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Composition of polymeric proteins and bread-making quality of wheat lines with allelic HMW-GS differing in number of cysteines

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Abstract

Three lines of Australian wheat variety Avocet, two biotypes of Australian variety Halberd, and the Italian bread wheat cultivar Fiorello and its derived line were used to study the possible role of the *Glu-B1* HMW-GS in polymeric protein composition and flour functionality. These sets of lines contain HMW-GS 7 + 8, 7 + 9, 20x + 20y or 26 + 27. Subunit Bx7 has four cysteine residues, while subunit Bx20 has only two. Compared to Avocet A (7 + 8), line Avocet C (20x + 20y) exhibited an increase in polymeric protein, a decrease in the gliadin-to-glutenin ratio, and a marked decrease in unextractable polymeric protein (UPP). Avocet C doughs exhibited greater extensibility and shorter Mixograph dough development times, and baked into smaller loaves compared to those from Avocet A. Similar differences were observed in comparisons between the two Fiorello lines and between the two Halberd biotypes that had differing *Glu-B1* alleles. These results show that in closely related genotypes, the presence of the Bx7 + By8 or Bx7 + By9 HMW-GS instead of Bx20 + By20 or Bx26 + By27 is associated with higher UPP, higher dough strength and better bread-making performance. It is suggested that this could be due to the greater number of cysteines in the Bx7 subunit that are available for forming higher-molecular-weight glutenin polymer. This could give the possibility to manipulate dough properties and bread-making quality by utilizing HMW-GS alleles with varying numbers of cysteine residues.

Keywords: Wheat; Glutenin polymers; Cysteine residue; Flour functionality

1. Introduction

Allelic variation at *Glu-1* loci, containing genes encoding HMW-GS, has been shown to largely contribute to differences in bread-making quality (Payne, 1987; Kasarda, 1989). Each *Glu-1* locus has been shown to contain two tightly linked genes, one encoding a HMW-GS of high M_r designated x-type and the other a subunit of lower M_r designated y-type. Correlative studies have shown the relative importance of certain subunits compared to others. Qualitative effects have been related to the number of HMW-GS present in a given cultivar or structural differences existing between them.

The number and position of cysteine residues available for intermolecular bonds have been suggested to be responsible, to a certain extent, for qualitative differences of different allelic subunits (Bietz, 1987) through their effect on the amount of large-sized glutenin polymer (Weegels et al., 1996).

One cysteine residue is generally present in the C-terminal domains of all of the subunits, whereas a single-cysteine residue is present within the repetitive domains of all y-type subunits. The subunit 1Dx5 contains an additional cysteine residue in the first repeat block adjacent to the N-terminal domain (Anderson and Green, 1989; Kasarda, 1989). Amino acid substitutions have resulted in the replacement of cysteines by tyrosines in

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a few Bx-type subunits (1Bx20, 1Bx14 and 1Bx26) (Tatham et al., 1991; Buonocore et al., 1996a; Margiotta et al., 2000; Li et al., 2004).

The role of the number of cysteine residues on dough properties has been addressed in a few studies. Gupta and MacRitchie (1994) used a series of recombinant inbred lines and biotypes differing in their allelic composition at the *Glu-B1* locus (1Bx17 + 1By18 vs. 1Bx20 + 1By20) or at the *Glu-D1* locus (1Dx5 + 1Dy10 vs. 1Dx2 + 1Dy12). They demonstrated that superiority of subunits 1Bx17 + 1By18 and 1Dx5 + 1Dy10 was associated with the production of greater amounts of large-sized glutenin polymers. These differences were postulated to be associated with the different number of cysteine residues existing between 1Dx5 (five) and 1Dx2 (four) and between subunit 1Bx17 (four) and 1Bx20 (two).

Previous work with some wheat lines and their biotypes confirmed that the number of cysteine residues affects the size of glutenin polymers and mixing properties (Margiotta et al., 2000). The present study aimed to extend the previous work in assessing the role of the number of cysteine residues present in subunits encoded at the *Glu-B1* locus, on the protein composition and flour functionality.

