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Comparative Study of the Effect of Incorporated Individual Wheat Storage Proteins on Mixing Properties of Rice and Wheat Doughs

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ABSTRACT: The aim of this work was to compare the effects of incorporated wheat storage proteins on the functional properties of rice and wheat flours. The advantage of rice as a base flour compared to wheat is that it does not contain any wheat flour components and, therefore, has no interactive effect between wheat glutenin proteins. The incorporation of individual HMW glutenin subunit proteins (Bx6, Bx7, and By8) in different ratios had significant positive effects on the mixing requirements of both rice and wheat doughs. Reconstitution experiments using two x+y type HMW-GS pairs together with a bacterially expressed LMW-GS have been also carried out in this study. The largest effects of polymer formation and mixing properties of rice flour dough were observed when Bx and By subunits were used in a 1:1 ratio and HMW and LMW glutenin subunits in a 1:3 ratio. However, using the same subunit ratios in wheat as the base flour, these synergistic effects were not observed.

KEYWORDS: rice, wheat, dough, HMW-GS, LMW-GS, incorporation, reconstituted dough

■ INTRODUCTION

The mixing requirement of wheat flour and the rheological properties of the resulting dough largely depend on the composition of gluten proteins. It is generally accepted that both the qualitative and quantitative aspects of protein composition are relevant to determine the wheat dough properties.[1] The large variation of storage protein composition of wheat is caused by the significant polymorphism of the 12 prolamin coding loci. Alleles of Glu-1 and Glu-3 loci of the A, B, and D genomes define the presence of HMW and LMW glutenin subunits, whereas the Gli-1 and Gli-2 loci code for the gliadin proteins, present.

The extensive polymorphism at certain loci (particularly at the Glu-B1 and Glu-B3 loci, and at each of the Gli-1 loci) with the possible allele combinations offers great potential for biodiversity. This qualitative aspect of protein composition is further influenced by the expression levels of genes determining the absolute and relative amounts of different gene products. The effects of growing conditions (E) on the expression levels and the different sensitivities of the expression levels on the individual genes (G × E effects) provide a basis for wide variation in protein composition.[2] The high level of polymorphism in wheat prolamins (gliadins and glutenins) has a special effect on the overall functional properties of wheat dough. During dough formation, when prolamin proteins are hydrated and form the gluten network, numerous structurally similar but slightly different proteins produce a mass in which several characteristics (such as size, polarity, charge distribution, solubility, and viscosity) show a continuous distribution over a relatively large interval. It is this structural feature that makes gluten proteins unique from any other protein system.[1,3]

From previous work it was observed that allelic variation in the composition of HMW and LMW glutenin subunits of bread is associated with differences in the viscoelasticity of gluten.[4,5]

This has led to the interest in comparing the extent of different glutenin alleles/individual glutenin polypeptide contributions to the functional properties of dough, which can then be exploited in plant breeding programs.

Most of our knowledge about the "genetics of quality" or about the relationships between chemical composition and quality attributes derives from two different experimental approaches: (i) direct measurements of quality traits on samples with systematically altered chemical composition;[6] and (ii) relating quality and chemical composition/genetics of large sample populations using statistical methods. This latter approach led to the development of several predicting procedures such as the classic Payne score,[7] followed by several more recent attempts using mathematical models in which both the HMW-GS and LMW-GS compositions are considered.[8−10] The results of all of these models emphasized the fact that glutenin polypeptides do not act individually to determine the viscoelastic properties of the dough but in interaction with the other glutenin proteins present.

Using the direct measurement approach, significant information about the effects of individual glutenin subunit proteins on dough properties has been obtained by a specially designed reconstitution study: the alteration of the rheological properties of a so-called "base-flour" dough after supplementation/ incorporation of the glutenin protein of interest provided novel information about the role of these individual proteins.[24] Small-scale dough-testing equipment such as the 2-g Mixograph, the microextension tester, and microbaking facilities[11,12] have been used for these studies, allowing these experiments using only some milligrams of isolated proteins to be carried out.

Table 1. Amount of Glutenin Subunits Incorporated into Rice and Wheat Flours as a Percentage of the Total Incorporated Amount[a]

added protein	Bx6	Bx7	By8	total HMW-GS	total LMW-GS
Bx6+Bx8	100			100	
	75		25	100	
	50		50	100	
	25		75	100	
			100	100	
Bx7+Bx8		100		100	
		75	25	100	
		50	50	100	
		25	75	100	
		100		100	
Bx6+Bx8+LMW	50		50	100	0
	37.5		37.5	75	25
	25		25	50	50
	12.5		12.5	25	75
					100
Bx7+Bx8+LMW		50	50	100	0
		37.5	37.5	75	25
		25	25	50	50
		12.5	12.5	25	75
					100

[a] Bx6, Bx7, and By9, purified native HMW-GS proteins; total HMW-GS, total amount of added HMW glutenin subunits; total LMW-GS, total amount of added bacterially expressed and purified LMWG-1D1 protein.

