.1	17.2
Min	17.8	13.5
Max	23.8	21.7
LSD		3.2

TABLE VI
Simple Correlations (r) Between Quality Data^a and Starch Properties of Tetraploid Wheat Accessions and Commercial Cultivars^b

	GP%	CL%	PV-s	B-s	FV-s	Setb-s	PV-st	B-st	FV-st	T_o	T_c	T_p
RVA												
PV-s		-0.38										
Breakdown-s		-0.57	0.83									
FV-s	-0.49	0.40		-0.33								
Setback-s	-0.56	0.48		-0.42	0.98							
PV-st	0.33	-0.61	0.62	0.76		-0.34						
Breakdown-st		-0.45		0.63	-0.58	-0.59	0.74					
FV-st		-0.45	0.56	0.51			0.87	0.40				
Setback-st							0.75	0.56	0.83			
DSC												
T_o	0.59	-0.55		0.55	-0.60	-0.64	0.47	0.38	0.32			
T_c	0.39	-0.54		0.55	-0.56	-0.59	0.37			0.74		
T_p					-0.41	-0.40				0.65	0.58	
ΔH				0.38	0.43					0.57	0.44	0.43
% Amylose		0.37	-0.58	-0.47							-0.39	

^a Sissons and Hare (2002).

^b Only significant correlations ($P < 0.05$) are shown. GP%, grain protein percentage; CL%, cooking loss percentage of initial pasta weight; RVA PV, peak viscosity; B, breakdown; FV, final viscosity and setback of semolina (-s) and starch (-st), respectively; T_o , onset temperature of gelatinization, T_c , completion temperature of gelatinization; T_p , peak temperature of gelatinization; ΔH , enthalpy change.

TABLE VIII
Differential Scanning Calorimetry Measurements (DSC), Onset (T_o), Peak (T_p), and Completion (T_c) Temperatures and Gelatinization Enthalpies (ΔH) of Starch Isolated from Tetraploid Wheat Accessions and Commercial Cultivars