2. Experimental

2.1. Materials

The material used included wheat lines and biotypes of two Australian varieties, *Avocet*, and *Halberd*. These were natural mutants differing in HMW-GS at *Glu-B1*, which were selected using SDS–polyacrylamide gel electrophoresis (PAGE) and multiplied as described by Lawrence (1986) and Lawrence et al. (1987). The other pair of lines was that of the Italian bread wheat cultivar *Fiorello* and its derived line in which the pair of subunits 7 + 8, present at the *Glu-B1* locus, was replaced by the pair of subunits 26 + 27 present in the bread wheat cultivar Cologna (Pogna et al., 1989).

This line was obtained by crossing Fiorello and Cologna and backcrossing the F1 generation with Fiorello. The seeds obtained from the first backcross were analyzed by SDS–PAGE and those possessing the pair of subunits 26 + 27 were crossed again with Fiorello. After three backcrosses, the seeds were taken to the F5 generation and then planted in Viterbo. The BC3F6 seeds were used for all the analyses. Wheat samples were harvested in the 2000 crop year, and three field replicates were provided for each sample. The HMW-GS of the lines are shown in Table 1.

2.2. Methods

2.2.1. Protein composition and quality tests

The protein content was determined by the nitrogen combustion method (LECO). Total nitrogen was converted to protein using *N* factor 5.7. One-dimensional SDS–PAGE was used to characterize the glutenin protein subunits. The procedure of Laemmli (1970) was used for sample and gel preparation, slightly modified to use 10% acrylamide separating gels. Loading samples were prepared with 50 mg of flour in approximately 1 mL of reducing sample buffer. Sample loading volume was approximately 6 μL.

SE-HPLC was carried out using an 1100 Hewlett Packard chromatography station with a UV detector set at 214 nm. Protein extracts (20 μL) were injected into a Biosep-SEC-S4000 size-exclusion column, with temperature controlled at 40 °C. A 1:1 mixture of 0.05% trifluoroacetic acid (TFA) in acetonitrile and 0.05% TFA in deionized water was used as eluting solvent. Extraction of total protein, and extractable and unextractable fractions followed the method described by Gupta et al. (1993), modified to use 15 and 25 s sonication for total and unextractable fractions, respectively. The percentage of total extracted protein that was present in the polymeric form (PPP), and percentage of unextractable polymeric protein in the total polymeric protein (UPP) were calculated according to Gupta et al. (1993). Gliadin-to-glutenin ratio was also determined, to estimate the molecular weight distribution (MWD) of the flour.

The mixing characteristics of the samples were assessed with a computerized 10-g Mixograph, following AACC method 54-40A (American Association of Cereal Chemists, 2000), modified according to Gupta et al. (1993). This method uses a constant ratio of NaCl solution to flour (60 ml solution/100 g flour at 14% mb with 2 g NaCl/100 g flour). The inclusion of NaCl was considered to be more suitable than the straight AACC procedure as it gives good dough handling properties and is more relevant to bakery procedure. In regard to the effect of flour protein content on water absorption as allowed for in the AACC method, the critical comparisons of Mixograph data are between biotypes of each individual variety and these are seen to have protein contents that are close to each other (Table 1). Extensional properties of the dough were evaluated by a TAXT-2 Texture Analyzer, equipped with a Kieffer dough and gluten extensibility rig, according to the method described by Suchy et al. (2000), modified to the use of a 10-g Mixograph. Baking test procedure was based on the standard no-time micro-baking test, as described by Pirozi (2003).

2.3. Statistical analysis

A randomized complete block design was used for Mixograph and extension tests. A completely randomized design was used for baking test, protein content and SE-HPLC data. All data was analyzed using the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Pairwise comparisons were made using Fisher's LSD. Some explanation of the relatively large LSD values in the flour protein determinations seems needed. Each grain sample had three field replicates. Replicates by LECO between

individual samples were very close (±0.2%) but the LSD values given in Table 1 were calculated for the field replicates.

3. Results and discussion

3.1. Protein composition of lines

The wheat lines evaluated in this study possess the *Glu-B1* HMW-GS subunits 7+8 and 7+9, or the equivalent 20x+20y and 26+27, respectively. The two former pairs of subunits are known to provide a higher number of cysteine residues than the two latter, due to the differences existing between the Bx type subunits. Gene sequences of subunits 8 and 9 are present in databases and confirm that each has the same number of cysteine residues. It may be expected, therefore, that the lines containing the pairs of subunits 7+8 or 7+9 would exhibit higher UPP and stronger properties, compared to their corresponding biotypes.