However, the principal limitation of these incorporation studies is that the results of these experiments are largely dependent on the allelic composition of the base flour. The resulting changes in the rheological properties of the wheat dough are partly dependent on the direct contribution of the incorporated protein and also on the protein's interaction with other prolamin type proteins present in the base flour.

Rice flour does not contain any wheat prolamin type proteins, and because of this it provides a new approach to investigate the functional properties of wheat proteins. By incorporating these proteins into rice dough, we can investigate the direct effect of the protein on the functional properties of the rice dough in the absence of the interactions with the other prolamin type proteins.

Previous studies described a reduction/oxidation procedure, optimized for rice flour, that is suitable for incorporating purified wheat protein fractions into rice dough, and their effects can then be monitored on the resultant mixing properties. It was confirmed that dough with reasonable strength and stability can be made from rice flour supplemented by wheat gluten.[13]

The aim of this work was to use rice and wheat flours in incorporation experiments to determine and compare the effects of purified wheat glutenin subunit proteins individually or in combination on the functional properties of reconstructed rice and wheat doughs. We hope that by using this approach unknown or hidden properties and protein interactions can be monitored. To survey these features, dough reconstruction experiments were carried out in parallel using wheat and rice base flours.

■ MATERIALS AND METHODS

Flours. Flours produced from 'Illadong' (*Orysa sativa* L.) rice cultivar with 7% protein content and 'Hombar' (*Triticum aestivum* L.) medium-quality bread wheat cultivar with 12.1% protein content were used as the base flours in this study. Rice and wheat grains were ground with a laboratory mill (METEFEM Ltd., Hungary), and flour was sieved to obtain particles smaller than 250 μm. Moisture contents of base flours were determined according to AOAC Official Method 44-15,[14] whereas protein contents were measured with the Dumas method (N × 5.95) using an automated protein analyzer (LECO FP-528, USA).

Wheat Proteins. 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 HMW glutenin subunits coded by the B-genome.[15] LMW-GS protein was expressed in *Escherichia coli* according to the method of Ciaffi et al.[16] The coding region, without the signal peptide, of the LMW-GS gene contained in the 3.6 kb genomic fragment from *T. tauschii* was used for bacterial expression.

Isolation of HMW and LMW Glutenin Subunits. Individual HMW-GS polypeptides were isolated from Galahad flours as previously published.[13] The protein components of isolated fractions were monitored by SDS-PAGE.

LMWG-1D1 subunit protein was expressed, extracted, and purified as described by Ciaffi et al.[16] The protein was finally precipitated by dialysis with a 12 kDa cutoff dialysis tube (Sigma) against distilled water. The size and purity of the extracted protein were analyzed on SDS polyacrylamide gel.[17]

Dough Mixing. Microscale mixing experiments were carried out using rice and wheat flours as base flour on a prototype micro z-arm mixer (METEFEM Ltd.) using 4.0 g of flour per test. Individual glutenin protein isolates and their mixtures were incorporated into the base flours, systematically altering the composition of the supplemented proteins (Table 1).

The x to y ratio of the HMW-GSs was altered using either Bx6 or Bx7 subunits combined with By8 subunit. The HMW-GS to LMW-GS ratio was also varied using the particular x to y ratio at which the highest mixing requirement was measured in the previous experiments. In each of these experiments, the supplemented protein represented 10% of the original protein content of the base flours. The incorporation conditions developed earlier both for wheat[18] and for rice[13] flours were applied. During mixing, the resistance values were sampled every 0.1 s and stored electronically. Software[19] was used to determine the following mixing parameters: maximum resistance (PR), dough development time (DDT), breakdown in resistance (BD), and stability (ST). Each mixing experiment was carried out in triplicate. Doughs were frozen in liquid nitrogen and freeze-dried for further experiments.

SE-HPLC Analysis. Size exclusion liquid chromatography was carried out to determine the protein size distribution in both the unextractable and extractable protein fractions of the dough according to the method of Oszvald et al.[20] Total proteins were extracted as described by Oszvald et al.[20] 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%) was determined by applying the calculation method developed for rice flours and wheat dough, respectively.[20,21]

Statistical Analysis. All measurements were carried out in triplicate. Analysis of variance (ANOVA) was then carried out on the mean values. The Statistica 10.0 program (StatSoft, Inc. 2006) was used for statistical evaluation.
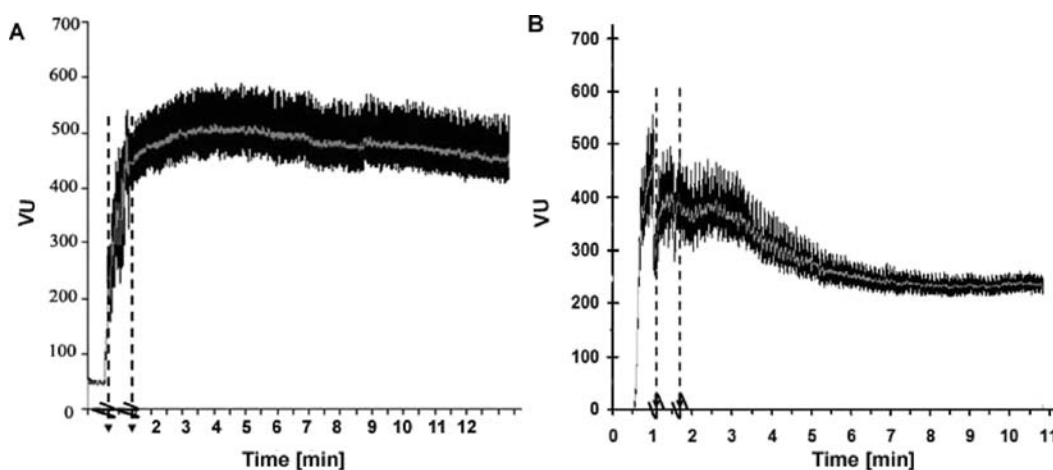
Figure 1. Micro z-arm mixer curve of 'Illadong' rice (A) and 'Hombar' (B) wheat doughs from the base flours.

