PERIODICA POLYTECHNICA SER. CHEM. ENG. VOL. 46, NO. 1–2, PP. 39–49 (2002)

PREDICTION OF WHEAT QUALITY – SUCCESS AND DOUBTS

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Received: March 30, 2003

Abstract

Since the discovery of gluten by Beccari in 18-th century, the wheat producers, millers and bakers tried to find relatively simple methods of prediction of wheat quality particularly during breeding process. Taking in mind that endosperm proteins play the governing role in determination of wheat quality the prediction is generally based on these proteins.

Early researches concentrated on relations of macrofractions, e.g. gliadin:glutenin ratio, ratio of acetic acid soluble proteins, etc. Later the gliadin polypeptides, the genes coding for these polypeptides and the variety identification came into focus of studies. More recent predictions are based on high-molecular-weight glutenin subunits.

Although the latter prediction is successfully applied in many countries in wheat breeding, the low correlation between predicted and practical quality observed in many cases suggests that further studies are needed to improve the reliability of prediction. Consideration of role of other proteins and non-protein constituents of wheat may be mentioned among potential ways of further research. From the methodological point of view the use of non-destructive methods is promising.

Keywords: wheat, wheat quality, quality prediction, gliadin, glutenin.

1. Introduction

Since the discovery of gluten by Beccari in 18-th century, the wheat producers, millers and bakers tried to find a relatively simple method to predict the bread-making quality of wheat. Particularly, the breeders were interested in developing such methods taking in mind the long process of traditional breeding and also the fact that in early stages of breeding process only small quantities of grain are available which do not allow normal baking test to be done.

As it is generally accepted, the endosperm proteins play the most important role in determination of wheat quality. So it is understandable that the studies related to development of quality prediction methods concentrated on these proteins.

2. Early Research

Early researches focused the efforts on study of the ratio of protein macro fractions. Results of these early investigations suggested that the ratio of gliadin to glutenin type proteins is the main factor influencing the gluten properties. These views were based on the fact that strength of the gluten complex is dependent on glutenin and the extensibility on gliadin component. A ratio of 1:1 was kept to be the optimal. Later the ratio of readily dispersible proteins (using weak organic acids such as lactic acid or acetic acid) has been measured. The results indicate that the amount of readily dispersible components negatively correlates with the rheological quality of gluten and dough.

An increase in the ratio of nondispersible protein has an improving effect. These observations are in good agreement with the practical work, and some methods for the determination of gluten quality and baking value of wheats (e.g. swelling test of gluten, sedimentation test of Zeleny) are based on these observations. The proportions of acetic acid-soluble and acetic acid-insoluble proteins (residue proteins, gel protein) of gluten have been investigated by many researchers (for reviews see [1] and [2]). More recent studies have suggested that the solubility and molecular weight distribution of the gluten proteins are major factors in determining gluten quality. Using reconstitution technique [3] has shown that dough strength appears to be a function of the molecular weight distribution of gluten proteins. More insoluble, high molecular weight protein fractions were found to increase farinograph dough development time and extensigraph height and area and to reduce farinograph dough breakdown.

3. The Era of Gliadin

The development of gel electrophoresis and its wide application in cereal protein research together with the increased knowledge of the genetics of wheat opened up new possibilities in the investigation of the correlation between protein composition of gluten and baking value of wheat and flours.

The investigations focused on gliadin because its extraction and purification was relatively easy and reproducible as well as the effectivity of separation by gelelectrophoresis was quite good. The researchers have found a consistent relationship between dough properties and presence of some gliadin components in durum wheat [4].

Concerning bread wheats correlations were also found between some allelic blocks and technological quality. As known, gliadins are inherited in the blocks associated with six loci on chromosomes 1A, 1B, 1D, 6A, 6B, and 6D. The gliadin composition of any wheat could be defined by the numbers of these six blocks. SOZINOV [5], based on investigation of 50000 samples, ranked the blocks according to their beneficial effect on grain quality (*Table 1*). More recent investigations, however, suggested that gliadins play a secondary role due to a genetic linkage

to another protein that more directly influences the functional properties. This suggestion stimulated the more intensive study of glutenin components of wheat gluten.

Table 1. Gliadin blocks arranged according to the degree of influence on the grain quality [5]

GLD1A7 > GLD1A4 > GLD1A2 > GLD1A5 > GLD1A3 > GLD1A1 > GLD1A6
GLD1B1 > GLD1B2 > GLD1B7 > GLD1B5 > GLD1B4 > GLD1B3 > GLD1B6
GLD1D4 > GLD1D6 > GLD1D1 > GLD1D2 > GLD1D3
GLD6A3 > GLD6A1
GLD6B2 > GLD6B1
GLD6D2 > GLD6D1 > GLD6D3

4. Glutenin Subunits and the Quality Prediction

Investigations mentioned above substantially extended our knowledge concerning the gluten complex and correlations between gluten composition and bread making quality of wheats.