Cultivar/Accession	Gelatinization Parameters								
	Subspecies	1997 Season				1999 Season			
		T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o	T_p	T_c	ΔH
Wollaroi	<i>durum</i>	52.5	59.1	64.9	3.2	50.7	61.3	65.5	3.8
Kamilaroi	<i>durum</i>	51.6	58.3	64.0	3.1	49.9	60.1	66.4	3.3
Yallaroi	<i>durum</i>					49.7	58.1	66.0	3.5
Mean		52.1	58.7	64.5	3.2	50.1	59.8	66.0	3.5
AUS285	<i>durum</i>	54.3	59.9	65.6	3.7	54.0	57.7	68.1	3.8
AUS297	<i>durum</i>	52.0	59.2	67.6	4.2	51.6	58.0	66.1	4.5
AUS7812A	<i>durum</i>	55.3	60.6	66.4	3.4	53.2	57.5	66.7	4.1
AUS7829	<i>durum</i>	51.2	58.2	66.4	2.7	53.6	57.8	67.9	3.5
AUS7842	<i>durum</i>	53.9	59.1	66.2	3.4	54.6	63.0	68.0	3.9
AUS7922	<i>durum</i>	53.9	60.2	68.5	3.4	55.0	61.0	68.6	4.3
AUS16041	<i>durum</i>	53.2	59.5	65.6	3.2	51.6	58.3	63.6	3.6
AUS17045	<i>durum</i>	55.2	60.1	64.6	3.3	56.4	59.4	67.0	4.9
AUS17294	<i>durum</i>	51.9	58.8	66.1	3.1	51.5	58.2	65.6	4.0
AUS20677A	<i>durum</i>	51.7	59.1	64.5	3.0	52.8	58.4	66.3	3.7
AUS20677B	<i>durum</i>	51.6	58.6	65.7	3.3	53.3	59.0	65.4	4.1
AUS22300	<i>durum</i>	54.1	60.0	68.0	3.4	55.4	60.5	68.0	4.4
AUS22303A	<i>durum</i>	53.9	60.5	68.5	3.3				
AUS3549A	<i>persicum</i>	52.8	58.9	66.5	3.2	52.3	60.4	68.3	3.5
AUS3830	<i>persicum</i>	55.8	61.9	68.8	3.3	57.3	59.2	69.3	4.6
AUS3838	<i>persicum</i>	53.6	60.4	69.6	3.6	55.8	59.6	70.9	3.9
AUS3824	<i>polonicum</i>	53.7	60.0	65.8	3.1	53.4	59.5	67.5	4.1
AUS3917	<i>polonicum</i>	54.1	60.3	68.8	3.2	55.0	59.9	68.2	4.2
AUS4049	<i>polonicum</i>	52.7	59.6	66.0	3.1	52.7	59.8	66.7	4.5
AUS5533	<i>polonicum</i>	53.5	59.8	66.5	3.3	53.4	57.2	67.2	3.8
AUS16133	<i>polonicum</i>	53.6	58.9	67.5	2.9	53.0	58.2	67.2	4.2
AUS22342A	<i>polonicum</i>	52.7	59.2	67.2	2.9	53.9	57.9	66.7	4.2
AUS22342B	<i>polonicum</i>	54.4	60.5	68.4	3.2	54.3	58.3	67.4	3.9
AUS3603	<i>R turgidum</i>	52.3	58.1	66.0	3.3	53.6	60.5	66.7	3.8
AUS15914	<i>R turgidum</i>	52.0	58.3	65.3	3.1				
AUS18721A	<i>R turgidum</i>	54.1	59.4	65.2	3.3	52.2	60.9	65.5	4.1
AUS18721B	<i>R turgidum</i>	51.8	58.1	64.6	3.1	50.3	58.8	63.6	4.0
AUS7810	<i>turanicum</i>	50.5	57.4	63.8	3.2				
AUS7812B	<i>turanicum</i>	55.1	60.7	67.0	3.6	54.6	57.1	68.1	4.0
AUS13539	<i>turanicum</i>	50.8	57.5	64.2	2.9	52.4	59.4	65.9	3.6
AUS15198	<i>turanicum</i>	51.1	57.4	64.4	3.1	50.8	60.2	65.0	3.6
AUS17210	<i>turanicum</i>	50.2	57.0	61.7	3.1	51.8	59.4	64.7	3.3
Mean		53.0	59.3	66.3	3.3	53.4	59.1	66.9	4.0
Min		50.2	57.0	61.7	2.7	50.3	57.1	63.6	3.3
Max		55.8	61.9	69.6	4.2	57.3	63.0	70.9	4.9
LSD		0.9	0.7	1.3	0.8	0.8	0.9	1.7	0.9

is stickier than when durum wheat is used. More investigation is needed to determine whether DSC measurements on durum starch have any value in a breeding program to produce wheats with unique starch properties.

The effects of protein on the quality are significant and override any effect of the variation in starch properties. To determine any benefit that might arise from a wider diversity in starch, reconstitution experiments need to be conducted with a common protein base (Sissons et al 2002).

CONCLUSIONS

A much greater diversity in protein composition, glutenin size distribution, paste viscosities, and gelatinization properties were found in the accessions compared with the cultivated wheats. The %UPP correlated well with dough strength and would be a good predictor of strength for screening larger populations of tetraploids in the search for higher gluten strength. In this set, most of the accessions had lower gluten strength, although several had low firmness and gluten elasticity that may suit specific applications. Those accessions all had a low %UPP and so this biochemical marker may prove useful for selecting these characteristics too. High RVA peak viscosity and breakdown were associated with lower cooking loss, a positive benefit for good pasta quality. With this set of samples whose protein content and composition varied

substantially, it is not possible to determine which starch characteristics are useful as predictors of good pasta quality. A set of samples differing in quality but at a constant protein content and composition and with varying starch characteristics is needed to identify the value of specific starch characteristics. Wild wheat accessions have been identified as valuable sources of genes for traits such as disease resistance, morphology, yield improvement, and agronomic properties (Valkoun 2001). Identification of tetraploid relatives with one or more of the above advantages with desirable quality attributes may simplify the release of new cultivars with the correct quality.

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