■ RESULTS

Altering the x to y Ratio of the Incorporated HMW-GSs.
For functional studies, purified individual Bx6 or Bx7 as well as By8 HMW glutenin subunit proteins were incorporated into 4.0 g of flour in different ratios, representing 10% of the total protein content of the rice and wheat flours (Table 1). Blends of proteins were prepared by adding 28 and 48 mg HMW-GS fractions to rice and wheat flours, respectively. The protein content of the purified fractions was 94—96% for each type of glutenin subunit.

Two mixing curves, characteristic for the wheat and rice base flours, are demonstrated in Figure 1. Differences observed between the wheat and rice dough mixing curves can be attributed to the slow hydration process in the rice flour. This early interval in the mixing curve produced substantially noisier plots than usually obtained with wheat flour dough.[13] The incorporation of glutenin subunit proteins increased the mixing requirement (curve shifted to the right), resulting in more stable dough and increased bandwidth of the mixing curve in case of both base flours.

Incorporation of HMW-GSs with any x to y ratio increased the amounts of larger polymers in both doughs, resulting in increased mixing requirements and more stable dough. To better compare the data obtained in the different types of experiments, the measured values have been expressed as a percentage of those from the control experiment sample.

The response of the different parameters to changes in the x to y ratio is shown in Figures 2 and 3. The dough development time to reach the maximum consistency of reconstructed rice dough increased the most (by 32%) when the Bx7 to By8 ratio was 1:1 (Figure 2B). Increasing the amount of y type HMW subunits decreased the DDT value of rice dough. The same tendency was demonstrated using the Bx6+By8 HMW-GS pair. The maximum value of DDT was 30% higher compared to the control rice dough (Figure 3B).

The incorporation of x and y HMW glutenin subunits had a positive effect on the tolerance to overmixing indicated by the significantly lower BD values in the rice base flour. The highest effect on this parameter was observed by using a 1:1 ratio of Bx7 and By8 subunits for incorporation into rice flour (Figure 2C). To a lesser extent a similar effect was detected on this type of mixing parameter after incorporation of the same amount of Bx6 and By8 HMW glutenin subunit proteins (Figure 3C), this value

being 15% lower when incorporated with 7+8 HMW subunits than with 6+8 HMW subunits.

The stability of the reconstructed rice dough increased by 41, 50, 58, 44, and 39% with x:y% ratios 0:100, 25:75; 50:50; 75:25; 100:0, respectively, when 7+8 HMW subunits were incorporated, compared to the control dough (Figure 2D). The incorporation of 6+8 HMW subunit proteins showed similar effects on stability as the 7+8 protein mixture.

To study the effects of the incorporated glutenin subunits on the distribution of polymeric protein in rice dough, SE-HPLC analyses were carried out. Compared to the control dough the UPP% increased with the incorporation of the mixed individual HMW-GSs. The amount of unextractable polymeric protein fractions had shown the highest value when the incorporated HMW glutenin subunit ratio was 1:1 (Figures 2A and 3A).

Similar effects (improvements to the functional properties of the dough), but with remarkable differences, were detected for the mixing parameters of wheat dough compared to rice dough after incorporation of Bx and By HMW glutenin subunit proteins. The DDT value monotonically increased, reaching a plateau, when the amount of incorporated Bx7 subunit was increased in the HMW mixture (Figure 2B). The same tendency was observed in the case of the Bx6 and By8 HMW glutenin subunits (Figure 3B).

The HMW glutenin subunits had significant effects on other mixing parameters of wheat dough as well. The BD value of wheat dough almost monotonically decreased as the amount of either Bx7 or Bx6 subunit was increased (Figures 2C and 3C) compared to the By8 subunit, improving the tolerance to overmixing of the reconstituted wheat dough. The improved stability was confirmed by the ST parameters for both 7+8 and 6+8 HMW subunit pairs (Figures 2D and 3D). The wheat dough stability of the 7+8 HMW x+y subunit mixture at a 1:1 ratio was 10% higher than the 6+8 HMW subunit mixture.

The amount of unextractable polymeric protein fraction in reconstructed wheat dough increased monotonically, reaching a plateau when the amount of incorporated Bx subunits increased. No significant differences were observed between the effects of 7+8 and 6+8 subunits (Figures 2A and 3A).