Summarizing our recent knowledge concerning protein composition and bread making quality a generally accepted conclusion may be drawn [1], [2] that properties of the storage proteins of wheat govern its suitability for processing into bread. Among cereals only bread wheats – and to lesser extent triticale – possess storage proteins which interact with water to yield doughs having necessary cohesiveness and elasticity for making high specific volume leavened breads.

The correlations between the protein content, protein composition properties of gluten complex and the rheological properties of wheat flour doughs have been thoroughly investigated. Some basic results and conclusions may be summarized as follows:

- Insoluble protein matrix is an essential pre-requisite of the formation of a cohesive dough.
- There must be a sufficient amount of insoluble protein to form a continuous protein phase in the presence of starch and water.
- The ratio of high and low molecular weight gluten proteins has a significant effect on the rheological properties of dough.

Progress in separation techniques and molecular biology made possible the isolation of individual polypeptides forming the gluten complex and also the identification of genes coding for these proteins. This fact allowed a deeper study of

correlations between individual gluten proteins and baking quality. Recently, the main attention is paid to the glutenin components.

According to recent views of specialists, summarized in reviews of SHEWRY and MIFLIN [6], MÜLLER et al. [7], SHEWRY et al. [8], and SHEWRY and TATHAM [9], glutenin is composed from subunits linked by disulfide bonds. The subunits are divided into two groups: (1) high molecular weight subunits (HMW or HMW-GS) and (2) low molecular weight subunits (LMW or LMW-GS). The HMW subunits are identified according to electrophoretic mobility within the group and according to chromosome coding for individual polypeptide. A catalogue of genes coding for HMW subunits of wheat is given by PAYNE and LAWRENCE [10]. The HMW glutenin subunits are classified into two subgroups: X-type and Y-type subunits.

Based on worldwide observations relating to correlations between HMW-GS patterns and wheat quality, PAYNE [11] proposed the use of so called, 'Glu-1 quality score' for prediction of baking quality of wheat varieties (*Table 2*). Although this system is used by breeders in many countries, some newer data suggest that its general validity is doubtful and other factors should also be taken into consideration by evaluation.

Table 2. Glu-1 quality scores assigned to high molecular weight glutenin subunits [11]

Chromosome	Subunit	Score			
		1	2	3	4
1A	1			+	
	2			+	
1B	17 + 18			+	
	7 + 8			+	
	13 + 16			+	
	7 + 9		+		
	7	+			
	6 + 8	+			
	20	+			
1D	5 + 10				+
	2 + 12		+		
	3 + 12		+		
	4 + 12	+			

5. What Makes the Criticism of Glu-1 Score Justified? What Should Be Clarified?

5.1. The Role of Absolute Quantity of Protein

One of the earliest criticisms has been expressed by HUIFEN and HOSENEY [12] 'In taking mind all overall view, we must consider that because the total protein is highly correlated with loaf volume potential, then it appears unlikely that quality is controlled by any one or even a small number of proteins or peptides. If that were true, we must assume that the 'critical' protein(s) is highly correlated with total protein content of the sample. This appears to us to be an illogical assumption.'

In every case it is sure that any correlation of wheat quality on chemical components of grain is influenced by the quantity of protein. Consequently, any correlation relating to wheat quality is generally valid for a given range of protein content.

5.2. Effect of Quantitative Ratios of Individual HMW-GS

By calculation of 'Glu-1 quality score' generally only the qualitative distribution of individual HMW-GSs are taken into consideration. It is a logical suggestion that the quantity may also play a role in formation of gluten complex. It seems that some of first studies on this topic [9], [13], [14] confirm the importance of quantitative ratios. Hopefully, a more detailed study of amounts of different subunits will help the explanation of big differences, in some cases, between the predicted (based on Glu-1 score) and actual quality of some cultivars. E.g. according to 'Glu-1 score' the combination of subunits 2 and 12 (2 + 12) or 4 + 12 is of low quality value. However, newer studies (in Hungary and Croatia) of an old Hungarian variety called Bánkuti, known as high quality hard winter wheat, have shown that this wheat contains 2 + 12 subunits. (Other varieties grown in Hungary containing 2 + 12 subunits have really low scores: 4–6, as expected). It should also be noted that according to KHAN et al. [15] one of HRS varieties (HY 320) grown in North Dakota with 2 + 12 subunits had a score as high as 8.

