Application of a Micro Z-Arm Mixer to Characterize Mixing Properties and Water Absorption of Wheat Flour

R. Haraszi,[1,2] P. W. Gras,[1] S. Tömösközi,[3] A. Salgó,[2] and F. Békés[1]

ABSTRACT

Cereal Chem. 81(5):555–560

This study applied the use of a new small-scale apparatus, the micro Z-arm mixer, which has analogous mixing action to that of the traditional valorigraf and farinograph. A novel methodology has been developed for prediction of water absorption replacing the traditional titration method. The basis of this technique is a common characteristic of wheat flour samples: a reasonably constant slope (20–25.7 BU%) of the relationship between dough resistance and the amount of water present during mixing. Using an average slope value, prediction of water absorption was possible from a single measurement using a simple equation and with a standard error of 1.65%. Applications of the new mixer to cereal research are highlighted, including investigation of the effects of flour protein content and protein composition on mixing properties and water absorption. When protein content and protein composition have been systematically altered by the addition of isolated proteins into the flour, both dough development time (DDT) and water absorption increased when protein content was increased by glutenin addition and decreased when protein content was decreased by starch addition. Gliadin addition decreased DDT; gluten addition slightly increased DDT; glutenin addition significantly increased DDT. Water absorption was not affected by altering the glutenin-to-gliadin ratio, but it changed in proportion to the amount of protein added. The effect of HMW-GS composition on the mixing requirement obtained with the micro Z-arm mixer and with the 2-g mixograph was also investigated using a set of single-, double-, and triple-null lines for HMW-GS coding genes. While subunits coded on the *GluD1* locus were most important for determining the mixing requirement in both cases, the sample ranking was different in the two mixing actions. A better differentiation ability of the micro Z-arm mixer was established for triple- and double-null lines.

The development of small-scale dough testing equipment and the associated electronic data capture and automated data analysis of the mixing curves has provided better reproducibility and removed operator bias, resulting in a more objective assessment of experimental variables (Békés and Gras 1999). This has facilitated a wide range of research and several applications in breeding, in which only limited amounts of test material have been available (Békés and Gras 1999, 2000; Gras et al 2001a).

Until recently, the only small-scale dough mixers available were based on the conventional pin mixer design exemplified by the mixograph. Successful application of the 2-g mixograph in breeding has been reported (Gras and O'Brien 1992). There are a wide range of research studies concentrating on the mixing properties provided by this type of mixer, such as assessing the effects of environment on wheat quality (Rogers et al 1998), or investigating the quality-related effects of genes introduced in wheat transformation programs (Barro et al 1997). One of the most important roles of the 2-g mixograph is to pretreat dough for fundamental rheology studies and end-product quality evaluation in a reproducible, well-documented way.

Investigation of the role of water and the determination of the optimum amount of water needed to obtain a certain dough consistency is essential for both a basic understanding of the dough formation process and the application of flour as a source material. Due to the lack of suitable equipment and methodology in small scale, investigations to date have not included water absorption, one of the most important dough quality parameters.

Water absorption is the amount of water needed by flour to give dough with optimal handling characteristics and bread capable of being processed to give a maximum bread yield and suitable for rheological testing (Stevens 1987). The methodologies applying rheological procedures for the determination of water absorption provide the amount of optimal water level for practical applications. However, these methods have severe limitations in fully explaining the complex nature of the interactions between added water and flour components. Early farinograph studies showed that there is competition between the different chemical components of the flour for the water in the dough (Sandstedt 1955; Greer and Stewart 1959). Numerous studies have been published on flour water absorption from different perspectives, such as the role of bound and free water in dough (Hlynka 1959; Gutierrez et al 2002), the importance of water activity in dough products (Czuchajowska et al 1989), the relationship of water absorption to baking properties (Larsen and Greenwood 1991), the influence of polysaccharides (Andersson et al 1994), starch damage (Dexter et al 1994), and pentosans (Bushuk 1966; Yin and Walker 1992) to water absorption and the relationship between kernel size and water absorption (Morgan et al 2000).

The relationship between the chemical composition of the flour and the amount of water used to produce dough are complex and can be investigated in many ways. Direct addition of purified or expressed proteins to a base flour can indicate the roles of the individual protein components in dough function. However, the production of highly purified proteins, either by expression or by conventional purification procedures, is lengthy and expensive. To date, studies on the effects of individual protein components on water absorption have been impractical because of the large amounts of protein required for conventional dough mixing equipment.

