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ARTICLE

Effects of Wheat Storage Proteins on the Functional Properties of Rice Dough

Mária Oszvald,[†,§] Sándor Tömösközi,[§] László Tamás L,[†] and Ferenc Békés*[,#]

[†]Department of Plant Physiology and Molecular Plant Biology, Eötvös Loránd University, Budapest, Hungary, [§]Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Budapest, Hungary, and [#]CSIRO, Plant Industry, Canberra, ACT 2601, Australia

The objective of this work was to develop a rice flour based procedure for in vitro structure−function studies of wheat proteins. Rice flour has an advantage over wheat flour, because the signal/noise ratio should be higher after the incorporation of the wheat prolamins into the protein matrix of the dough. A reduction/oxidation procedure has been developed to incorporate glutenin subunits into the polymeric structure of rice dough protein. The results indicated that incorporation of bulk fractions of HMW and LMW glutenin subunits increased the mixing requirements of the dough, whereas simple addition resulted in weaker dough. The incorporation studies of individual HMW subunits (Bx6, Bx7, and By8) demonstrated that rice flour can be used to study and compare the functional properties of different glutenin subunits.

KEYWORDS: Wheat glutenin subunits; rice dough; incorporation; functional properties; dough reconstitution

INTRODUCTION

A unique property of wheat flour is its ability to form dough when it is mixed with water. Dough formation is largely determined by the ability of the hydrated protein components to form the gluten network stabilized by both covalent and noncovalent interactions among wheat flour proteins (*1*). The fundamental properties of wheat flour dough are mostly governed by the polymeric characteristics of the proteins present: dough strength mostly depends on the size distribution of the polymeric proteins, whereas dough extensibility is highly correlated with the relative amount of the polymeric proteins in the dough (*2*). Interpolypeptide disulfide bonds, formed among glutenin subunits, play a crucial role in the formation of polymeric glutenin. Structural differences, such as the number and position of the cysteine residues in the polypeptides, cause differences in the capabilities of forming polymers among the different polypeptides in glutenin subunits. Therefore, the relationships between the HMW and LMW GS allelic composition and dough properties are well documented.

Most of our knowledge on the relationships between protein composition and functional properties is derived either from indirect correlative studies or from direct reconstitution experiments (*3*). Recent correlative results on large sample populations underscored the significant contribution of allele−allele interactions determining functional properties: the combinations of glutenin alleles present rather than individual glutenin alleles determine mixing requirement, dough strength, and extensibility (*4*).

*Corresponding author [e-mail ferenc.bekes@csiro.au; telephone (+61)-2-6246-5032; fax (+61)-2-6246-5000].

Using dough reconstitution studies, the direct effects of supplemented constituents can be monitored by systematically altering the chemical composition of a "base flour" (*5*). In the case of subunit type of constituents, the procedure must involve an incorporation step when the supplemented polypeptide is built into the polymeric protein fraction: the polymeric glutenin of the base flour is partially reduced followed by an oxidative step in the presence of the supplemented constituent (*6*).

By application of the in vitro incorporation procedure for wheat flour, meaningful estimation of the contribution of individual wheat glutenin subunits on the functional properties can be achieved. However, one of the limitations of the so-called base flour method is that the supplemented constituents obviously interact with the original components of the flour; thus, depending on the base flour used, different "noise" is superposed on the measurements. An ideal solution to avoid this problem would be the use of base flours not containing any wheat flour components.

Unlike other cereals, which accumulate prolamins as their primary nitrogen reserve, the major storage proteins in rice are the glutelins, which are homologous to the 11S globulin proteins (*7*,*8*). These differences provide the molecular background of the observed physical/functional differences between wheat and rice flours and their utilization in different products. Despite the obvious differences in the structure and functionality of wheat and rice proteins, the fundamental relationships found in wheat between polymeric characteristics of proteins present and dough properties can be adopted for rice flour doughs: rice flours containing a larger overall polymeric size of proteins form stronger, more stable doughs (*9*, *10*).

The aim of this work was to use rice flour as a base flour in incorporation experiments to determine the effects of individual

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glutenin subunits on dough functionality, without any interactive effects of other gluten proteins present.

MATERIALS AND METHODS

Rice Flours. Flours produced from two ('White rice' and 'Illadong') rice (*Oryza sativa* L.) varieties were used as the base flours in this study. Rice grains were ground with a laboratory mill (Metefem Ltd.), and flour was sieved to obtain particles smaller than 250 μm. The moisture contents of rice flours were determined according to AOAC Official Method 44-15 (*11*), whereas protein contents were measured according to the Dumas method (N × 5.95) using an automated protein analyzer (LECO FP-528).

Wheat Proteins. Flours from good (Mv Suba) and medium (Mv Matyó) quality Hungarian bread wheat cultivars with 15.7 and 14.2% protein content, respectively, were used to isolate bulk fractions of HMW and LMW glutenin subunits. The wheat grains were grown in Martonvasár (Hungary) in 2006. The HMW-GS and LMW-GS allelic compositions of the two varieties—a, c, d, c, j, and b for Mv Suba and b, c, d, c, b, and b for Mv Matyó (*12*)—provided the possibility to isolate bulk glutenin subunit proteins with slight but important differences in allelic composition.

Samples of wheat lines Galahad 6, Galahad 7, and Galahad 8 were kindly provided by P. I. Payne, PBI Cambridge Ltd., U.K. This set of wheat lines contained null alleles for the (*Glu-1*) gene on both the A and D genomes and expressed only single x type 6, 7 or y type 8 subunits coded by the B-genome (*13*).

