

Modelling Water Absorption of Wheat Flour by Taking into Consideration of the Soluble Protein and Arabinoxylan Components

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Damaged starch, protein and arabinoxylan (AX) content and composition have been related to water absorption (WA) in a large set of samples. We tested 20 modern bread wheat cultivars bred in Hungary, 20 old Hungarian landraces, and 17 cultivars with special biochemical/functional characteristics from all around the world, this last set for international comparison. Grain was field grown in the 2011 and 2012 harvest seasons. A linear mathematical model has been developed to estimate WA from protein content, starch damage, AX content and the relative amount of soluble proteins with strong correlation ($r^2 = 0.65$) between measured and estimated data. The introduction of a new parameter, related to the cultivar dependent quantitative composition of soluble proteins and determined by lab-on-a-chip (LOC) analysis, largely improved the predictability of WA. Based on the large variation among the level of AX and certain soluble protein components in wheat flour and their significant contribution to WA determination, it was concluded, that these properties could be appropriate target traits to alter them during wheat breeding programs to improve the WA of wheat flour.

Keywords: wheat, water absorption, arabinoxylan, soluble protein, modelling

Abbreviations: AP – sodium chloride extractable proteins; APR – albumin protein ratio = A1 + A2 protein fractions on LOC; E – environment; G – genotype; Glu/Gli – quantitative ratio of the glutenins and gliadins; LOC – lab-on-a-chip analysis; T – technology; TOTAX – total arabinoxylan; TOT A/X – quantitative ratio of total arabinose and xylose residues; UPP – unextractable polymeric proteins; WA – water absorption; WEAX – water extractable arabinoxylan; WE A/X – quantitative ratio of water extractable arabinose and xylose residues

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Introduction

In the last decade the requirements for bread-making quality of wheat cultivars varied significantly. Until recently, the term ‘superior bread-making quality’ mostly covered only parameters such end-product quality attributes as loaf volume and textural parameters. These characteristics are directly related to the balance of dough strength and extensibility. Our knowledge from their relationships to the composition of storage proteins altered significantly in the last decade (Wrigley et al. 2006). Together with this new level of knowledge on the traditionally considered quality parameters, however, several new aspects of ‘quality’ became more important and require deeper understanding of the genetics, and of the composition of wheat flour. Traits, directly related to the economics of production such as the milling yield, the amount of water and energy required to dough with optimal consistency became significantly more important than earlier.

Water absorption (WA) – the amount of water needed to hydrate flour components to produce dough with optimum consistency – is one of the most fundamental quality parameter of wheat flour (Bushuk and Békés 2002). Based on this definition, WA can be interpreting as the function of the relative amounts of the components capable to be hydrated (starch, proteins, pentosans) and their specific water binding capacity.

During the milling process, a part of the starch granules, depending on the hardness of the grain, is damaged with the result that they absorb more water than native starch, and become more readily available to starch degrading enzymes. In conclusion, damaged starch is one of the characteristics which determined not only by the interaction of the genetic makeup and the growing conditions but is highly variable caused by the milling technology used to mill the flour (GxExT). Early experimental data of the protein and damaged starch components that contributed to the Farinograph WA of the flour varied from one to two times of the dry weight, occasionally even more (Moss 1961). These observations led to the development of several regression models predicting WA as the function of either protein and damaged starch content or protein content and wheat hardness (reviewed by Roels et al. 1993). Wheat hardness was used for decades in wheat breeding to select for WA. Slowly reaching the limits of grain hardness measurements, other approaches, leading to improved WA, were required. One of the possibilities is the better utilization of the water binding potential of soluble protein- and pentosan-components. Experiments carried out with flour supplements of different protein classes, resulted the following observation: mixing requirements, dough strength and extensibility depended significantly on the glutenin to gliadin ratio, while the water absorption was not sensitive to that ratio (Uthayakumaran et al. 1999), but was sensitive to the ratio of the gluten- to soluble-proteins (Tömösközi et al. 2004). Supplementing wheat flour with soluble proteins of different origin and polarity showed that polarity/hydrophobicity as well as the charge distribution of albumins and globulins are the key features changing the amount of water needed for hydration (Tömösközi et al. 2002).