While there are methods for determining water absorption following the moisture content of the flour (Bull 1991) or different empirical models or equations (Stevens 1987; Given 1991; Ruan et al 1999), the most relevant methodology to estimate water absorption is obtained using a Z-arm mixer such as the valorigraf or farinograph. These mixers form the dough with a pack-squeeze type of gentle kneading or shearing action. These methods squeeze the dough between the mixer blade and the mixer body (Landis and Freilich 1935). By developing dough in such a manner, there is a linear relationship between the maximum resistance during mixing and the amount of water added into the flour, providing an empirical definition of water absorption: amount of water required to reach a certain dough consistency (500 BU) (D'Appolonia and Kunerth 1984; ISO 1988).

A micro Z-arm mixer was developed based on the design of the traditional instruments employing the Z-arm mixing action. This instrument can provide information on both the mixing and water absorption properties of flours using only 4 g of flour sample, which is comparable to the traditional farinograph and valorigraf capacities (Haraszi et al, *submitted*). This study introduces a new, small-scale Z-arm mixer as a research tool. Experiments were

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[1] CSIRO Plant Industry, North Ryde, NSW, and Canberra, ACT, Australia.
[2] Corresponding author. Phone: 02-6246-4975. E-mail: reka.haraszi@csiro.au
[3] Dept. Biochemistry and Food Technology, BUTE, Budapest, Hungary.

Publication no. C-2004-0712-01R.

conducted to investigate the relationship between mixing parameters and the amount of water in doughs produced with Z-arm mixing action, and also to observe the alterations of the mixing properties and water absorption caused by changing flour protein content and composition.

MATERIALS AND METHODS

Materials

The sample set of 12 Australian bread wheat cultivars (provided by BRI Australia, North Ryde, NSW) was produced on a Buhler laboratory mill, and characterized with a 50-g farinograph (Brabender GmbH, Germany) using a standard method (ISO 5530-1, 1988). The moisture content of flour samples was 13.8–14.7%, the protein content was 10.86–13.2%, and the water absorption was 57.4–63.5%, representing a wide range of values (Table I). This sample set was used for the validation of the relationship between the amount of water present and dough resistance. In this experiment, water amounts below, identical, and above the optimal amounts were applied for each flour sample.

The effects of protein content and composition on water absorption and mixing parameters were characterized by systematically altering the flour composition of selected samples. Gluten, starch, and glutenin-rich and gliadin-rich fractions were produced from flours of cultivars Janz (9.2% protein, 14.32% moisture), Hartog (11.9% protein, 13.98% moisture), and Eradu (13.9% protein, 14.12% moisture) using the isolation and characterization methods described by Uthayakumaran et al (1999).

The effects of protein content of flour on mixing parameters were determined using flours from three cultivars (only Hartog is presented). Protein contents were adjusted to 90, 95, 105, and 110% of the original protein content by the addition of their own starch or gluten (Uthayakumaran et al 1999). The effects of glutenin-to-gliadin ratio were determined by the addition of glutenin, gluten, or gliadin fractions, increasing the original protein contents by 10 and 20% (Uthayakumaran et al 1999).

The effects of HMW-GS composition on parameters using a micro Z-arm mixer were investigated with a set of nearly isogenic lines with Olympic/Gabo background and null lines (referred as Lawrence lines) for genes coding for HMW-GS (Lawrence et al 1988) and compared with results obtained with a 2-g mixograph. In these experiments, protein content and glutenin-to-gliadin ratio of each test flour was kept constant at 9.2 and 0.9%, respectively, by the addition of starch and gliadin isolated from the non-null sibling line (Uthayakumaran et al 2000, 2002; Beasley et al 2002). Each mixing experiment with altered protein content and composition was conducted in duplicate.