The DDT, ST and UPP% values in the wheat flour experiments were observed to monotonically increase when the x to y ratio was increased, and each parameter in the rice flour experiments had a maximum value at the 1:1 x to y ratio. The BD values, however, showed an inverse effect, that is, a local minimum for
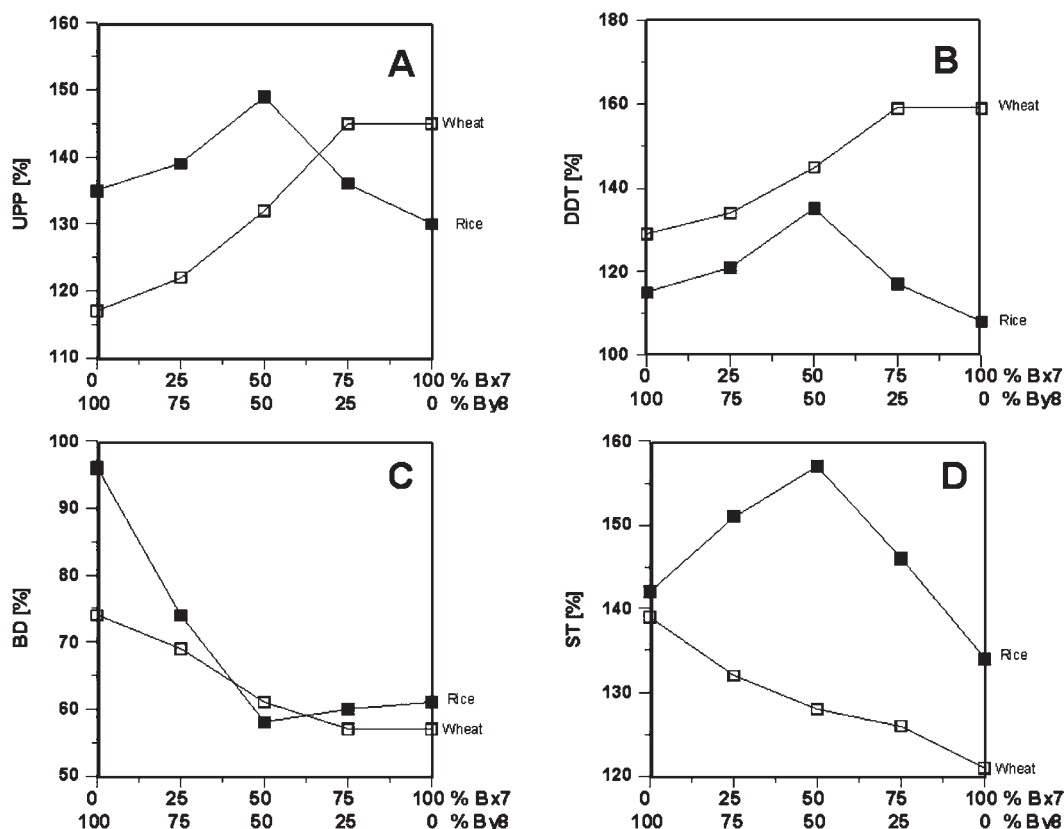
Figure 2. Effects of incorporated Bx7 and By8 individual HMW glutenin subunits on the mixing properties and polymer size distribution in the protein matrix in rice and wheat doughs: (A) unextractable polymeric protein (UPP); (B) dough development time (DDT); (C) breakdown in resistance (BD); (D) stability (ST); (■) rice base flour; (□) wheat base flour; Bx7, By8 isolated native HMW glutenin subunits. 100% value represents the parameters of the control dough.

rice flours and an almost monotonic decrease for the wheat flours (Figures 2 and 3).

Altering the HMW- to LMW-GS Ratio in the Reconstituted Dough. Incorporation experiments were carried out with native HMW glutenin subunits purified from wheat flour and the LMWG-1D1 glutenin subunit expressed in bacterial systems to alter the HMW-GS/LMW-GS ratio. The x to y ratio was chosen to be 1:1, as it was found to have the maximum effect on rice dough properties. Results of using the Bx7+By8 and Bx6+By8 pairs of HMW-GSs mixed with LMW-GS are shown on Figures 4 and 5, respectively.

The dough development time of reconstructed rice dough increased the most (by 33%) when the HMW/LMW-1D1 ratio was 1:3. Incorporating only the LMW-1D1 subunit, the DDT value was drastically reduced (Figures 4B and 5B). No differences were measured in the effect of 7+8 and 6+8 pairs combined with the LMW-GS.

With both HMW-GS pairs the LMW glutenin subunit had a positive effect on the tolerance to overmixing indicated by the decreasing BD values. The minimum value of the curve could be detected when the HMW to LMW ratio was 1:3. For both HMW-GS pairs the change in the amount of LMW-1D1 subunit had a significant effect on the BD value, particularly when incorporated with the 6+8 subunits (Figures 4C and 5C).