Isolation of HMW and LMW Glutenin Subunits. HMW-GS and LMW-GS bulk fractions were isolated from Mv Suba, Mv Matyó, and Galahad flours according to the method of Verbruggen et al. (*14*) with slight alterations. Gliadins were removed from the defatted flour with extraction (three times) by 50% (v/v) propan-1-ol for 30 min. After centrifugation, the supernatant was discarded. Glutenin subunits were isolated from the last residue flour with 50% (v/v) propan-1-ol containing 1% (w/v) DTT at 65 °C for 30 min. HMW-GS and LMW-GS bulk fractions were selectively precipitated from the supernatant by the addition of different volumes of propan-1-ol in two consecutive steps. HMW-GS proteins were precipitated first from 60% propan-1-ol solution, whereas LMW-GS proteins were precipitated afterward from 85% propan-1-ol solution at 4 °C. Both pellets were washed twice with 60 or 85% propan-1-ol solution, respectively,and freeze-dried.

The protein components of both isolated bulk fractions were monitored by SDS-PAGE according to the method of Laemmli (*15*) (**Figure 1**). Protein content of the purified fractions was 94−96% for each type of glutenin subunit.

Incorporation and Addition. The method of Békés et al. (*8*), developed originally for the incorporation of HMW glutenin subunits into wheat dough using a 2 g Mixograph, was adapted for rice dough prepared in a prototype micro z-arm mixer (Metefem Ltd.).

The concentrations of reducing agent and oxidant were optimized to obtain partial reduction and optimum restoration of the dough properties of rice flour. Rice flour, with or without added glutenin subunits, was mixed with 2.55 mL of deionized water and 100 μL of DTT solutions (2 mg/mL) for 45 s. The homogenized mixtures were allowed to react without mixing for 4 min. The partially reduced dough was then treated with 250 μL of KIO_3 (5 mg/mL), and the dough was mixed for 30 s and allowed to rest and react without mixing for 6 min. The dough was mixed again for an additional 10 min.

The same protocol was followed in separate experiments when the glutenin subunits were simply added to the flour without the reduction/oxidation protocol. In this case, the DTT and KIO_3 solutions were replaced by water. In both addition and incorporation experiments, the amount of water was adjusted proportionally with the increased protein content of the rice flour−supplement mixture (*16*).

SE-HPLC Analysis. SE-HPLC was carried out to determine the protein size distribution in both the unextractable and extractable protein fractions of the dough according to the method fo Oszvald et al. (*10*). The conditions of protein separation were identical as previously described by Larroque and Békés (*17*). Freeze-dried dough samples were ground, and proteins were extracted in two consecutive steps (*2*), without and with sonication, separating the soluble and unextractable proteins, respectively. The first SDS-soluble fraction is called extractable proteins.

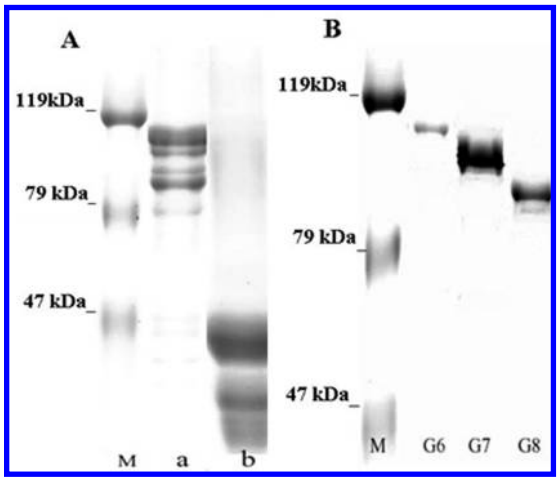

Figure 1. (**A**) SDS−gel electrophoresis of HMW-GS and LMW-GS extracted from Mv SUBA. (**B**) SDS-PAGE of HMW-GS 6, 7, and 8 extracted from Galahad 6, 7, and 8. G6, HMW-GS Bx6; G7 = HMW-GS Bx7; G8, HMW-GS By8.

Table 1. Mixing Properties of the Two Rice Flours Used as Base Flour in This Study

rice variety	DDT[a] (s)	BD[b] (VU·s)	ST[c] (s)	UPP[d] (%)
White rice	310	294	35	58.6
Illadong	240	95	102	54.7

[a] Dough development time. [b] Bandwidth. [c] Stability. [d] Unextractable polymeric protein determined by SE-HPLC.

Total proteins were extracted from freeze-dried and ground dough by sonication for 15 s in 0.05 M sodium phosphate buffer (pH 6.9) containing 0.5% (w/v) SDS. Samples were than centrifuged at 12000*g* for 15 min and the supernatants filtered through 0.45 μm PVDF membranes and transferred to clean vials. Protein fractions were separated on a Phenomenex Biosep-SEC 4000 column, 300 × 7.8 mm (5 μm clone), for 10 min.

The percentage of unextractable polymeric protein (UPP%) (*2*)—a simple but effective measure of the size distribution of the polymeric proteins—was determined by applying the calculation method developed for rice flours and dough (*2*, *10*).

Dough Mixing. Microscale mixing tests were carried out on a prototype micro z-arm mixer (Metefem Ltd.) using 4 g of flour per test. The resistance values were sampled every 0.1 s and stored electronically. The following parameters were determined from the mixing curve: maximum resistance (VUmax), dough development time (DDT), breakdown (BD), and stability (ST) (*16*).

Statistical Analysis. All measurements were carried out in triplicates. Analysis of variance (ANOVA) was then carried out on the mean values. The Statistica 7.0 program (StatSoft, Inc., 2006) was used for statistical evaluation.