Methods

Mixing experiments with the micro Z-arm mixer were conducted using 4 g of test flour per mix (Haraszi et al, *submitted*). Constant angular velocity (with shaft speeds of 96 and 64 rpm, respectively, for fast and slow blades) was used during all mixes. Mixing was performed in duplicate, each for 20 min. Before adding water to the flour, the baseline was automatically recorded (30 sec) by mixing only the solid components. Water addition was performed in one step using an automatic water pump. The mixing parameters determined by taking the averages from individual mixing experiments were dough development time (DDT); time to peak resistance (sec); peak dough development (PDD) in a Z-arm

TABLE I
Protein Content (%),Moisture Content (%), Average Farinograph Water Absorption Values (%), and Dough Development Times (sec) of Wheat Flours Used in This Study

Flour	Protein (%)	Moisture (%)	Farinograph Water Absorption (%)	Farinograph Dough Development Time (min)
Sunco	13.20	13.84	61.5	6.7
Frame	10.86	14.16	62.6	3.7
Babbler	11.12	14.34	61.8	5.4
Sunbrook	12.82	14.33	60.6	6.7
Suneca	13.05	14.72	57.4	6.7
Whistler	12.07	14.44	62.9	5.5
Braewood	12.67	14.70	59.3	6.4
Wylah	12.04	14.13	60.6	5.8
Cunningham	11.76	14.52	60.7	5.5
Chara	11.15	14.64	59.0	8.2
Janz	11.43	14.45	60.9	6.8
Meering	11.90	14.56	60.5	5.5

TABLE II
Slope and r^2 Values of Relationships Between the Amount of Water Added and Mixing Parameters[a]

	PDD		DDT		ST		BD	
	Slope (BU/%)	r^2	Slope (sec/%)	r^2	Slope (sec/%)	r^2	Slope (BU × sec/%)	r^2
Sunco	–20.02	0.97	21.53	0.96	8.75	0.95	–562.3	0.76
Frame	–22.58	0.99	14.77	0.97	1.464	0.67	–504.1	0.46
Babbler	–23.81	0.94	15.44	0.87	7.382	0.91	–655.8	0.61
Sunbrook	–24.49	0.97	27.43	0.93	7.247	0.89	–980.7	0.79
Suneca	–25.24	0.99	21.38	0.88	6.302	0.83	–860.8	0.64
Whistler	–25.27	0.99	15.65	0.99	5.15	0.94	–1,336	0.91
Braewood	–25.68	0.97	25.3	0.89	9.423	0.76	–534.7	0.70
Wylah	–24.61	0.95	13.81	0.96	13.84	0.91	–1,842	0.91
Cunningham	–21.68	0.96	15.24	0.91	11.15	0.91	–868.8	0.68
Chara	–25.54	0.87	16.52	0.81	3.837	0.81	–535	0.65
Janz	–23.03	0.96	10.29	0.96	2.5	0.67	–625.1	0.34
Meering	–25.55	0.99	14.93	0.94	3.081	0.82	–1,073	0.50
Average	–23.96	0.96	17.69	0.92	6.68	0.84	–864.8	0.66
SD	1.797	0.033	5.079	0.052	3.719	0.097	443.8	0.189

[a] PDD, peak dough development; DDT, dough development time; ST, stability; and BD, breakdown.

mixer (either analogue-digital converter units [AD] or BU) (Haraszi et al, *submitted*); stability (ST), time between first and last excursions over 500 BU line (sec); resistance breakdown (BD), area 10 min after PDD between the midline of the recorded data and the 500 BU line, which is crossing the curve maximum (BU × sec).

RESULTS AND DISCUSSION

The micro Z-arm mixer (along with other small-scale equipment such as a 2-g mixograph) provides a practical method for determining the contributions of particular polypeptides to mixing behavior on a scale never before possible. Two types of experiments to relate protein composition to water absorption were conducted in this work: 1) investigation of mixing properties resulting from varying water addition, and 2) study of the effects on mixing properties and water absorption caused by the systematic alteration of the chemical composition of the flour.