SDS–PAGE analyses confirmed the HMW-GS composition for all lines and biotypes (Fig. 1), as stated in Table 1. The Halberd lines appear identical except for the HMW-GS at the *Glu-B1* locus. In the case of Avocet, apart from the differences at the *Glu-B1* locus, some small differences are seen in other proteins in the patterns. In view of the quality differences that have been consistently established between HMW-GS alleles, it seems more probable to tentatively conclude that differences in quality of the Avocet biotypes are due to allelic variation at *Glu-B1*. No electrophoretic differences are detectable in the BC3F6 line obtained from the cross between Fiorello X Cologna and Fiorello itself, except for the presence of the pair of subunits 26+27 in place of the 7+8 associated at the *Glu-B1* locus (compare lanes 1 and 2) present in the latter. In addition, it must be stressed that the bread wheat cultivar Fiorello has been obtained using Cologna as one of the parents and this would make three backcrosses sufficient for the scope of the present work. The protein content and SE-HPLC measurements for all the lines are presented in Table 1. Mixograph, extension and baking test data are recorded in Table 2.

3.2. Avocet lines

Compared to Avocet A, line Avocet C (20x+20y subunits) showed increase in PPP and decrease in gliadin-to-glutenin ratio that were accompanied by a marked decrease in the UPP, in spite of the fact that, compared to the former, the latter possesses an additional HMW-GS (subunit 1 at the *Glu-A1* locus). Similar findings were reported by Gupta and MacRitchie (1994). Buonocore et al. (1996b), through peptide sequencing obtained from subunit Bx20, and Shewry et al. (2003), through DNA sequencing of corresponding gene, have provided evidence

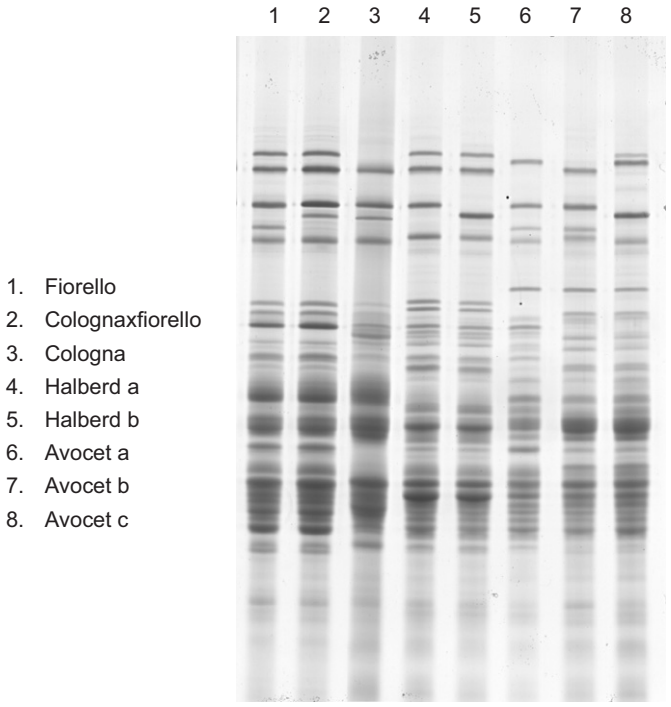

Fig. 1. SDS–PAGE patterns for Fiorello, Halberd and Avocet lines.

Table 1
LECO protein content and SE-HPLC results of Avocet, Fiorello and Halberd lines[1]

Lines and their biotypes	Protein content (14%mb)	SE-HPLC		
		PPP	Gliadin-to-glutenin ratio	UPP
Avocet A null, 7+8, 2+12	9.6c	37.4c	1.32b	33.9c
Avocet B null, 7+8, 5+10	8.9d	39.7a	1.13c	39.3a
Avocet C 1, 20x+20y, 2+12	8.9d	40.2a	1.09c	24.4e
Fiorello 1, 7+8, 5*+12	10.5a	38.6b	1.25b	40.0a
Fiorello 1, 26+27, 5*+12	10.6a	37.5bc	1.32b	28.6d
Halberd 1, 7+9, 5+10	10.2ab	34.3d	1.56a	40.9a
Halberd 1, 20x+20y, 5+10	9.9bc	34.3d	1.56a	36.0b
LSD	0.5	1.1	0.07	2.0

[1]Means followed by different letters are significantly different ($P<0.05$).