RESULTS AND DISCUSSION

Wheat Protein Incorporation into Rice Dough. The selection of base rice flours used in both addition and incorporation experiments was based on a previous paper (*10*) in which significant differences have been demonstrated in the mixing properties of these two rice flours (**Table 1**). Because of the slower hydration process of rice flours and the different mixing action in the z-arm mixer, the reduction/oxidation procedure required different conditions compared to wheat flour. Under the optimized mixing conditions, no significant differences were detected either in the mixing properties of the control and the reconstituted—reduced and then reoxidized—doughs or in the size distribution of the proteins isolated from both dough samples.

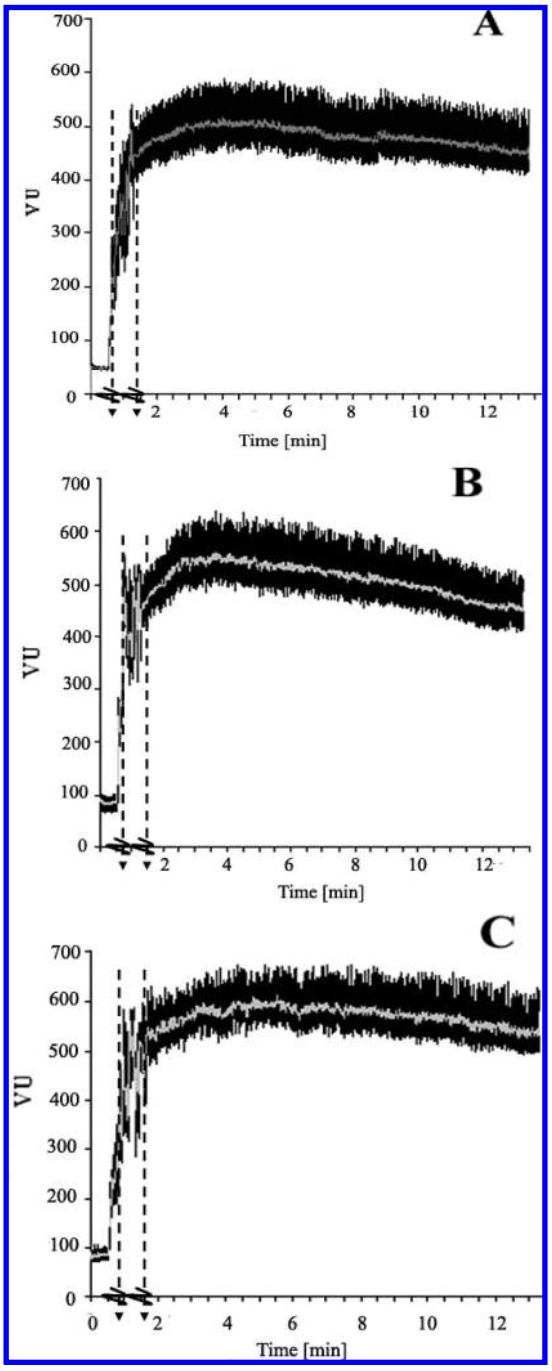

Figure 2. Mixing curves registered during dough making in the bowl of the z-arm mixer: (**A**) control base rice flour; composite rice flour with the addition (**B**) and incorporation (**C**) of 56 mg of HMW-GS bulk protein. Broken lines indicate the times when the mixer was switched off to allow reduction and oxidation reaction. Mixing properties: DDT, 310, 216, and 420 s, respectively; BD, 294, 443, and 156 Vu · s; and ST, 35, 19, and 53 s, respectively.

Mixing Experiments. For functionality studies, 14, 28, and 56 mg bulk fractions of HMW-GS and LMW-GS proteins, in dry form, representing 5, 10, and 20% of the total protein content, were added into 4 g flour samples in both addition and incorporation type experiments.

Three mixing curves of the 'White rice' base flour and the composite flours are demonstrated in **Figure 2**. Simple addition of the HMW glutenin subunit protein fractions decreased the mixing requirements (curve shifted to the left) (**Figure 2B**) compared to untreated rice dough (**Figure 2A**). However, the incorporation of the same protein fraction increased the mixing requirement (curve shifted to the right) and resulted in a more stable dough, represented by a flatter slope after the peak and increased bandwidth of the mixing curve (**Figure 2C**). The characteristics of changes in the mixing curves after the addition and incorporation of wheat storage proteins into the 'Illadong' rice flour were found to be identical.

The mixing parameters and the values of UPP% for both rice varieties with added/incorporated wheat glutenin proteins are summarized in **Figures 3** and **4**. For better comparison of the data obtained in different types of experiments, the measured values have been expressed as a percentage of those from the control experiment with untreated sample.

The statistical analysis of data shown in **Figures 3** and **4** was carried out by ANOVA to study the contribution of the four different treatments applied in this study. The individual effects of the treatments (as F values and corresponding probability percentages) as well as the least significant differences (calculated by Student's t test) for the mixing parameters and UPP% values of rice doughs are summarized in **Table 2**.

The data clearly demonstrate that the effects of the proteins on the functional properties of rice doughs strongly depend on the nature of supplementation as well as the amount of the wheat proteins. Alteration in the HMW and LMW glutenin allele composition also resulted in different effects on the dough properties after addition and incorporation.