Altering Volume of Water Added

Peak resistance has previously demonstrated a linear relationship with the amount of water present in wheat flour dough mixed with Z-arm mixers (Gras et al 2001b). Results of this study were validated in the current work using a larger experiment (Table I). Highly significant relationships were found between the amount of water added and peak dough development (Fig. 1). The average Pearson's correlation coefficient (r^2) of the linear relationship between these two parameters was 0.96, while the slopes of the linear relationships calculated for the individual flour samples was 20–25.7 BU/% (Table II), indicating a very good similarity. The strong relationship and the remarkably similar slope values found between PDD and added water provided the basis for a simpler and faster way for screening samples for water absorption. Instead of applying repeated mixings until resistance reaches the 500 BU line, water absorption (WA) can be estimated from one single measurement. This provides a method for ranking samples for breeding. The estimation uses the average slope and maximum resistance value (that is an expression of 500 BU in AD units) derived from the mix using any water amount. This can be defined with a simple equation

$$\text{WA (\%)} = \text{added water (\%)} + \{[\text{PDD (AD units)} - 500 \text{ BU in AD value}]/\text{average slope}\}$$

The predictability of water absorption was defined as the water absorption calculation at any added water level. In the 12 samples used in this study, the standard error of prediction from a single measurement was 1.65% compared with that of a multiple ($n = 5$) determination of 1.15%. The accuracy of the estimated water absorption values had the coefficient of variation of 0.95% (in a traditional farinograph this is 0.25%) (ISO 5530-1, 1988). If better estimates of water absorption are required, these can be obtained by interpolation or extrapolation of the linear relationship from the results of mixings with ±5% differences in the volume of water added.

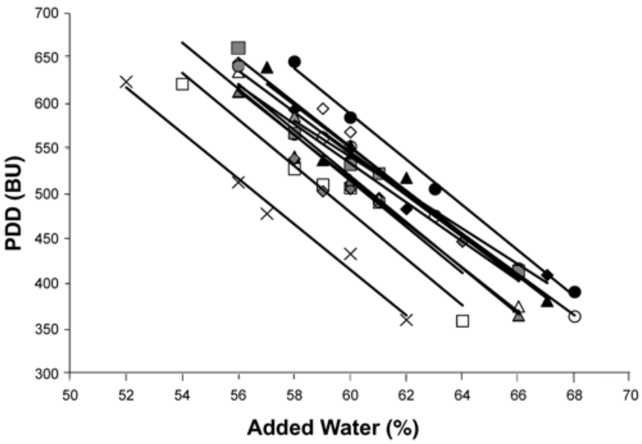

Fig. 1. Relationship between added water and dough resistance. Flours: Sunco (■), Frame (▲), Babbler (◆), Sunbrook (○), Suneca (×), Whistler (▲), Braewood (△), Wylah (●), Cunningham (□), Chara (◇), Janz (●), Meering (◆). PDD is peak dough development in the Z-arm mixer.

The experiments also confirm that each of the mixing parameters were sensitive to the amount of water used. DDT (Fig. 2A) and stability (Fig. 2B) showed strong positive relationships, while BD (Fig. 2C) showed significant negative relationships with the amount of water added. The reproducibility of DDT is 6.01% (in a farinograph it is 9%) (ISO 5530-1, 1988). Stability and BD obtained