5.3. Role of LMW-GS and Gliadins

It is generally accepted that the LMW-GS have a role in formation of gluten complex and in formation of its rheological properties. Earlier investigations [13], [16] showed that LMW-GS quantity and distribution influence the molecular weight distribution and quantity of polymeric proteins and gluten viscoelasticity of durum wheat.

Nevertheless, until now the role of LMW-GS is not yet fully clarified. There are some views and hypotheses concerning the structure of gluten which suggest

a secondary role for these subunits. However, data about quantitative ratios of different gluten subunits show that the quantity of LMW-GS and gliadins is about three times higher than that of HMW-GS. The recent work of GROSCH and WIESER [14] revealed that the attack of low molecular weight thiol compounds such as reduced glutathion (GSH) is primarily directed to intermolecular – S – S – bonds between LMW-GS and HMW-GS. As known GSH may cause drastic changes in consistency of dough.

It is also an important observation that some D-type (coded by chromosome D) LMW-GSs contain only one SH- group and can act as terminators in the potential polymerization process [17]. May be its quantity is a factor which should not be omitted.

The role of gliadin polypeptides needs also further studies. It seems that the hypothesis that gliadins act as fillers, plasticizers in the gluten complex should be modified. It was shown e.g. by KECK et al. [17] that some peptides obtained by enzymic hydrolysis of purified glutenins contain gamma-gliadin components.

5.4. The Effect of Dough Formation and Mixing

It is important to know that under conditions of bread making technology, the disulfide bond system of proteins may be viewed as a dynamic system. Their number and distribution may change depending on conditions of mixing, presence of low molecular weight thiol (disulfide) compounds, enzymes etc. [14]. E.g. the breakage of disulfide bonds during mixing of dough and their reformation during resting period has been experimentally shown by several researchers. Low molecular weight thiol compounds e.g. GSH may cause disulfide-thiol interchange by formation of Protein-S-S-G molecules.This disruption of interprotein bonds may cause weakening of the dough structure.

5.5. The Mechanism of Disulfide Bond Formation and Polymerization of Polypeptides

One of the open questions concerning the disulfide bond system of gluten complex is the mechanisn of disulfide bond formation *in vivo*. In the framework of earlier experiments [1] it has been shown that using the same mixture of polypeptide (produced by splitting the disulfide bonds of gluten by beta-mercaptoethanol) masses quite different rheological properties may be produced by reoxidation under different conditions (concentration, pH, presence of urea etc.). These results suggest that the process of formation of disulfide bonds and probably the polymerization process of glutenin subunits may be regulated. At present we know that all wheat gluten proteins are synthesized on the rough endoplasmic reticulum (RER), with a signal peptide that is cleaved as it directs the nascent polypeptide into the RER lumen. Protein folding and formation of disulfide bonds then occur within the RER. Among

enzymes playing role in posttranslational transformations of proteins primarily the protein disulfide isomerase (PDI) may be of interest in elucidation of mechanism of disulfide bond formation in gluten forming polypeptides. This enzyme is located within the lumen of ER and has been demonstrated to catalyze disulfide bond formation in secretory proteins in a range of biological systems [19]. The presence of PDI in wheat endosperm, aleurone layer and embryo was confirmed by several researchers [20]–[22]. BULLEID and FRIEDMAN [23]–[24] and BULLEID et al. [25] showed that PDI is able to catalyse the formation intrachain disulfide bonds in gamma-gliadin synthesised *in vitro* and also in some HMW-GS and LMW-GS. However, none of the proteins were observed to form stable disulfide-linked oligomers. On the other side, experiments made with procollagen [26] showed that in the presence of PDI the formation of disulfide-stabilised trimers is much faster. Consequently, the role of PDI in formation of interchain disulfide bonds in gluten could not be excluded. When we mention that wheat grain contains a lot of other redoxy-enzymes and other enzymes with the possible role of molecular chaperons, it is clear that a lot of further investigations are needed to give a fully correct answer on the questions raised in this paper.

Although the important role of disulfide bonds in formation and determination of physical properties of gluten is well known and our knowledge concerning formation and breakage of intra- and intermolecular disulfide bonds increased considerably in the last decade, full explanation of the mechanism of disulfide bond formation and interaction of polypeptides needs further research.

In the framework of studies conducted in our laboratory the effect of conditions of *in vitro* polymerization by oxidation of different mixtures of gluten subunits was investigated. Concentration of polypeptides, pH, ionic strength, presence of low molecular weight thiol compounds and urea highly influenced the degree of polymerization and also the degradation during mixing. Preliminary studies also showed that the quantitative ratio of subunits in addition to the qualitative composition play a significant role in determination of properties of polymer obtained by reoxidation.