The addition of different amounts of bulk HMW-GS or LMW-GS fractions had significant negative effects on the mixing requirements of both rice doughs. The extent of the alterations in functional properties was significantly larger when LMW-GS proteins were added into rice flour compared to added HMW-GS proteins and proportional with the amounts of supplemented LMW-GS or HMW-GS (**Figure 3A**−**C**). The differences between the observed effects of the two subunit types on the mixing properties of rice dough are related to the larger shift of the size distribution of proteins when LMW-GS was supplemented. Incorporation of 5, 10, and 20% wheat HMW-GS bulk fraction increased the mixing requirement of both rice doughs. The incorporated 14, 28, and 56 mg of HMW-GS protein fraction, isolated form Mv Suba wheat cultivar, raised the DDT value of reconstructed 'White rice' dough by 8.4, 12.4, and 26.4%, respectively. A similar effect was observed on the rice dough using 'Illadong' rice flour after incorporation of the same amount of HMW-GS proteins. The DDT increased by 9.2, 12.7, and 32.8%, respectively (**Figure 4A**). The DDT values between the two rice base flours did not show significant differences.

The incorporation of HMW glutenin subunits isolated from Mv Suba wheat cultivar (containing Ax1 subunit) resulted in stronger doughs compared to the same amount of HMW-GS proteins extracted from Mv Matyó (containing Ax2* subunits). The stability of the dough increased by 13.7, 19.0, and 35.8% in 'White rice' dough and by 12.1, 21.5, and 38.2% in 'Illadong' dough, respectively (**Figure 4C**).

Similar, but proportionally smaller, effects were observed on dough properties when LMW-GS proteins were incorporated. The incorporated 5, 10, and 20% LMW-GS proteins isolated from Mv Suba increased the DDT value by 8.0, 17.7, and 28.5%, respectively, whereas the LMW-GS proteins from Mv Matyó altered the mixing requirement to lesser extents (3.8, 8.3, and 21.3%, respectively) (**Figure 4A**). The incorporation had a positive effect on the tolerance to overmixing indicated by the significantly lower BD values for both HMW and LMW glutenin protein subunits (**Figure 4B**).

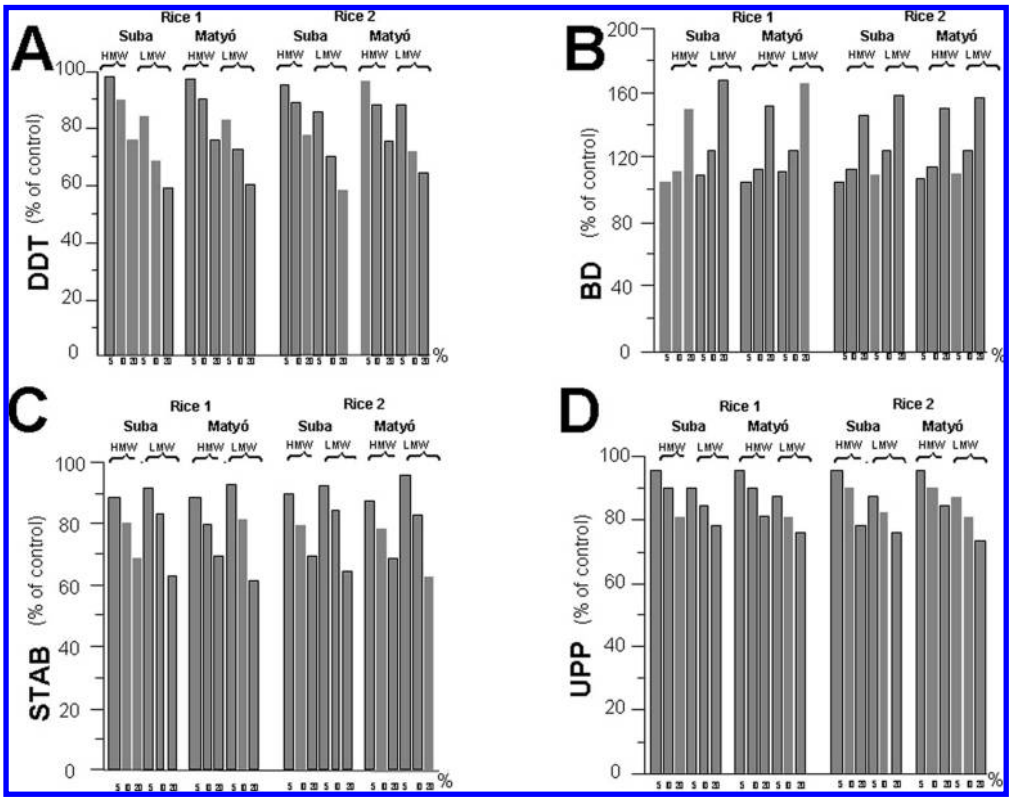

Figure 3. Effects of added bulk glutenin subunits on the mixing properties and polymer size proteins of rice doughs: (**A**) dough development time (DDT); (**B**) bandwidth (BD); (**C**) stability (STAB); (**D**) unextracable polymeric protein (UPP). Rice 1, cv. White rice; rice 2, cv. Illadong used as base flours; Matyó and Suba are wheat cultivars from which the bulk HMW and LMW Gs have been isolated.