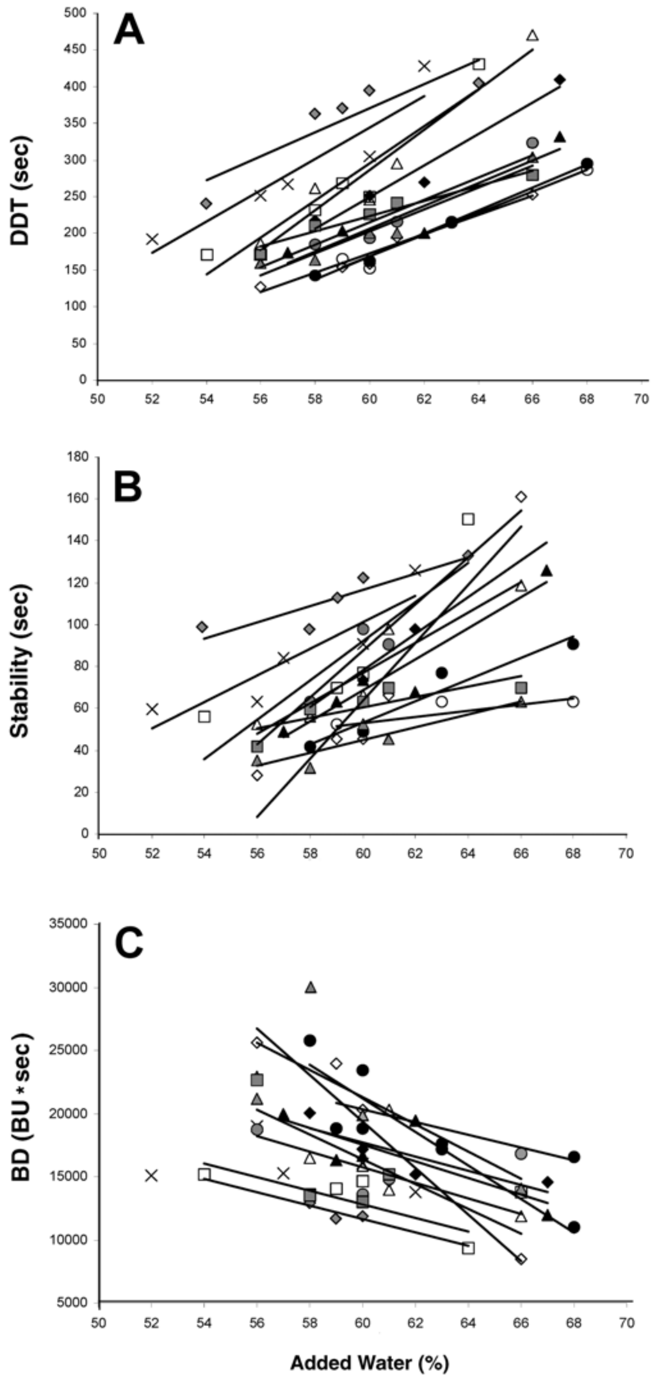

Fig. 2. A, Dough development time (DDT); **B,** stability; and **C,** breakdown (BD) measured on a micro Z-arm mixer. Flours: Sunco (■), Frame (▲), Babbler (◆), Sunbrook (○), Suneca (×), Whistler (▲), Braewood (△), Wylah (●), Cunningham (□), Chara (◇), Janz (●), Meering (◆).

from a micro Z-arm mixer have quite large standard errors (17.84 and 12.32%, respectively), while the value for BD in a farinograph is 7% (ISO 5530-1, 1988). The value for stability is not available from the standards. However, collaborative studies show ≈11% coefficient of variation (D'Appolonia and Kunerth 1984). Compared with standard errors of a farinograph and considering that the smaller size mixers generally have larger variance in mixing parameters (ISO 5530-1, 1988), the standard errors of a micro Z-arm mixing are suitable for screening applications. While large variances in slopes were observed for stability and BD, a highly significant relationship (average $r^2 = 0.92$) and a relatively narrow range (10.3 and 27.4 sec/%) of slopes between DDT and water amount were found among the samples (Table II). Similar to the procedure for the prediction of water absorption, this relationship has the potential to estimate DDT from one single measurement providing useful information about dough behavior. These estimates would be valuable in breeding for early selection of mixing requirement. A preliminary prediction procedure established for the DDT at a nominal water level using an average slope value (17.69 sec/%) has some limitations, but for future application, improvement of prediction seems to be required. One way could be evaluating specific prediction models for sample sets different in protein composition.

The computer software developed for the micro Z-arm mixer automatically provides an estimate for water absorption on both a 14% moisture basis and on a dry weight basis, and for DDT. The method thus provides a useful addition to the tools available for early generation screening for flour quality.