Table 2
Results from extension test, Mixograph and baking tests of Avocet, Fiorello and Halberd lines[1]

Lines and their biotypes	Extensibility (mm)	Peak force (g)	MDDT (min)	Loaf volume (cm^3)
Avocet A null, 7+8, 2+12	119.3b	8.4c	3.2c	162.3b
Avocet B null, 7+8, 5+10	90.5c	17.5a	4.9a	187.0a
Avocet C 1, 20x+20y, 2+12	145.9a	8.7c	2.4d	150.7b
Fiorello 1, 7+8, 5*+12	113.1b	13.6bc	3.4bc	184.0a
Fiorello 1, 26+27, 5*+12	154.74	9.3c	2.5cd	168.8b
Halberd 1, 7+9, 5+10	93.2c	15.1b	5.3a	189.3a
Halberd 1, 20x+20y, 5+10	107.6bc	11.7c	4.0b	179.0ab
LSD	17.5	1.9	0.7	12.0

[1]Means followed by different letters are significantly different (*t*-test, $P<0.05$).

that this subunit has only two cysteine residues, one at each of the N- and C-terminal regions of the molecule. This is two less than found more commonly for other subunits such as Bx7.

It is postulated that the lower number of cysteine residues in subunit 20x could influence the polymerization of glutenin subunits causing the MWD to be skewed to lower values than the line with subunits 7+8. Results for mixing and baking properties are consistent with this (Table 2). Even with the extra HMW-GS 1 at *Glu-A1*, the Avocet line C (with HMW-GS 20x+20y) showed weaker dough properties and poorer bread-making performance. Mixograms of all lines are shown in Figs. 2 and 3, and photographs of all baked loaves are shown in Fig. 4.

Comparison between Avocet A and Avocet B confirmed the superiority of the allelic pair 5+10 in the accumulation of polymeric protein and in dough strength and bread-making quality.

3.3. Fiorello and Halberd lines

Similarly to the Avocet lines, decrease in UPP was clearly observed in the Fiorello line carrying subunits 26+27 and in the Halberd line carrying 20x+20y subunits. The effect of the decrease in UPP is observed in all quality tests performed (Table 2 and Figs. 3 and 4), essentially shorter mixing times, lower resistance to extension and lower bread volume. Mixing properties and UPP results in these lines were similar to those reported by Margiotta et al. (2000).

Like subunit 1Bx20, subunit 1Bx26 has been shown to have only two cysteine residues, one in the N-terminal region and the other one in the C-terminal region (Margiotta et al., 2000). Shewry et al. (2003) demonstrated