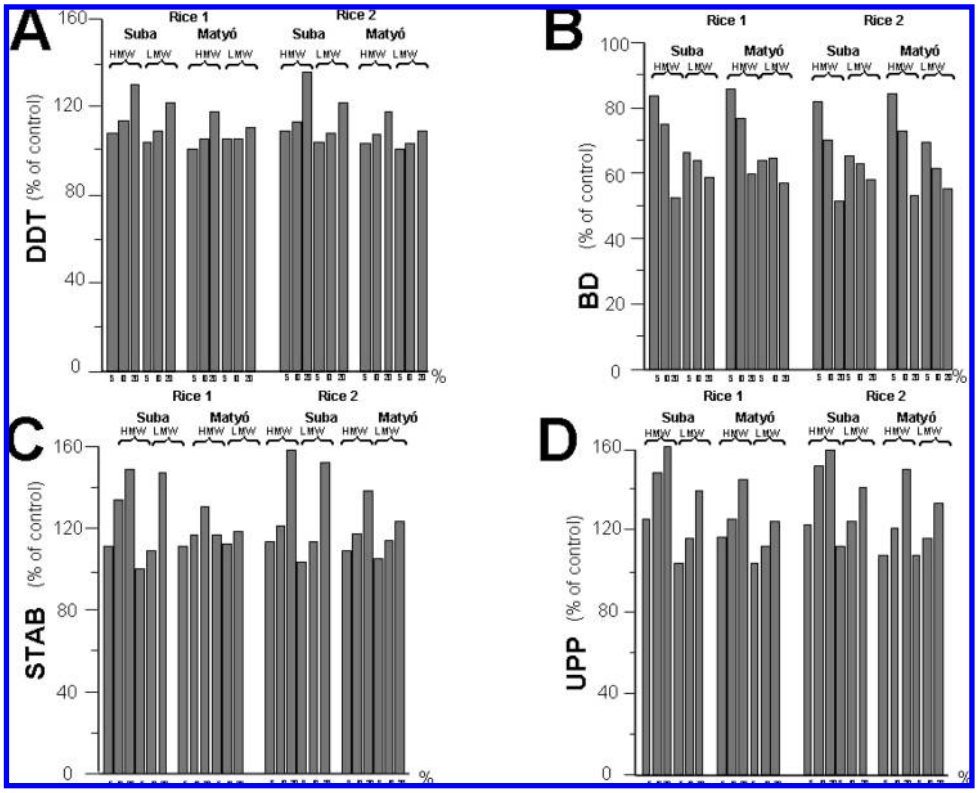

Figure 4. Effects of incorporated bulk glutenin subunits on the mixing properties and polymer size proteins of rice doughs: (**A**) dough development time (DDT); (**B**) bandwidth (BD); (**C**) stability (STAB); (**D**) unextracable polymeric protein (UPP). Rice 1, cv. White rice; rice 2, cv. Illadong used as base flours; Matyó and Suba are wheat cultivars from which the bulk HMW and LMW Gs have been isolated.

The effects of individual glutenin subunits coded on the 1B chromosome on the mixing properties of rice dough were also studied. Reconstitution type experiments were carried out using 14 and 28 mg of purified Bx6, Bx7, and By8 HMW subunits.

Similarly to the results using bulk HMW-GS, individual HMW-GS proteins also showed significant positive effects on the dough stability and tolerance to overmixing after incorporation. The effects on mixing characteristics of rice dough were determined by the type and amount of the protein fractions. Incorporation of 10% single HMW proteins had greater effects than 5% proteins in every case, however, to a slightly different extent. The dough development times after incorporation of 28 mg of Bx6, Bx7, and By8 were 9, 17, and 21% larger, respectively, compared to the control sample (**Figure 5A−C**).

SE-HPLC Analysis of the Protein Matrix in Rice Dough. A typical elution profile of the total protein extract of rice flour is shown in **Figure 6A**. Four fractions were distinguished and quantified for each sample: P-I, corresponding to large glutelin polymers; P-II, smaller glutelin polymers; P-III, monomeric forms of α and β subunits of rice glutelin; and P-IV, monomeric albumin, globulin, and prolamins (*10*). Wheat storage proteins were superimposed over the rice protein peaks, according to their size, modifying the intensity of the appropriate peak.

The amount of the large polymer (P-I) fraction was increased and the relative amounts of P-III and P-IV were reduced in the reconstituted dough in incorporation experiments (**Figure 6B**), whereas the relative amount of P-III significantly increased in simple addition experiments compared to the control rice dough (**Figure 6C**).

Results on the alteration of the size distribution of polymeric proteins caused by addition and incorporation of isolated HMW and LMW glutenin proteins and the individual HMW-GS proteins are presented in **Figures 3D**, **4D**, and **5D**. The statistical analysis of UPP% data after the addition and incorporation of different types glutenin proteins is shown in the last columns of **Table 2**. Addition of either HMW or LMW glutenin subunit fractions significantly reduced the UPP%. In the case of incorporation experiments a significant increase of UPP% values was observed to a larger extent for HMW-GS than for LMW-GS for both rice flours used as base flours.

SE-HPLC analysis performed on rice doughs incorporated with single HMW subunits showed that the incorporation of the same amount of By8 subunits caused significantly larger changes in the amount of unextractable polymeric proteins than Bx6 or Bx7 subunits ($p < 0.05$) (**Figure 5D**).