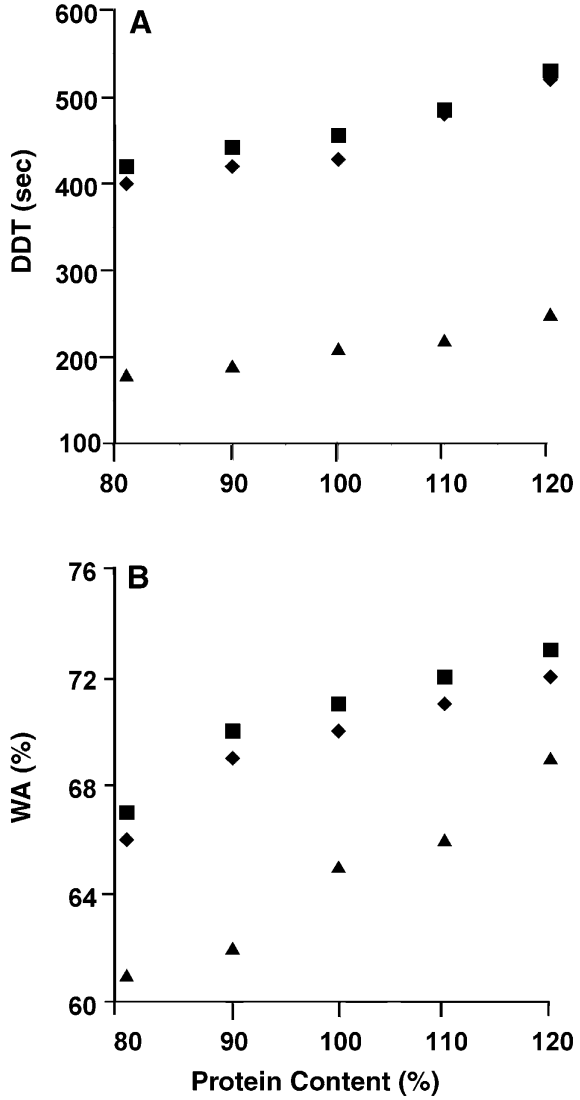

Fig. 3. Effects of protein content caused by starch or gluten addition. **A**, dough development time (DDT); **B**, water absorption (WA) of flours from different cultivars measured on the micro Z-arm mixer. Janz (◆), Hartog (■), Eradu (▲). Protein content was expressed as the percentage of the protein content of the original flour. Symbols represent the mean values of two replicate measurements with the average coefficient of variation values of 4.6 and 7.8 for DDT and WA, respectively.

Altering Protein Composition

Differences in dough properties are largely determined by superposition of the effects of protein content, glutenin-to-gliadin ratio, and the size distribution of the polymeric glutenin (Uthayakumaran et al 1999). Size distribution of the polymeric glutenin largely depends on the HMW-to-LMW ratio, the allelic composition of HMW and LMW glutenins, and the relative amounts of the different glutenin subunits. To establish cause and effect relationships between chemical composition and functional parameters, experiments are needed where, ideally, only one parameter is altered while the others are kept constant. Such experiments are difficult because in a fixed-mass system an increase in the amount of one component necessarily results in a change in the relative amounts of the other components.

One experimental approach involves systematically altering the chemical composition of the flour by supplementing it with just one of its own components and determining the changes in the functional properties (Uthayakumaran et al 1999, 2002). Applying this technique, we investigated the independent contributions of protein content and then glutenin-to-gliadin ratio in defining the mixing requirement and water absorption using flours of three cultivars (Janz, Hartog, and Eradu).

Effect of Protein Content on Mixing Properties and Water Absorption

Small differences in the flour protein content were reflected in changes in water absorption and DDT obtained with a micro Z-arm mixer (Fig. 3). Both water absorption and DDT increased if protein content was increased by gluten addition and decreased if protein content was decreased by starch addition. For water absorption, the diluting effect of starch with a larger amount of starch resulted in slopes different than those observed for the effect of enrichment with gluten. The extent of this effect seems to be different among the three samples investigated and presumably related to the different levels of starch damage in these samples. This observation is a clear indication of the direct effect of added starch on water absorption, besides decreasing the protein content (Fig. 3B).

DDT changed significantly, depending on whether gliadin, gluten, or glutenin was added (Fig. 4A). Gliadin addition decreased the DDT, while gluten addition slightly increased it and glutenin addition significantly increased it. These findings are analogous to those obtained from studies made on pin mixers (Uthayakumaran et al 2001).

While mixing requirement was a function of both the amount and size of the different gluten components present, water absorption increased in direct proportion to the amount of protein added, independently from the size distribution of the supplemented proteins (Fig. 4B). This finding indicates that, for water absorption, the contribution of proteins present in the flour is related to other structural features of the proteins rather than their polymeric characteristics. Compared with the globular, albumin, or globulin proteins, most of the wheat endosperm storage proteins show typical prolamin characteristics in their solubility behavior (Gianibelli et al 2001), which is mostly related to their surface properties, such as polarity or hydrophobicity. In the light of results shown in Fig. 4B, it seems that, in relation to water absorption, these common characteristics of glutenin and gliadin proteins are more important than their differences in polymeric characteristics. The importance of polarity or hydrophobicity of proteins altering water absorption have been directly demonstrated in experiments where supplementation of different dairy protein isolates and cereal germ proteins with different hydrophobicity properties resulted in significant changes in the water absorption of the flour (Haraszi 2002). Thus, the variation in glutenin-to-gliadin ratio on a given protein level does not lead to changes in water absorption. It is the reason that several empirical equations estimated water absorption of pure flours (containing nonprotein additives) by measuring protein content, starch damage, and pentosans to provide meaningful measure of the protein contribution in the flour used in industry (Stevens 1987).