Calculating the stability of the reconstituted rice dough revealed similar effects from the LMW subunit as the DDT mixing parameter. The peak value was reached at 1:3 HMW to LMW ratio. Incorporation of LMW subunit alone drastically (50%) reduced the

strength of the dough in both cases, but was still, however, 20% higher than the control dough (Figures 4D and 5D).

The amount of polymeric proteins in rice dough was also increased after incorporation of the LMW-1D1 glutenin subunit. The amount of unextractable polymeric protein was at its maximum value at 1:3 ratio of the HMW/LMW-1D1 protein mixture. A similar but slightly larger effect was observed on the UPP% value when the 6+8 HMW glutenin subunit pair was used instead of the 7+8 pair (Figures 4A and 5A).

Conversely, increasing the proportion of the LMW subunit in the wheat base flour resulted in a slight monotonic decrease to DDT and increase of BD values of the dough (Figure 4B,C). The incorporated LMW-1D1 subunit did not modify the strength of the wheat dough significantly according to the stability (ST) data (Figure 4D). The amount of polymeric proteins in the reconstructed wheat dough did not change significantly during the increase of the LMW to HMW subunit ratio, but it was monotonic (Figure 4A). No major differences were observed on the wheat dough properties using either the Bx6 and By8 pair or the Bx7 and By8 HMW glutenin subunit pair (Figures 4 and 5).

It has to be noted that the bacterially expressed and purified LMW-1D1 subunit itself had positive effects on the mixing parameter and UPP% of the wheat dough compared to the control.

■ DISCUSSION

Most of the knowledge regarding the functional properties of gluten proteins is derived either from indirect correlative studies
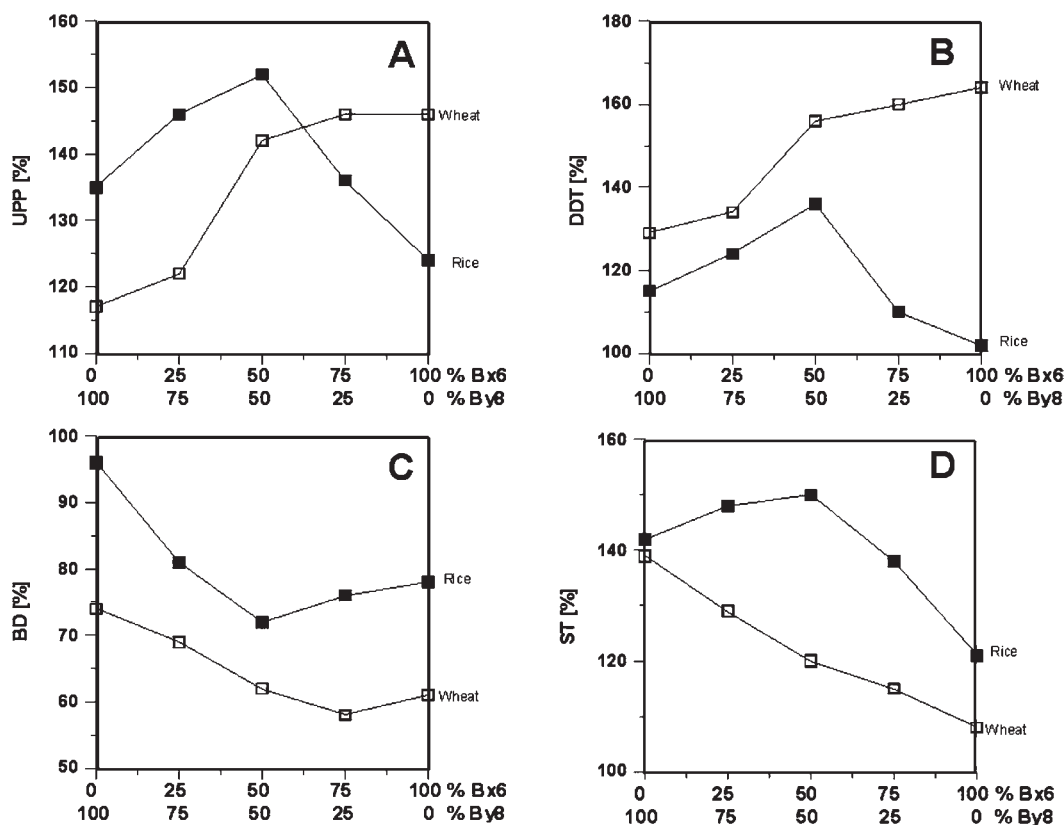
Figure 3. Effects of incorporated Bx6 and By8 individual HMW glutenin subunits on the mixing properties and polymer size distribution in the protein matrix in rice and wheat doughs: (A) unextractable polymeric protein (UPP); (B) dough development time (DDT); (C) breakdown in resistance (BD); (D) stability (ST); (■) rice base flour; (□) wheat base flour; Bx6, By8 isolated native HMW glutenin subunits. 100% value represents the parameters for the control dough.

or from direct reconstitution experiments.[6] One of the limitations of the "base flour" method is that the supplemented constituents obviously interact with the components of the flour, so depending on the choice of the base flour used, different "noise" is superposed on the measurements. Rice flour, due to the absence of wheat type storage proteins, could provide a special background to observe physical/functional variations between wheat and rice flour and to utilize different products.[22] Because of these different features of the rice flour, a rice-based model system has been developed recently in our laboratory to study the functional properties of wheat storage proteins.[13,20] Three single HMW glutenin subunits and one LMW-GS were combined in different ways and ratios to investigate their interactions in rice and wheat flour, measuring dough mixing parameters and protein size distribution in the developed dough.

