# In vitro and in vivo studies of wheat storage proteins on dough quality in model system

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## INTRODUCTION

A unique property of the wheat flour is its ability to form dough when it is mixed with water. This property is largely determined by the ability of the hydrated component proteins to form inter-chain disulphide bonds, with the glutenins consisting entirely of disulphide stabilised polymers. Most of our knowledge on the functional properties of the gluten forming proteins is derived either from indirect correlative studies, or from direct reconstitution experiments (Payne, 1987). One of the limitations of the so called 'base flour' method is that the supplemented constituents obviously interact with the components of the flour, so depending on the choice of base flour used different levels of 'noise' are superimposed on the measurements. An ideal solution to avoid this problem would be to use base flour not containing any wheat flour components. Because of the fact that the structure and properties of the storage proteins of wheat and rice are different enough (Muench and Okita, 1997), in our studies rice was chosen as a base flour to explore the functional characteristic of wheat storage proteins in our studies (Oszvald et al. 2006).

Rice is one of the most important crops all over the world. In countries where rice production is more suitable than wheat because of the climatic conditions, it could be an interesting approach to partially substitute rice flour for wheat flour in bread and bakery products. However, dough-making quality of rice flour is very poor compared to wheat, mainly because rice endosperm lacks the proteins responsible for this trait.

The structure and properties of the storage proteins of wheat and rice are significantly different. While the rice proteins are mostly globulins, the major seed storage proteins in wheat are prolamins. 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. These differences provide the molecular background of the observed physical/functional differences between wheat and rice flour and their utilization in different products.

Studies of structure-function relationship of the wheat storage proteins can be carried out by altering the composition of the base flour, either by the addition or the incorporation of different protein components (single protein or purified fractions) and measuring the effects on the dough mixing properties. Direct addition of the monomeric glutenin subunits to the flour does not provide credible information of their functional roles, since they do not become integrated part of the polymeric protein matrix (Schropp and Weiser, 1996). Therefore, incorporation technique was developed to get appropriate information on their function in dough formation (Butow et al. 2003). Due to the development of '*in vitro'* incorporation procedure for wheat flour, meaningful data on the functional properties of individual wheat glutenin subunits became available. The effect of reducing agents on the dough properties can be attributed to the opening of the disulfide bonds within the protein matrix. The effects of oxidizing agents are the results of cross-linking of the sulfhydryl groups, forming disulfide bonds, which increases the average molecular weights of the polymeric proteins in the dough (Békés et al. 1994; Tamás et al. 1998).

The incorporation of HMW-GS proteins lead to greater mixing requirements (increased mixing time and peak resistance) and increased tolerance to over mixing in wheat dough. For individual wheat glutenin subunits coded on 1D chromosome, the effects of the various subunits have been studied to see if they differ in their contributions to wheat dough properties (Schropp and Weiser, 1996). The results show a significant positive correlation between subunit size and mixing parameters.

Supplementing rice flour components with wheat storage proteins – either by *in vitro* methods or by *in vivo* transformation – could improve our basic understanding about the possibilities of improving/altering the functional properties of rice/wheat flour.

In this study, components of wheat gluten such as glutenin rich fractions and individual HMW glutenin subunit proteins have been obtained in *in vitro* incorporation experiments. To study the relationship between HMW subunits and rice dough strength, gene encoding Dx5 HMW-GS protein was expressed in transgenic rice. To monitor chemical and functional alterations of the rice dough after incorporation of wheat proteins, analytical and functional methods was adapted from wheat flour studies.

## MATERIALS AND METHODS

A Hungarian type of wheat, Mv-Suba was used as a source of both HMW-GS and LMW-GS proteins bulk fractions, carrying the 2*, 7+9, 5+10 and c, b, b alleles, respectively. A set of double mutants Galahad wheat cultivars, containing single HMW-GS subunit, like Bx6,

Bx7 and By8, were also used to isolate HMW-GS proteins. HMW-GS and LMW-GS fractions were isolated from wheat flours as described previously (Verbruggen et al. 1998).

A reduction/re-oxidation procedure was adapted for rice dough based on the method originally developed for wheat flour dough (Oszval et al. 2006).

The effects of incorporation of wheat storage proteins on the functional properties of rice dough were investigated on a prototype micro z-arm mixer (Metefem Ltd, Hungary) (Haraszi et al. 2004).