Effect of HMW-GS Composition on Mixing Properties

The strong effects of HMW-GS composition on mixing properties and dough strength have been well demonstrated (Lawrence et al 1988). In recent studies (Beasley et al 2002; Uthayakumaran et al 2002) investigating relationships between the rheological properties of wheat flour dough and end-product quality in a set of near isogenic lines differing only in the number of HMW-GS (Lawrence et al 1988), the differences in properties were caused by synergistic and additive effects of the three HMW glutenin loci. The effects of HMW-GS composition on mixing properties determined by a micro Z-arm mixer were comparable to those found for doughs mixed on the pin mixer (Beasley et al 2002; Uthayakumaran et al 2002). However, there are differences in the relative levels and even the sequences of the mixing requirements among the eight samples. DDT was not significantly affected in single null lines missing HMW-GS 1 or 17+18 (Fig. 5), while DDT was significantly shorter for the *Glu-D1* null line missing HMW-GS 5+10 than in a 2-g mixograph. Similarly, for double null lines, lines not containing subunits 5+10 showed significantly shorter DDT than those combinations missing the other glutenin subunits. The relative contributions to DDT of the three HMW glutenin alleles present in the sample set can be summarized as 5+10 > 17+18 > 1. The sequence of the triple- and double-null lines (marked × in Fig. 5) shows this ranking is identical in both mixers. The micro Z-arm mixer is better able to differentiate among these samples than is the 2-g mixograph. This observation might be related to the findings of Kilborne and Tipples (1972), who found that the pin mixing action does not readily develop weak doughs. The insignificant contribution of Ax1 is understandable given the fact that no Ay subunits are expressed in contradiction to other HMW glutenin alleles.

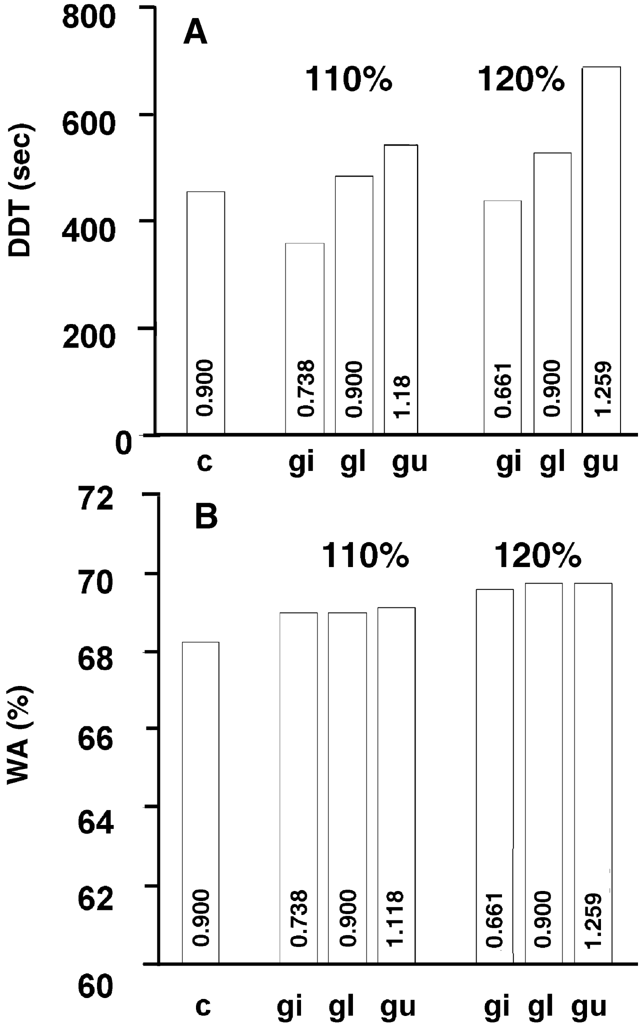

Fig. 4. Effects of glutenin-to-gliadin ratio on (**A**) dough development time (DDT) and (**B**) water absorption (WA) of cultivar Hartog determined on a micro Z-arm mixer. Control flour (c) with 11.9 protein content supplemented with gliadin (gi), gluten (gl), and glutenin (gu) to 110 and 120% of the original flour protein content. Numbers in the bars represent the actual glutenin-to-gliadin ratios in the sample. Bars represent mean values of two replicate measurements with average coefficient of variation values of 4.6 and 7.8 for DDT and WA, respectively.