The largest effects on polymer formation and mixing properties of reconstructed rice flour dough were observed when Bx and By HMW glutenin subunits were used in a 1:1 ratio. Although in agreement with previous studies, this synergistic effect of these two incorporated protein types was not observed when wheat flour was used as a base flour.[23] Uthayakumaran et al.[24] have already demonstrated that the effects of the incorporation of individual Dx or Dy HMW-GSs into a *Glu-D1* null wheat flour were lower than the effects of the Dx+Dy pairs. The synergistic effects were much higher for 5+10 than for 2+12 allele pairs, but both were peaking at a 1:1 molar ratio.[24,25] The molecular reason for this superior contribution is the extra cysteine in the Dx5 HMW glutenin subunit, which provides a branching point to the

polymer formation.[26,27] However, in the case of Bx and By type subunits, additive instead of synergistic effects were observed. Separately incorporated Bx7 HMW-GS had stronger effects than By8 or Bx7+By8 together.[26,28]

The observed changes in the functional properties of rice and wheat doughs by incorporation of the subunit type proteins are directly related to modification of the relative amount and the size distribution of the polymeric proteins in them. In the case of rice flour, the increased UPP% and mixing requirements clearly indicate the individual effects of the incorporated protein on the polymeric structure. Using wheat flour, the effects of interactions of the incorporated proteins affect the internal prolamin interactions in the base flour. Thus, the synergistic effects of x and y HMW glutenin subunits can be monitored only in the case of the very strong subunits of the Glu-D1 loci, whereas using the subunits coded by the Glu-B1 loci, the interactive effects are hidden by the complex effects observed in in vitro studies applying wheat flour as the base flour. Replacing wheat by rice flour, the synergistic effects can be clearly monitored during functional studies because of the lack of other wheat prolamin proteins. In light of the different results provided by using rice and wheat flour doughs to observe the synergistic effects of co-incorporating x and y type HMW subunits, the apparently conflicting results of using the D or B genome derived HMW-GSs can be explained.

Most of the reported x type subunits possess four conserved cysteine residues (three in the N-terminal domain, one in the C-terminal domain), and the majority of the y type subunits
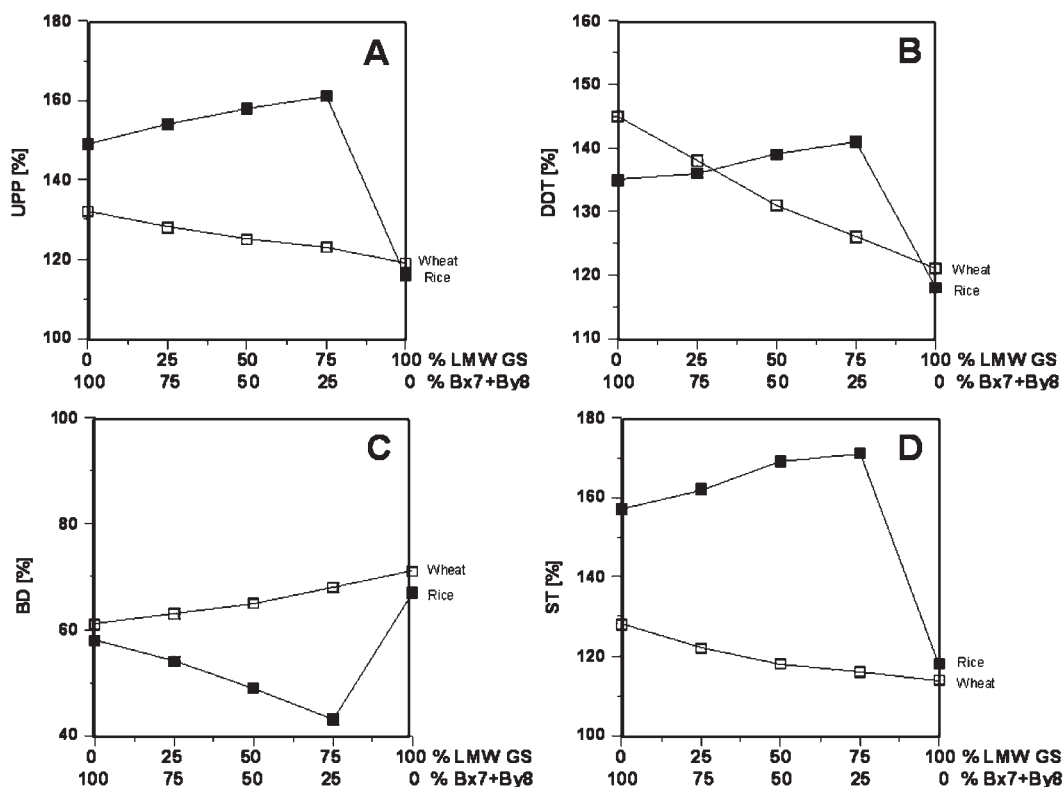
Figure 4. Effects of incorporated Bx7 and By8 individual HMW and LMW glutenin subunits on the mixing properties and polymer size distribution in the protein matrix in rice and wheat doughs: (A) unextractable polymeric protein (UPP); (B) dough development time (DDT); (C) breakdown in resistance (BD); (D) stability (ST); (■) rice base flour; (□) wheat base flour; Bx7+By8 isolated individual HMW glutenin subunits; LMW-GS bacterially expressed and purified LMWG-1D1 protein. 100% value represents the parameters for the control dough.