Table 2. ANOVA Tables of the Effects of Addition/Incorporation of Bulk Glutenin Subunit Isolates on the Mixing Properties of Rice Flour[a]

	DDT		BD		ST		UPP	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Addition								
type of rice	0.69	0.4113	2.65	0.1103	0.12	0.7253	0.33	0.5656
type of wheat	2.67	0.1086	0.85	0.3625	0.07	0.7883	0.05	0.8180
glutenin subunit	607.01	**0.0000**	67.47	**0.0000**	0.15	**0.0390**	31.34	**0.0325**
amount	446.08	**0.0000**	637.93	**0.0000**	66.36	**0.0000**	41.57	**0.0219**
LSD (calcd by *t*)	1.247		2.331		3.562		2.896	
Incorporation								
type of rice	0.27	0.6047	1.25	0.2696	0.32	0.5766	0.04	0.8481
type of wheat	18.85	**0.0000**	0.59	0.4464	19.6	**0.0000**	0.52	**0.0473**
glutenin subunit	18.78	**0.0000**	28.44	**0.0000**	18.16	**0.0000**	75.46	**0.0000**
amount	76.05	**0.0000**	52.08	**0.0000**	99.37	**0.0000**	110.37	**0.0000**
LSD (calcd by *t*)	2.662		3.167		3.729		3.405	

[a] DDT, dough development time; BD, bandwidth; ST, stability; UPP, unextractable polymeric protein determined by SE-HPLC; LSD, least significant difference (calculated from Student's *t* test). The significance has been investigated on a $p < 0.05$ level for each parameter. Bold values indicate significant relationships.

GENERAL DISCUSSION

The application of in vitro functional studies on small-scale dough testing equipment carried out with either isolated protein fractions or proteins produced by bacterial expression provided a deeper understanding of the structure−function relationships in cereal science.

The incorporation of either purified bulk fractions (*18*) or individual HMW-GS (*19*), LMW-GS (*20*), and glutenin analogue proteins (ANGs) (*21*) led to greater mixing requirements (increased mixing time and peak resistance) and increased tolerance to overmixing in wheat dough. Simply adding the same

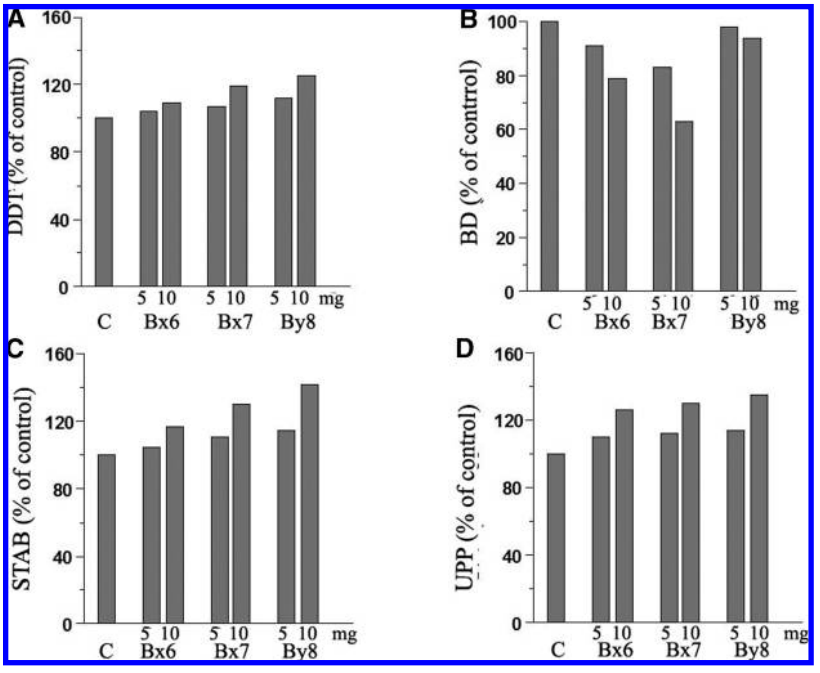

Figure 5. Effects of incorporated individual glutenin subunits on the mixing properties and polymer size proteins of rice doughs. DDT, dough development time; STAB, stability; BD, bandwidth; UPP, unextractable polymeric protein.

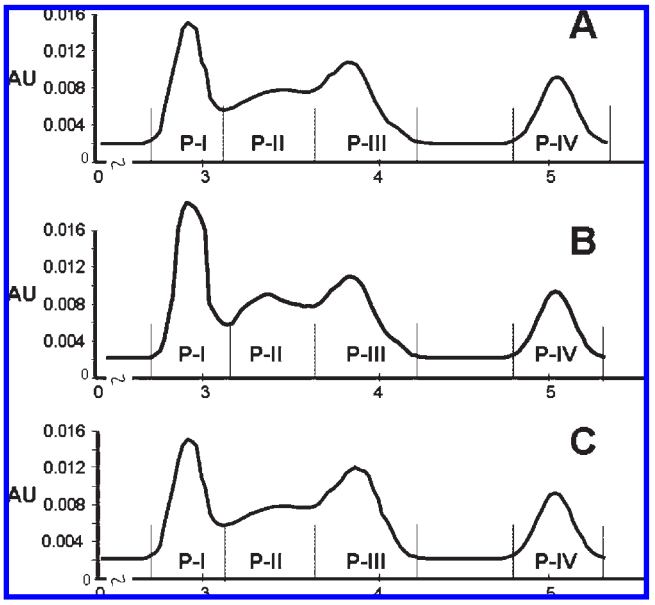

Figure 6. SE-HPLC chromatogram of the proteins extracted with sonication from the control dough. Regions used for evaluation of the dough-mixing experiments are marked on the chromatogram. P-I, large polymeric glutelin proteins; P-II and P-III, small polymers; P-IV, albumin/globulin and monomeric prolamins fraction.

proteins without the partial reduction/reoxidation based incorporation had the inverse effect: shorter mixing time, less stable dough. Studies on the effects of individual wheat glutenin subunits coded on the 1D chromosome (*22*, *23*) and the ANGs (*21*) on the functional properties of wheat dough illustrated the role of certain structural features of proteins (size, number, and positions of cysteine residues) in the extent of changes in dough properties. Altering the amounts of supplemented proteins underscored the importance of the quantitative aspects of these changes in dough properties and indicated the effects of the total amount and relative ratios of different proteins in the gluten polymer on dough strength and extensibility (*24*).