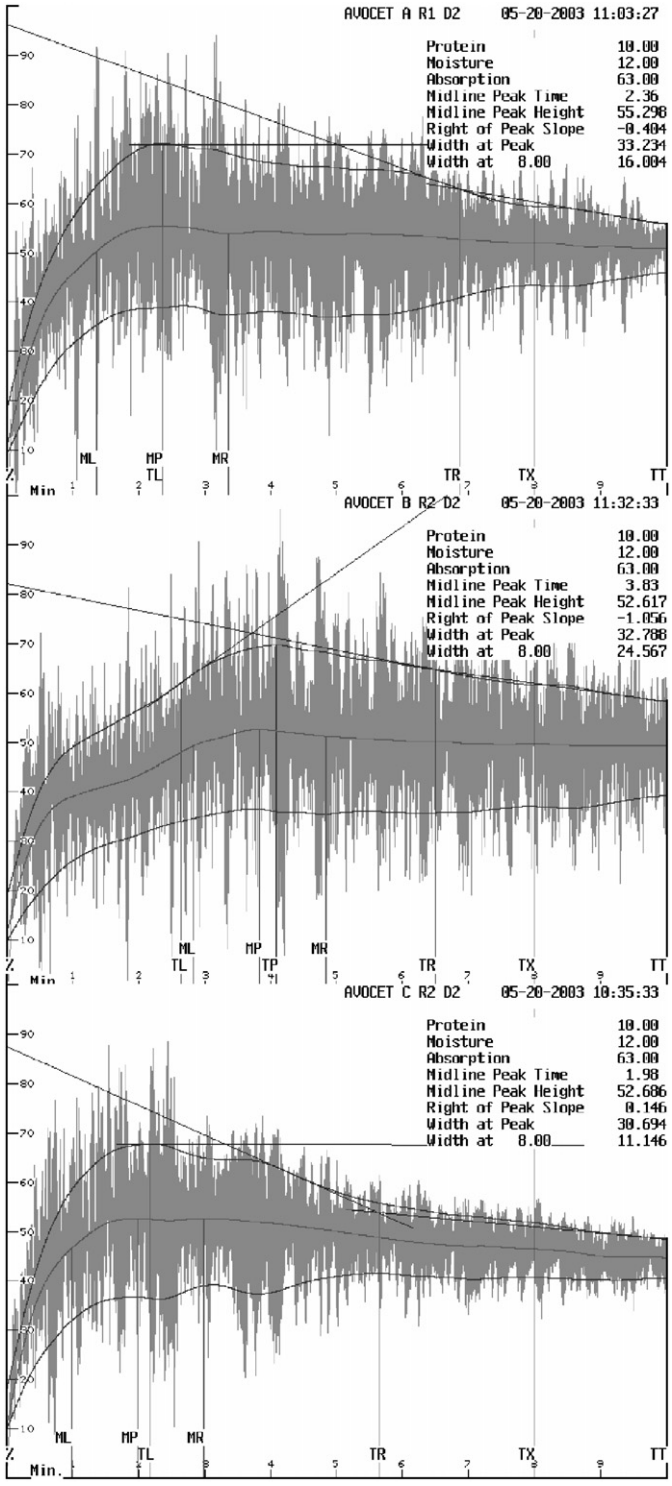

Fig. 2. Mixograms of Avocet lines. From top to bottom: Avocet A, Avocet B, and Avocet C.

that subunit 1Bx20 differs from subunit 1Bx7 by two tyrosine residues that are present in the former in place of the cysteine residues in the latter. The authors concluded that this substitution could affect the pattern of disulfide crosslinks in the glutenin polymers, and negatively impact the dough strength. A similar explanation might be given