Pentosans form a small fraction of the flour (2 to 3%), but have a large effect on its water absorption (Holas and Tipples 1978). Their water absorption capacity is estimated to be 10 times of their own weight and so, they hold one-fourth of the dough water (Kulp

1968). The limited information that we have on the relationships between the pentosan composition and water absorption of flours indicated that arabinoxylans (AX) components have the major effect on the WA, especially the soluble small and medium sized AXs (Primo-Martin and Martínez-Anaya 2003). The active research interest on AX in recent years focused on its effects having on the nutritional and some functional properties of wheat flours (Biliaderis et al. 1995; Skendi et al. 2010; Duyvejonck et al. 2011; Saeed et al. 2011; Rakha et al. 2013). Based on the variation of AX content caused by genetic and environmental factors it is supposed that quality improvement could be achieved by considering this minor component during wheat breeding (Saulnier et al. 2007; Dornez et al. 2008).

The current research summarized in this paper is part of a larger study investigating the $G \times E$ effects on the chemical composition and functional properties of old and modern Hungarian wheat cultivars and landraces in comparison with the international findings. The particular aim of the work presented here was to collect elementary information on the variation of soluble protein and AX components of the flours and use them to find the best model for the estimation of wheat flour water absorption.

Materials and Methods

Sample population

In the 2010 season, 402 landraces and 955 wheat cultivars and breeding lines have been grown at the Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary. Fifty-seven cultivars have been selected based on the agronomic field performance, bread-making quality and laboratory tests – with emphases on HMW and LMW glutenin allelic composition (Baracskaei et al. 2011; Kovács et al. 2013). These include 20 modern bread wheat cultivars, 20 old Hungarian cultivars and landraces as well as 17 international cultivars with special compositional and functional characteristics (Table S1*). The selected cultivars were field grown in triplicates in small plots in the 2011 and 2012 and harvested seeds were conditioned to 15.5% moisture content and milled with a Chopin CD1 mill to produce white flour.

Size exclusion high performance liquid chromatography (SE-HPLC)

The albumin/globulin content of the samples was determined in triplicate applying the extraction protocol of Singh et al. (1990) followed by the separation procedure of Batey et al. (1991).

LOC based analysis of soluble proteins

Proteins were extracted from flour samples according to the sequential procedure of Uthayakumaran et al. (2005) with some modifications (Balázs et al. 2012). Flour (40 mg) was extracted with 250 μ l MilliQ water and then with 250 μ l of 0.5M NaCl successively at

* Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

room temperature for 30–30 minutes for albumins and globulins. Centrifugation of the samples were carried out at 1500 rpm for 10 min to provide clear supernatants, and extracts (4 µl each) were added to Agilent sample buffer and transferred to the 10 sample wells of a Protein 230 series II LabChip of the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) according to the manufacturer's instructions. Each sample contained internal standards, with upper marker of 240 kDa and lower marker of 4.5 kDa. The evaluation was performed with Agilent 2100 Expert software.

Arabinoxylan analysis

The GC based analytical method of Courtin et al. (2000) has been adapted to determine the total and water soluble arbinoxylan contents of the flours (TOTAX and WEAX, respectively), their ratio (WEAX/TOTAX) and the arabinose to xylanose ratios in them (TOT A/X and WE A/X, respectively.)

Functional analyses

The following methods and instruments were used to determine the functional properties of the lines: Protein content: Kjelttec 1035 Analyzer (AACC 46-10), Wet gluten spread: MSZ 6369/5-87 Hungarian standard, Zeleny sedimentation test (AACC 56-61.02 or ICC 116/1), Farinograph (ICC115/1, MSZ6369/6-1988) and Chopin Alveograph (ICC 121). Additionally, a prototype Micro-Zeleny tester (Tömösközi et al. 2009) has also been used to get micro-scale sedimentation value and in case of the 2011 samples the MixoLab equipment (Koksel et al. 2009) and the Kieffer rig (Kieffer et al. 1998) were also applied to characterize mixing properties and determine dough resistance (Rmax) and Extensibility (Ext).

Statistical analysis

Statistical analysis was carried out using STATISTICA 9.0 (StatSoft, Inc. 2006, Tulsa, OK, USA). Significant differences among samples and treatments were characterized by ANOVA method.

Results

Overall means of all studied compositional and functional traits together with the minimum and maximum values determined from the two consecutive growing seasons are summarized in Table S2.