characterized so far contain seven conserved cysteine residues (five in the N-terminal domain, one in the repetitive domain, and one in the C-terminal domain).[29,30] These cysteine residues are involved in the formation of disulfide bonds within and between subunits and are thus important for the high-order structure and functionality of these proteins in shaping the elastic properties of the gluten complex in wheat dough.[31] These subunits are able to form intermolecular interactions with other prolamin proteins, resulting in modified rheological properties of the dough. In our previous study[13] the incorporation of the By8 subunit resulted in a stronger rice dough compared to the effects of the incorporated x type subunits. This could be attributed either to the structure of the By8 subunit or to the different interactions between the wheat and rice proteins.

The differences in the relative contribution of the B subunits to the functional properties of the doughs, particularly the relatively stronger effects of By8 in rice flour, can be elucidated. By using the wheat flour as a base flour, the alterations are related not only to the individual but also to the interactive effects caused by the incorporated protein, whereas changes in the rice dough are due to the direct effects of the individual incorporated wheat protein alone.

The LMWG-1D1 together with the HMW glutenin subunits increased the size of the polymeric proteins, the dough mixing requirements, and the dough stability, compared to both control doughs. No synergistic effects were observed during reconstruction experiments in wheat flour by any incorporated HMW: LMW glutenin subunit ratio. Compared to wheat, the rice dough mixing parameters showed maximum or minimum values when the HMW/LMW ratio was 1:3. This means that this ratio of LMW to HMW glutenin subunit proteins in rice increases the probability of the incorporation of the wheat proteins into the glutenin network, forming larger polymers, which contribute to higher quality dough properties. This ratio is within the range measured for HMW and LMW glutenin subunits that are usually deposited in the starchy endosperm of the wheat.[32]

The secondary structure of the LMW-GSs[33] is a key factor responsible for the variation in the dough development time as these proteins contain amino acid substitutions that affect polarity, charge, and side-chain structures.[29,31,34] Some noncovalent bonds in LMW subunits may also assist in creating a favorable arrangement of the proteins, which affects the mixing requirement. These may influence protein interactions in the dough system and in gluten network formation and, thereby, modify the physical properties of the dough.[33]

It has already been revealed that the HMW-GS loci differ in their contribution to dough properties in the order Glu-D1 > Glu-B1 > Glu-A1 and that this relationship is independent of protein content.[35] There were significant differences in dough development times (indicative of dough strength) between the null lines containing no or one loci and those containing two or three loci coding for HMW-GSs. The relationship between the numbers of HMW-GS loci present and increased average polymer size can be explained in part by the increased HMW- to LMW-GS ratio in cultivars containing higher numbers of HMW-GS coding loci.[24] This may also explain the increase in DDT.

Different models have been proposed over the years to understand gluten structure.[2,36] The model described by
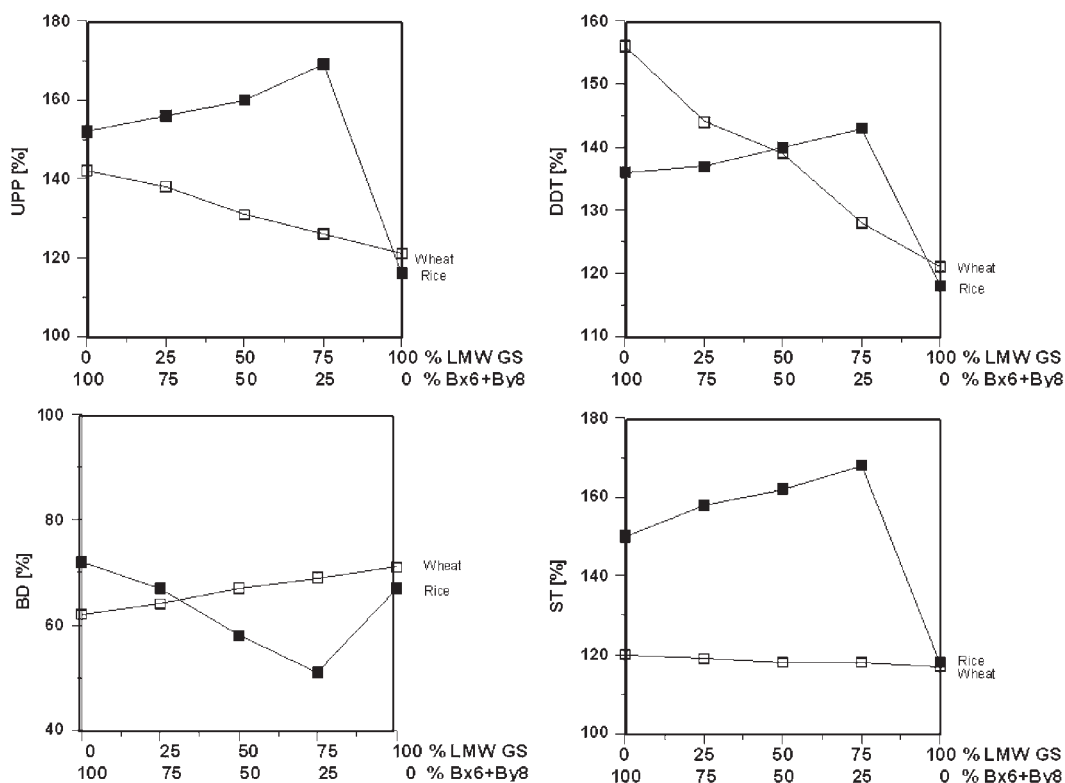
Figure 5. Effects of incorporated Bx6 and By8 individual HMW glutenin and LMW glutenin subunits on the mixing properties and polymer size distribution in the protein matrix in the rice and wheat doughs: (A) unextractable polymeric protein (UPP); (B) dough development time (DDT); (C) breakdown in resistance (BD); (D) stability (ST); (■) rice base flour; (□) wheat base flour. 100% value represents the parameters for the control dough.