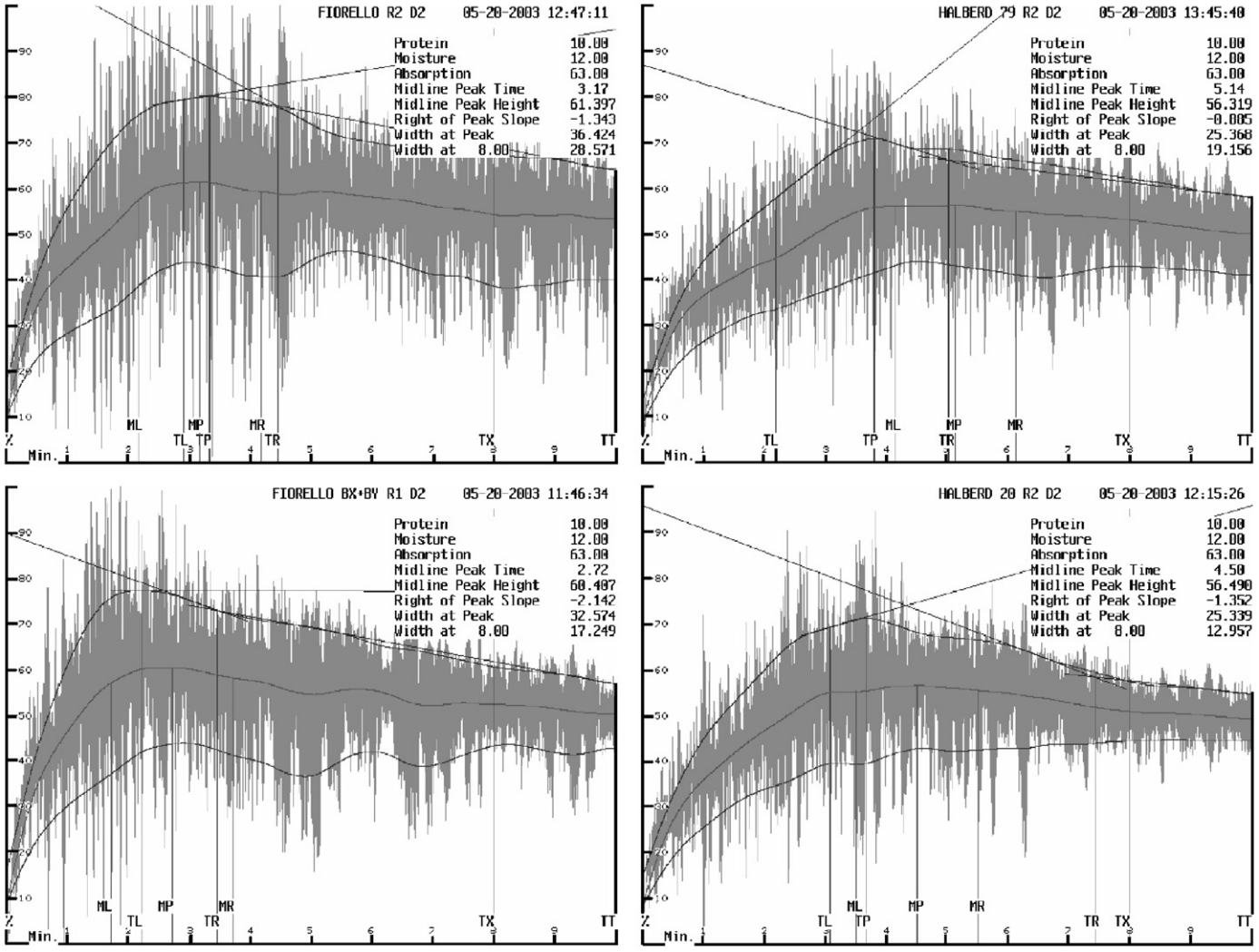

Fig. 3. Mixograms of Fiorello and Halberd lines. On the left: Fiorello 1, 7 + 8, 5* + 12 (top): Fiorello 1, 26 + 27, 5* + 12. On the right: Halberd 1, 7 + 9, 5 + 10 (top); Halberd 1, 20x + 20y, 5 + 10.

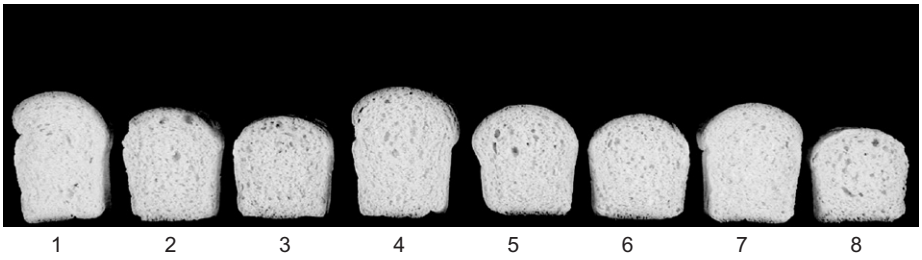

Fig. 4. Comparison of bread loaves of Halberd, Fiorello and Avocet lines. 1—Commercial bread wheat sample; 2—Fiorello 1, 7 + 8, 5* + 12; 3—Fiorello 1, 26 + 27, 5* + 12; 4—Halberd 1, 7 + 9, 5 + 10; 5—Halberd 1 20x + 20y, 5 + 10; 6—Avocet 7 + 8, 2 + 12; 7—Avocet 7 + 8, 5 + 10; 8—Avocet 1, 20x + 20y, 5 + 10.

for subunit 1Bx26. Variations in y-type subunits, if present, may also contribute to the observed differences.

4. Conclusions

The aim of this study was to evaluate the effect of the number of cysteine residues on the quantity and size distribution of polymeric proteins, and to assess effects on dough strength and bread-making quality. Comparison of lines differing at the *Glu-B1* locus showed that Bx-type HMW-GS having two less cysteine residues appeared to shift the MWD to lower values. These lines showed lower dough strength and poorer bread-making performance. There is a potential to manipulate dough properties and

bread-making quality by utilizing alleles with a varying number of cysteine residues, although the mechanism by which the number of cysteine residues affects the size of glutenin polymers, and consequently dough properties, is yet to be clearly explained.

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