The results of the statistical evaluations carried out on the data shown in Table S2 are tabulated in Tables S3 and S4. Table S3 shows the results of an ANOVA, comparing the soluble components of the flour and their water absorption in the three subgroups (modern Martonvásár-bred cultivars, old Hungarian cultivars and landraces and international samples set). The effects of genetic factors and harvesting years on the same parameters are characterised by the F and p values from ANOVA are illustrated in Table S4. These effects on the gluten proteins and dough properties in the same sample population have been investigated by Rakszegi et al. (2012).

The matrices of linear correlation coefficients describing the relationships between protein and AX composition parameters and functional characteristics of samples harvested in the 2011 and 2012 season are shown in Table S2.

Systematically varying the parameters considered, a multiple linear regression investigation have been carried out to find the best fitting model to estimate the water absorption from their protein content, damage starch level, soluble protein and total AX content of samples harvested in 2011. The linear correlations comparing the measured and calculated WA values for the different models are shown in Table S5. The best model (Model 15 in Table S5) – applying all the above parameters – has been validated on the sample population harvested in 2012.

The model has been improved using the 2012 sample set by a further parameter derived from the LOC based analytical characterization of the sodium chloride extractable proteins (AP). LOC separation profiles of some samples are shown in Fig. S1. Relating the relative amounts of proteins at different intervals to water absorption of the samples, it was found that the ratio of the areas under the profiles A1 (20.1–25 s) and A2 (38.5–41.5 s) – signed as “APR”, showed stronger relationship to WA than any individual chemical composition data ($r^2 = 0.503$, Table S4).

The multiple regression modelling has been repeated involving the APR ratio. The resulting correlations between measured and predicted WA values are shown in Table S5.

Finally the Model 15 on Table S5 was found to be the best for the prediction of the flour water absorption. This model takes into consideration such properties, as the albumin/globulin ratio, the starch damage, the TOTAX and the APR next to the protein content of the flour.

Discussion

Bread-making quality of wheat flour is determined by its constituents. While important factors of mixing properties and dough rheology such as mixing requirement, dough strength and stability, extensibility etc. are mostly determined by the qualitative and quantitative composition of gluten proteins, water absorption – as well as the texture of the end-product – strongly related to other components than gliadins and glutenins. An integrated approach is required, trying to fully cover the relationships between flour composition and quality with the aim of improving quality, where the variation caused by both genetic and environmental factors on the composition of soluble proteins, starch and non-starchy carbohydrates and their effects on particular quality attributes have to be investigated beside the traditional gluten protein based characterization of samples.

Comparison of variety groups based on their quantitative protein and AX composition

As it is illustrated in Table S2, the 57 cultivars grown in two consecutive seasons represent a population, which varied widely by both in chemical composition and quality. Comparing the overall mean values of the data measured in 2011 and 2012 (last columns in Table S1), 5.7, and 10.0 relative percentage differences have been found for the protein and TOTAX contents of flours, respectively. The distribution among the different protein

classes showed even larger variation from –2.5 and –4.5 to 19.2%, for glutenins, gliadins and soluble proteins, respectively. As a consequence of the variation in chemical composition, 2–25 relative % alteration was observed in the overall means for the different quality parameters, with the largest value (–25.2%) for gluten spread and the smallest (2.1%) for water absorption.

Statistical analysis, comparing the quantitative protein composition of the three subpopulations (Table S3) showed interesting trends for the specific characteristics of the Hungarian wheat cultivars compared to the subgroup of the international standard varieties. At the same time the achievements of breeding activity to improve quality of Hungarian varieties compared to the old landraces was also visible. This was partly demonstrated by the comparison results on the HMW and LMW glutenin allelic composition of old and modern Hungarian germplasms, which have already been discussed by Baracskaei et al. (2011) and Kovács et al. (2013) and the F and p values of the ANOVA analysis carried out here. While the WA showed an overall balanced picture, there were significant differences among the three subpopulations for each of the other investigated proteins and the AX parameters. Old Hungarian landraces and cultivars contains significantly higher protein content than the another two subgroups, while both old and modern Hungarian germplasm contained significantly less TOTAX than the international varieties. Old Hungarian samples contained comparable amount of WEAX to the international controls, while significantly higher values have been found for the modern Hungarian cultivars. Two varieties, Mv Suba and Mv Marsall from this latter subgroup specifically had significantly higher WEAX content in the entire population.