Rice dough samples were taken during mixing at peak and characterized by SE-HPLC using the method developed by Batey et al (1991).

Genetic transformation of matured rice was carried out using biolistic method (GENEBOOSTER, Gödölllő, Hungary) as described by Oszvald et al. (2007).

Transgenic rice lines were identified by using PCR and RT-PCR (D'ovidio et al.1994). Alcohol soluble proteins, extracted from the endosperm of three independent transgenic rice lines were separated on SDS-PAGE (Leammli, 1970).

## RESULTS AND DISCUSSION

### Protein incorporation into the rice flour

Micro z-arm mixer was used to determine the effects of the incorporation of wheat storage proteins into the rice dough, using reduction/oxidation procedure (Oszvald et al. 2006). The following parameters were determined: DDT (dough development time), VUmax (peak resistance), BD (resistance breakdown) and stability (ST). For functionality tests, 14, 28 and 56 mg of bulk fractions of HMW-GS and also LMW-GS proteins, corresponding to 5, 10 and 20% of the total protein content of the rice flour were incorporated into the dough. For individual glutenin subunits coded on 1B chromosome (Bx6, Bx7 and By8), the effects of the 14 and 28 mg of various subunits have been studied on functional properties of rice dough. Statistical comparison of the mixing parameters for the study is summarised in Table 1.

Table1. The effects on mixing parameters of incorporation of glutenin subunits into rice dough

| Sample | VUmax | | DDT [sec] | | BD [VU] | | ST [sec] | |
|---|---|---|---|---|---|---|---|---|
| | mean | SD | mean | SD | mean | SD | mean | SD |
| **Control** | 585 | 15.15 | 240 | 11.54 | 95 | 6.25 | 96 | 5.21 |
| **HMW-5%** | 546 | 20.21 | 262 | 21.36 | 78 | 5.54 | 109 | 4.21 |
| **HMW-10%** | 583 | 12.58 | 274 | 14.78 | 70 | 4.98 | 117 | 3.54 |
| **HMW-20%** | 596 | 11.12 | 326 | 20.87 | 49 | 5.69 | 152 | 6.78 |
| **LMW-5%** | 555 | 19.84 | 249 | 17.21 | 62 | 8.12 | 99 | 7.21 |
| **LMW-10%** | 564 | 16.27 | 260 | 13.25 | 60 | 3.24 | 109 | 7.89 |
| **LMW-20%** | 567 | 21.43 | 291 | 12.41 | 55 | 4.32 | 146 | 11.02 |
| **G6-5%** | 537 | 12.54 | 250 | 20.24 | 86 | 5.21 | 101 | 3.56 |
| **G6-10%** | 572 | 18.78 | 262 | 12.17 | 75 | 2.31 | 112 | 4.51 |
| **G7-5%** | 469 | 14.35 | 256 | 11.45 | 87 | 4.56 | 107 | 6.87 |
| **G7-10%** | 469 | 11.23 | 286 | 10.34 | 72 | 5.12 | 125 | 4.52 |
| **G8-5%** | 472 | 10.12 | 268 | 18.64 | 68 | 5.64 | 110 | 3.98 |
| **G8-10%** | 521 | 15.28 | 301 | 17.63 | 57 | 3.25 | 136 | 7.41 |

Incorporation of 5, 10 and 20 % wheat HMW glutenin subunits rich fraction increased rice dough strength raising DDT by 8.39%, 12.41% and 26.38%, respectively. Positive effect on tolerance to over mixing was also observed, which is indicated by the significantly lowered BD value. Using the same reduction/oxidation technique to incorporate bulk fractions of LMW-GS, it was found that their effects on mixing properties were proportionally smaller than for HMW-GS. The incorporation of 5%, 10% and 20 % LMW-GS proteins increased DDT by 3.61%, 7.69% and 17.52%, respectively.

The results show also a significant positive effect of incorporated individual HMW-GS proteins on rice dough mixing requirements increasing DDT, as well as the stability and the tolerance to over-mixing. Incorporation of 10% single HMW proteins had a greater effect than 5% subunits in every case, however in a slightly different extent. Differences were significantly bigger for the By8 HMW-GS (Fig. 1).