Shewry et al.[29] includes one interchain disulfide bond within the N-terminal domain of an x type subunit, two parallel disulfide bonds between the N-termini of y type subunits, an interchain bond between a y type subunit and a LMW glutenin subunit, and a bond linking y and x type subunits in a "head-to-tail" fashion. Although it is now widely accepted that disulfide-linked glutenin chains provide an "elastic backbone" to gluten, evidence from spectroscopic studies (using NMR and FTIR spectroscopy) of HMW subunits and of model peptides based on the repeat motifs suggests that noncovalent hydrogen bonding between glutenin subunits and polymers may also be important.[37,38]

If the HMW-GS proteins are not present in the flour, there is no "backbone", and therefore the LMW-GS proteins could form linear polymers. The real effects of linear LMW glutenin polymers can be measured after incorporation of LMW subunit into rice flour due to the absence of HMW subunits. By incorporating both HMW- and LMW-GS proteins, these subunits are able to build a polymer resembling its native form. The maximum value in the mixing parameters of rice dough (by the incorporation of x and y type HMW-GSs at a 1:1 ratio and LMW- and HMW-GSs at a 3:1 ratio) indicates that either the head-to-tail or the backbone polymerization of glutenin subunits is possible in rice flour.

LMW-GS had smaller effects on the functional properties of rice dough than HMW-GSs when applied in the same amounts and at the same polymer/monomer protein ratio. A possible explanation is that the magnitude of the effect is greater on polymers with longer backbones (caused by greater numbers of HMW-GSs) than longer branches (caused by greater numbers of LMW-GSs).

This is the first experimental result when synergic effects have been observed between Bx and By type HMW-GSs at a 1:1 ratio

and between HMW and LMW glutenin subunits at a 1:3 ratio when incorporated into the rice base flour. The observed optimum LMW to HMW ratio for the maximum polymerization of glutenin subunits, which results in the highest amount of UPP% in rice, is similar to the LMW:HMW ratio in common wheat flour.

By incorporating glutenin subunits into the polymeric structure of rice flour proteins, the mean of the size distribution of the polymeric protein increases. Furthermore, the conformation of the polymer might change due to the altered charge distribution and hydrophobicity, and therefore the number of interactions may change among the subunits through secondary forces. By monitoring the effects of incorporation through the alteration of mixing requirement, the overall results of these effects can be observed; however, the contributions of direct and interactive effects are unknown and hidden. By further using both wheat and rice flour doughs in incorporation experiments, some new information can be deduced from the results.

Our previous findings have shown the potential of using rice flour as a model sytem to characterize functional properties of wheat storage proteins;[13] the results presented here further support these findings.

The variation of wheat and rice flour as base flour provides a possibility to study the effect of a particular protein and determine the individual and interactive effects on two related but different aspects, namely, on polymer formation and on the contribution to the viscoelastic properties of the dough. This novel information provides a deeper understanding of the structural/functional relationships of wheat glutenin proteins and can be applied to improve wheat quality. For example, in

breeding programs, the real value of a certain allele has to be investigated in several backgrounds to be able to realize its interaction potential. Because of the large contribution from allele−allele interactions, the different allelic combinations, rather than the individual glutenin alleles, can be the real target in breeding situations for developing new lines with the desired functional qualities. The interactive effects of the alleles present in commercial wheat flour blends are responsible for the well-known industrial problem: dough properties, such as dough strength and extensibility, are not simply additive characteristics; they show usually nonlinear relationships with the blend formulation.[39]

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■ ACKNOWLEDGMENT

■ ABBREVIATIONS USED

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