As it was reported earlier (Juhász et al. 2000a, b), wheat cultivars of the Carpathian basin contain relatively higher amount of gliadins than the world average, so the glutenin to gliadin ratio is traditionally and characteristically low. This characteristic is an important contributor to the superior bread-making quality of these landraces, providing the basics for the good extensibility and appropriate dough strength, resulted by the special HMW glutenin alleles (*Glu-A1x* and *Glu-B1a1*) present in some old Hungarian landraces (Juhász et al. 2003; Bedő et al. 2005). These special characteristics are also giving also good reasons to use these landraces in further crossing programs during breeding to improve quality.

The wide interval of UPP% values observed for both old and modern Hungarian cultivars (Table S2) is clear indication of the fact that these cultivars are utilized as source materials for a range of baking products from traditional sponge and dough type of Hungarian bread to different buns and special egged noodle products, requiring significantly different dough strength and extensibility. Based on the variance and the overall mean of the UPP%, the modern Hungarian cultivars reached slightly surplus related to the values of the international standard group and some of these cultivars, like Mv Mazurka and Mv Karizma reached the UPP% level of the Canadian cultivar, Glenlea.

Analysis of variance carried out to demonstrate the effects of the genetic (G) and environmental (year) factors (E) on the quantitative chemical composition and water absorption of the flours (Table S4) indicated, that the actual values of each parameters were significantly ($p < 0.001$) affected by both of these factors. Based on the comparison of the

F values, the investigated parameters could be divided into three groups. The effect of G was larger than that of E on TOT A/X, WE A/X and UPP%. In the second group, the extent of the two effects were comparable on WEAX, GLU/GLI and WA, while in case of protein content, TOTAX, glutenin%, gliadin% and soluble protein% the effect of E factors were larger than those caused by G factors. It has to be noted that highly significant ($p < 0.001$) $G \times E$ effects have been found for each chemical parameter, but no interactive $G \times E$ effect has been observed for water absorption.

Modelling water absorption with the use of quantitative protein and AX composition

Linear correlation coefficients (r^2) tabulated in Table S6 represent a remarkable apparent conflict with the well-established knowledge about the relationships between certain quality attributes and chemical composition. Apart from a few cases (each of them ratios of two different protein classes, UPP and HMW/LMW ratio), no significant relationship was found between any functional parameter and protein or AX contents. The everyday practice of wheat quality evaluation and also numerous research works carrying out experiments by systematically altering the chemical composition of flour using supplementary flour components have already been determined certain effects of the protein content or the Glu/Gli ratio, etc. (Uthayakumaran et al. 1999; Wrigley et al. 2006). The relative amount of only certain supplementary components were altered in these special designed investigations, but the interference of many other components were not taken into consideration. The explanation of the observed conflicts between the results of the direct supplementation and the correlative studies was the existence of certain physical, physiochemical and even chemical interactions in the real flour affecting the final functional properties of wheat. To clearly demonstrate the effect of certain components in correlative studies, multiple regression models had to be used. In the particular case of water absorption, its combined relationships with each of the water binding compounds have been investigated by multiple regression analyses. It is known since decades that protein content and starch damage together provide a weak but meaningful estimate of water absorption. It is also well established that the hydration properties of the native arabinoxylans have a direct influence on the ability of flours to absorb water, while the high water-holding capacity of the cross linked polymers (obtained via oxidative coupling of feruloyl residues) affect the distribution of moisture among dough constituents (Izydorczyk and Biliaderis 1992; Wang et al. 2003). These effects alter not only the rheological properties of the dough but they reduce the availability of “free” water in the dough to hydrate starch and protein components, and to keep water soluble proteins fully in solution. This latter effect limits these proteins to act as key components in those thin liquid films, which are essential to build up the foam-like structure and being responsible for the gas holding capability of the dough (Hoseney 1984; Salt et al. 2005). The knowledge about ‘soluble proteins’ – albumins and globulins – significantly enlarged in the last decade, and the latest reviews (Shewry et al. 2009; Juhász et al. 2013a) on wheat proteins now provide detailed information about their numerous subclasses, structure, function in the grain and their contribution to end-use quality of wheat. This increased interest is partly related to the fact that the novel protein separation techniques such as 2-D DIGE,

2-D electrophoresis or MALDI-TOF (Gao et al. 2009) applied in proteomic experiments (reviewed recently by Juhász et al. 2013b) provide enough resolution to separate these proteins for their identification and this resolution shows inter-cultivars differences (Vu et al. 2013) and alterations during endosperm development (Tasleem-Tahir et al. 2012).