Differences in sample ranking mostly occur among the double- and triple-null lines. For example, the double-null line containing 5+10 only shows greater DDT than a single-null line with Ax1 and *GluB1* 17+18 composition, but this is not true when a 2-g mixograph was used. Dough behavior can be different in different mixers due to the effect of the mixing action on alleles and allelic interactions. In other words, the differences in mixing requirement result from the differences in mixing action.

CONCLUSIONS

The micro Z-arm mixer was a reliable research tool for investigating the composition and functional relationships in dough. The reduced sample size compared with traditional Z-arm equipment such as a valorigraf or farinograph allows experiments with purified flour components or protein sources which, until now, were not technically or financially feasible. Improved reproducibility and removed operator bias assists in objective analysis of the data. Water absorption can be predicted from one single measurement, and the potential to develop a similar predictive procedure for DDT was demonstrated.

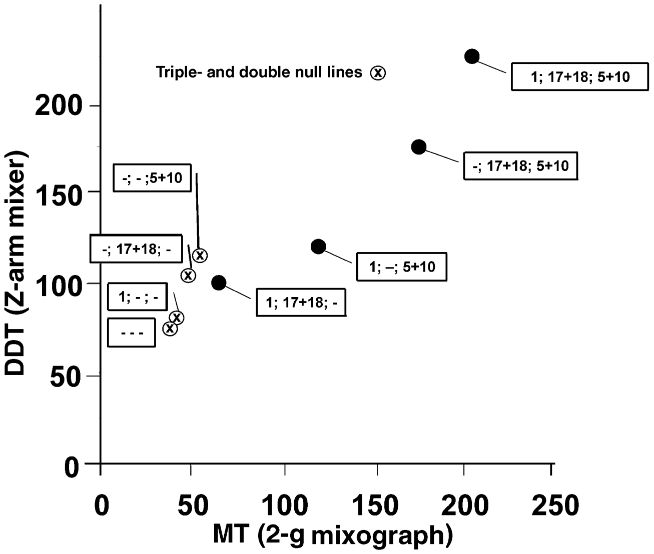

Fig. 5. Relationship between dough development time (DDT) obtained with a micro Z-arm mixer and mixing time (MT) obtained with a 2-g mixograph. Flours contain different HMW glutenin subunits.

In experiments systematically altering protein content and composition of flours and monitoring changes in mixing properties and water absorption, flours containing more proteins showed increased DDT and increased water absorption. Significant changes in DDT were observed with varying glutenin-to-gliadin ratios at the same protein content level. Water absorption did not show variation in experiments where flours were supplemented with gliadin, gluten, or glutenin, in contrast to observations with proteins with different polarity or hydrophobicity, resulting in significant alteration in water absorption. Therefore, it can be concluded that the overall polarity or hydrophobicity of the proteins present in flour plays a greater role in determining water absorption than does size distribution.

The effects of HMW-GS on DDT determined on the micro Z-arm mixer were similar to those found for mixing time determined on the 2-g mixograph. However, in weak doughs, there are differences in the relative levels and even in the sequences of the mixing requirements among the samples. These observations are clear indications of the differences in the mixing actions in pin mixers and Z-arm mixers. For a better understanding of the role of different HMW-GS in forming glutenin polymers and producing doughs with different rheological properties, further research is required to relate the mixing results on different dough mixers on well-defined germplasm populations.

ACKNOWLEDGMENTS

This research has been partly funded through an Australian Government award to R. Haraszi, made available through the Department of Education, Training and Youth Affairs (2000-2001). This work has also been supported by the Hungarian Scientific Research Foundation (OTKA, Project No. T-034486/2001). We would like to acknowledge the contribution of the value added by CRC (North Ryde, NSW, Australia). We also thank Matthew Morell for his valuable comments.

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[Received September 24, 2003. Accepted February 16, 2004.]