However, the extent of the effects of the supplementing wheat proteins on the functional properties of dough was found to be highly dependent on the choice of the base flour (*25*). The changes in functional parameters in these experiments were due to both the effect of the supplemented polypeptide and their interactions with the original components of the base flour. As it is known from correlative studies (*3*, *4*), only around 60 and 20% of variation in dough strength is related directly to the individual HMW-GS and LMW-GS alleles, the remaining ~20% is the contribution of the interactions among the six alleles present in a particular sample. In the case of extensibility, the contribution of interactions is even larger (*3*). To reduce the noise level induced by the protein components of the base flour in in vitro studies, wheat lines, null for certain genes—such as the lines containing single, double, and triple null alleles for HMW-GS genes, developed by Lawrence et al. (*26*)—have been successfully used in wheat reconstruction experiments. The "ideal base flour" for these experiments would be such base flours that do not contain any interacting prolamin proteins, such as lines triple null for both HMW-GS and LMW-GS.

Because the rice flour does not contain any wheat prolamin type proteins, it can theoretically provide a new approach to investigate the functional properties of wheat proteins in rice dough and compare it to wheat flours. Previous studies confirmed that dough with reasonable strength and stability can be made from rice flour supplemented with wheat gluten (*9*). The characterization of rice flour protein composition and the mixing properties of rice dough indicated that the rheological properties of rice dough are determined by the size distribution of the polymeric protein of rice flour (*10*). These results allow the development of strategies to improve the mixing properties of rice dough and furthermore to study the functional properties of the wheat glutenin proteins after they have been incorporated into the protein matrix of rice dough. The addition of individual or a bulk mixture of monomeric glutenin subunits into the rice flour effectively reduced the average molecular weight of the protein in the composite flour by altering the ratio of polymer and monomer proteins. From the results of this study, it is concluded that monomeric glutenin subunits, even in the amount of 5% of the rice flour protein content, caused significant changes in the functional properties of the dough and resulted in weaker and less stable dough. Direct addition of the monomeric glutenin subunits into the flour did not provide credible information on their real functional potential, because they did not become an integrated part of the polymeric protein matrix. Therefore, the incorporation procedure, developed originally for wheat flour dough (*6*), has been adapted to rice flour.

The glutelins—the major rice storage proteins—are soluble in diluted acid or alkaline solution. These globulin type subunits are able to form large macromolecular complexes stabilized by disulfide bonds and hydrophobic interactions (*27*). Partial reduction is presumed to open up the rice glutelin polymers through the breaking of the S−S bonds. The subsequent process of reoxidation is anticipated to restore S−S bonding and rebuild the polymer network. The results of the SE-HPLC analysis in this study indicated that careful selection of the parameters of the incorporation procedure can rebuild the polymers without altering the size distribution of the original rice polymer. Using the incorporation technique, wheat glutenin subunits can be incorporated into the polymeric network of the rice proteins, and the changes in the size distribution of the polymeric proteins as well as in the dough properties depend on the characteristics and the amount of the supplemented subunits.

The increased amount of large protein polymers in the rice dough after incorporation of wheat glutenin proteins significantly changed the functional properties of the dough. The mixing requirement of rice dough increased, and the dough was stronger and more stable compared to the control sample.

In agreement with previous studies using wheat flour as base flour in in vitro addition and incorporation types of experiments, it was found that the major governing factor to determine the mixing requirement of the dough is the size distribution of polymeric proteins. By plotting the dough development time data of the whole study as the function of the relative amount of large polymers in the dough (UPP%), this statement can be demonstrated (**Figure 7**).

In light of **Figure 7** the two in vitro experimental designs can be described simply as different ways of altering the size distribution of the polymeric proteins in the dough caused by the supplemented proteins. The extent of the alteration in polymer size is proportional with the changes in mixing properties. It is important to note that whereas the changes in mixing properties are largely dependent on the amount and nature of supplemented proteins, there is no significant effect of the rice flour used in the experiment.

The extent of the changes observed in rice dough properties was proportional to the amount of the proteins used and significantly depended on the type of the incorporated glutenin subunits (HMW-GS or LMW-GS). As found in previous wheat studies (*19*), the incorporation of bulk mixtures of HMW-GS had significantly larger effects on the mixing parameters of rice dough

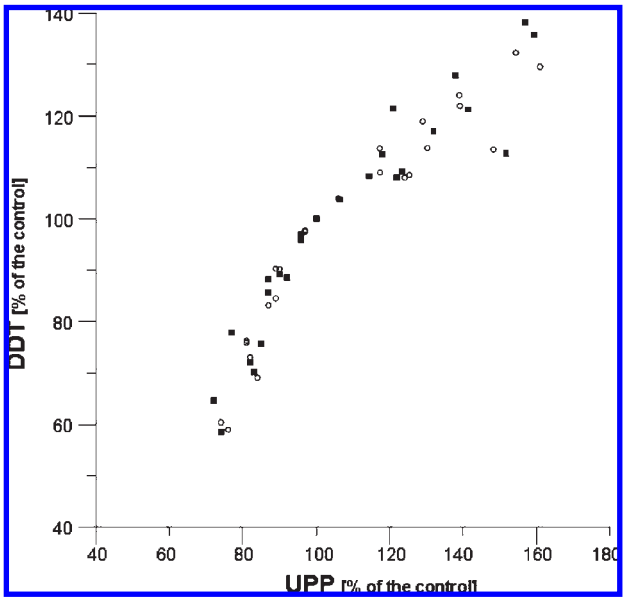

Figure 7. Effect of protein addition/incorporation on the size distribution of the polymeric proteins in the rice dough. Mixing requirement (DDT) as the function of polymer size (characterized by UPP%): (■) White rice flour; (○) Illadong rice flour.