Protein content, damage starch, total and water extractable arabinoxylan content as well as the relative amounts of glutenin, gliadin and soluble protein fractions in the total protein of flours had been related to water absorption by systematically selecting some of these parameters in the 2011 harvest season. Comparing the measured and calculated water absorption values by using the selected parameters (Table S5) it was found, that the glutenin and gliadin content of the total flour proteins did not improve the correlation, but the joint application of the remaining four parameters resulted in a remarkably good relationship ($r^2 = 0.643$). The model has been validated using the data of the 2012 season and the correlation coefficient was found to be 0.665 between the measured and the calculated water absorption values.

Trying to further improve the predictive power of the models, a parameter, related to the chemical composition of soluble protein fraction has been developed. Proteomic analysis of albumins and globulins present in starchy endosperm of wheat led to the identification of 487 proteins (Tasleem-Tahir et al. 2012). In order to relate water absorption to any soluble proteins there is no need for a very sophisticated protein separation techniques as the overall size and/or the surface properties of the proteins provide an adequate information about the amount of water required for the full hydration. The LOC technique is a relatively simple, fast and cheap method to get a characteristic profile, and this was applied in this study. More than 20 protein bands have been separated in this way from the water extracts with the size of 6–62 kDa, in three groups regions (15, 29 and 62 kDa). Based on these results, only minor qualitative, but significant and characteristic quantitative differences have been found among the profiles of the different varieties. Most of the quantitative differences appeared in those proteins that were larger than 30 kDa. The sodium chloride extract prepared from the residue followed by the extraction of albumins contained less number of proteins, mostly with the size smaller than 20 kDa and cultivar dependent intensity. The relative amounts of the soluble proteins and their ratios have been systematically related to WA measurements to look for the highest linear correlation coefficients. The ratio of areas in the LOC patterns between 21 to 25 s (A1) and 38 to 42 s (A2) showed a remarkably strong relationship ($r^2 = 0.579$, Table S5). The A1/A2 ratio, called “albumin protein ratio” (APR) was used together with the parameters used earlier to relate to water absorption using linear regression (Table S5). The resulting predictive equation with an $r^2 = 0.786$ (Table S5) seemed to be satisfactory for practical applications to estimate the water absorption, and it also clearly indicated that the simultaneous consideration of all water holding components of the flour were needed to get meaningful estimate of the water absorption.

In conclusion, the water absorption, one of the most important quality attribute of wheat flour, can successfully be predicted. Next to the total protein content and the amount of damaged starch, the consideration of the total arabinoxylan- and soluble protein-amounts are also essential to get meaningful estimates. The relative amount of certain

soluble proteins (APR) determined by LOC technique in this study seems to be one of the key factors determining WA. Our results indicate that both the amounts and composition of AX and soluble proteins show significant inter-cultivar differences; consequently they can be target traits to alter them during wheat breeding programs to improve water absorption.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at <http://www.akademiai.com/content/120427/>

Electronic Supplementary *Table S1*. Wheat cultivars and landraces investigated

Electronic Supplementary *Table S2*. Overall means, minimum and maximum values of compositional and functional parameters of samples (International set, Martonvásár varieties, Old Hungarian cultivars and landraces) from the two consecutive growing seasons

Electronic Supplementary *Table S3*. Comparison of the chemical composition and water absorption of samples in the three subpopulations by ANOVA

Electronic Supplementary *Table S4*. Characterising the G × E effects on the chemical composition and water absorption of samples in the total populations by ANOVA

Electronic Supplementary *Table S5*. Linear correlation coefficients (r^2) comparing measured and estimated water absorption data for models applying sets of different chemical composition parameters

Electronic Supplementary *Table S6*. Linear correlation coefficients (r^2) for the relationships between quantitative chemical composition parameters and functional properties of wheat flours

Electronic Supplementary *Figure S1*. Typical LOC separation of soluble flour proteins