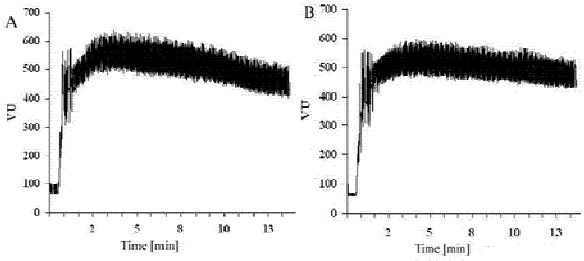


Fig.1. Mixing curve of (A) untreated rice dough and (B) rice dough with incorporated with 10% By8 HMW-GS.

The incorporation of bulk HMW-GS, LMW-GS fractions and the single Bx6, Bx7 and By8 HMW subunits into the protein matrix of the rice dough was clearly demonstrated by SE-HPLC analysis performed on reconstituted doughs. Unextractable polymeric protein (UPP%) values, a widely used parameter for characterizing the size distribution of polymerized proteins in wheat flour, were also determined for each rice dough samples. SE-HPLC analysis showed that the incorporation of each glutenin fraction increased the UPP% value of reconstituted doughs as compared to the control. According to the observations on size distribution of polymeric proteins, we can say that the incorporation of HMW-GS contributing the highest UPP% when compared with other flours (data not shown). The size distribution of polymeric proteins in reconstituted rice doughs using various glutenin subunits shows a positive correlation with the mixing parameters of doughs.

The incorporation of wheat storage protein subunits also increased the strength of the dough, as predicted from earlier wheat dough reconstruction studies (Butow et al. 2003), however the effect was weaker in case of rice dough. The only one exception was observed in case of the use of the single By8 HMW-GS. It had larger effect on mixing parameters in case of rice dough than the

single x-type subunits, could be the result of the altered interaction between rice and wheat storage proteins.

**Rice transformation**

In order to study the effects of individual HMW-GS on functional properties '*in vivo*', mature seeds of rice (*Orysa sativa* L.), genotype T-309, was transformed with a cassette containing the native gene encoding for the Dx5 HMW-GS protein. It was developed several independent PCR positive transgenic rice lines. Alcohol soluble proteins, extracted from the endosperm of three independent transgenic rice lines were separated on polyacrylamide gel. A new band appeared on SDS-PAGE, which confirmed the presence of an extra protein in the endosperm of the T1 transgenic rice seeds (Fig. 2). The relative electrophoretic mobility of Dx5 HMW-GS protein was similar to the corresponding endogenous wheat proteins. It was also confirmed that wheat endosperm specific promoter drives protein expression only in the endosperm of transgenic rice.

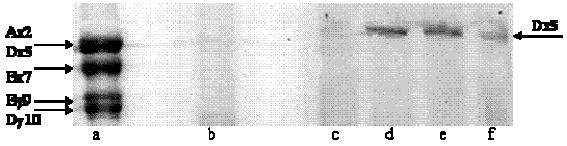


Fig. 2. SDS-PAGE of alcohol soluble seed proteins extracted from wheat flour (a), and rice flour (b) of controls, regenerated non-transgenic rice line (c) and transgenic rice lines (d-#21, e-#23, f-#26).

Amount of the expressed HMW-GS protein in transgenic endosperm was determined by densitometric analysis of polyacrylamide gels. Transgenic rice lines #21, 23 and 26 expressed HMW-GS 1Dx5 at level accounts for 0.75%, 3.19% and 3.81% of the alcohol soluble grain protein, respectively.

The effect of the transgene on dough mixing properties was determined using micro z-arm mixer. Results indicate that transgenic rice lines had increased dough strength (data not shown).

## CONCLUSION

This work describes that the reduction/oxidation procedure optimised for rice flour is suitable to incorporate purified wheat protein fractions into the rice dough and monitor their effects on the mixing properties of it.

It has been proved that wheat prolamin incorporation has significant effects on rice dough in small-scale experiments. It is now possible to ask specific questions about the functionality of glutenin subunits, individually or in combination.

The expression of wheat *Glu-1Dx5* HMW-GS gene in transgenic rice makes possible to investigate the interaction of the wheat protein with different rice protein molecules and provide an option to analyse the quantitative and qualitative effects on functional properties of rice dough.

An interesting aspect of the possible improvement of the baking quality of rice, is the fact that people allergic to wheat globulins, and therefore being on strict wheat-free diet, could possible enjoy baking products made from rice flour in the future.

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