than observed with LMW-GS. Our results show that by using this method it is possible to differentiate the effect of bulk glutenin subunits with only slightly different allelic compositions. The isolated bulk HMW-GS from Mv Suba containing Ax1, Bx7, By9, Dx5, and Dy10 HMW glutenin subunits had significantly greater effects on the mixing parameters of reconstructed rice dough than that of isolated from Mv Matyó, being different only by replacing the Ax1 subunit to Ax2*. The effect of 1B/1R translocation of wheat glutenin proteins on functional properties of rice dough was clearly demonstrated by incorporating bulk LMW-GS proteins isolated from the two samples used in this study containing identical *Glu3A* and *Glu3D* alleles. [Mv Suba contains 1B/1R translocation (Glu-3Bj allele), whereas Mv Matyó holds the Glu-3Bb allele.] It is important to note that the alterations in mixing properties of reconstructed rice doughs were demonstrated using two rice flours with significantly different mixing properties. No significant effects were observed between the two rice base flours in the reconstitution studies when the mixing parameters and value of UPP% were expressed as a percentage of the control, untreated flour.

The size of the HMW subunits and the number of reactive cysteines in the subunits play important roles in determining the functional properties of wheat dough. The incorporation of the longer size x-type subunits into wheat glutenin was found to be more effective than incorporation of the shorter y-type subunits altering mixing properties. The quantitative comparison of HMW-GS genes coded by the Glu-1B indicated a small, not significantly larger, effect of Bx7 on the Mixograph parameters of wheat flour doughs than the By8 subunit (*19*). In the case of Glu-1D HMW-GS the differences between the effects of x- and y-type of subunits are much stronger, not only for the Dx5 subunit, where the extra cysteine residue in the structure provides extra capability to form larger polymers, but for the Dx2 subunit, too (*22*).

As expected from the previous studies, the incorporation of single wheat storage protein subunits into rice dough increased the strength of the dough. The extent of the alteration seems to be generally smaller on rice dough functionality parameters than in case of wheat dough. The relative contribution of different subunits into mixing requirement of rice dough is different from that observed in case of wheat flour dough: the incorporation of the By8 subunit resulted in a stronger rice dough compared to those with incorporated x-type subunits. It was found in previous studies that the effects of incorporated subunits on the functional properties of wheat dough were strongly related to the number of available cysteine residues (*28*). One possible explanation for the stronger effects of By8 incorporated into rice dough could be the presence of an extra cysteine residue in the C-terminal of this subunit (*29*).

The smaller effects on dough properties and the different levels of contribution of subunits to dough strength when rice dough was used as a base flour instead of wheat dough clearly indicate the fundamental differences between the two experiments: in the case of rice dough—no another prolamin proteins being present—the direct contribution of the individual polypeptide was detected, whereas in wheat flour dough the interactive effects of the incorporated subunit are superposed onto the direct effect. It was found in correlative studies (*3*,*4*) that the interactive effects of a HMW-GS glutenin with different LMW-GS alleles are widely different and that the extent of these effects can be comparable with the direct effect of that allele. One of the explanations for the above results is that whereas the direct contribution of By8 subunit (because of its extra cysteine residue) is larger than those for Bx6 or Bx7 (in rice dough), the total effects of the x-type subunits in the presence of another prolamin proteins (using wheat flour dough) are larger, caused by the greater interactive effects.

The significant potential of using rice flour in incorporation experiments is that the direct and interactive effects of subunits can be investigated separately by supplementing the subunit alone and together with other subunits. It is now possible to ask specific questions about the functionality of glutenin subunits, individually or in combination, without the interference of any other prolamin type of protein present. Further research is in progress to apply this co-incorporation design to determine the interactive effects of a HMW-GS with a set of different LMW-GS.

Improving Rice Quality by Wheat Storage Proteins. Rice is one of the most important crops all over the world with rather poor dough quality compared to wheat. The molecular reason for this feature is probably due to the lack of prolamin proteins in rice endosperm. The results of this study on the incorporation of single glutenin subunits demonstrated a significant increase in the size distribution of the polymeric proteins, along with a positive relationship between the amount of glutenin proteins and the mixing parameters. These observations, in agreement with wheat studies, led to the conclusion that the increased amount of the macropolymer in the rice protein matrix leads to increased strength of rice dough. The amount and the chemical structure of the supplemented proteins are responsible for the increase in the molecular size of the polymer.

The results provided in this study also indicate that in countries where rice production is more suitable than wheat production, it could be an interesting approach to substitute rice flour with certain wheat flour constituents for the production of bread type and bakery products. This in vitro approach can be further developed with the successful introduction of glutenin genes into the rice genome by genetic engineering (*30*). It can initiate further research to develop novel rice varieties and/or novel technologies by utilizing in vivo or in vitro supplementation of wheat constituents into rice.

ABBREVIATIONS USED

BD, resistance breakdown; DDT, dough development time; DTT, dithiothreitol; HMW-GS, high molecular weight glutenin

subunit; KIO_3, potassium iodate; LMW-GS, low molecular weight glutenin subunit; PR, peak resistance; SE-HPLC, size exclusion HPLC; ST, stability; UPP%, unextractable polymeric protein.

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There was an error in Figure 5 in the version of this paper published ASAP September 23, 2009; the corrected version published ASAP October 12, 2009.

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