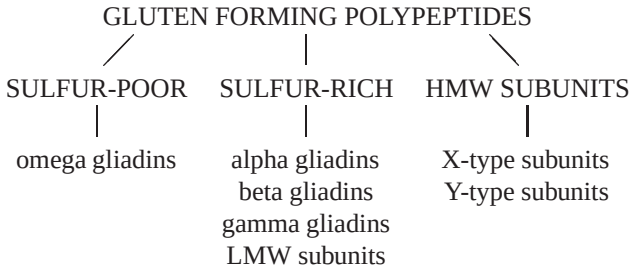

Fig. 1. Gluten forming polypeptides

As known, gluten forming proteins are coded on chromosomes 1A, 1B, 1D, 6A , 6B and 6D and synthesized as individual polypeptides. On the basis of their

amino acid sequences these are classified into sulfur rich, sulfur poor and high molecular weight (HMW) polypeptides [1], [2] (*Fig. 1*).

All three types of proteins have similar structures containing 250–300 amino acid residues. The N-terminal domain is composed from repetitive units formed mainly from glutamic acid and proline and has a short non-repetitive end. At the C-terminal another non-repetitive domain is located. The proteins of this group contain 6-8 cysteine residues. The LMW proteins are divided into three types on the basis of their electrophoretic mobility. However, only two groups of them are rich in sulfur: the group B and group C. The B-type subunits are in the major group with a molecular weight of 40–50 kD, while the C-subunits have a molecular weight of 30–40 kD. The number of cysteine residues per molecule varies between 6 and 8.

The HMW glutenin subunits are coded by six genes in chromosomes 1A, 1B and 1D. The subunits are divided into two types named x-type and y-type HMW polypeptide.The x-type polypeptides have molecular weights of about 83–88 kD and the y-types of about 67–74 kD. Some of these genes may be silent, resulting in the presence of only 3, 4 or 5 HMW subunits in different cultivars of bread wheats: these are 1Dx, 1Dy and 1Bx in all cultivars and, in some cultivars only, 1By and/or 1Ax subunits. In addition to this polymorphism arising from the presence of a small multigene family, there is also allelic variation in the structures and properties of the subunits encoded by the various genes. Thus, a number of different HMW subunits can be recognized, which are numbered according to their electrophoretic mobility. All HMW polypeptides have a similar three domain structure. Non-repetitive domains exist at the N-terminus (81 to 104 residues) and at the C-terminus (42 residues) flanking an extensive repetitive domain (481 to 681 residues). Most of the cysteine residues are found in the terminal domains of y-types and all the cysteines are in the termini of x-type subunits.

About 40–50% of synthesized polypeptides, mainly gliadins are present in monomeric form and have only intramolecular disulfide bonds. However, the second part of polypeptides (LMW and HMW subunits) is polymerized in a posttranslational process by intermolecular disulfide bonds forming glutenin. It is generally accepted that the presence of an adequate quantity of polymeric glutenin is one of the most important prerequisites of formation of elastic, strong gluten. Thus, the knowledge of all factors influencing the polymerization of monomeric polypeptides is of great importance in relation to breeding, growing and processing high bread-making quality wheats. Although our knowledge concerning the subunit composition of glutenin increased dramatically in the last decade (see minireview of SHEWRY and TATHAM [9]) and some correlations were found between subunit composition and bread-making quality [27], the mechanism of disulfide bond formation and the effect of different factors on polymerization (or depolymerization) is not fully clarified. It is generally accepted that the polymeric glutenin molecules have a linear character. This supposition may be supported by the following experimental facts:

- The splitting of disulfide bonds by reduction or oxidation results in drastic change in the molecular weight.

- The viscosity of the glutenin solutions decreases rapidly even by splitting small number of disulfide bonds.

Earlier experiments [28] have shown that using the same mixture of polypeptides (produced by reducing gluten with beta-mercaptoethanol) fractions with quite different rheological properties may be obtained by reoxidation under different conditions. This study and several other experiments have suggested that the arrangement or interactions between subunits in the polymer are not random or at least they may be influenced by some factors.

The prerequisite of formation of linear chains is that the polypeptide should have minimally two free thiol groups. Thus, polypeptides in which all thiol groups are involved in intramolecular disulfide bond formation cannot take part in a polymerization process. The presence of three (or more) free thiol groups makes possible the branching of chains or crosslinking between chains. Finally, when only one thiol group is free, the polypeptide can be linked to the chain and act as a terminator (*Fig. 2*).

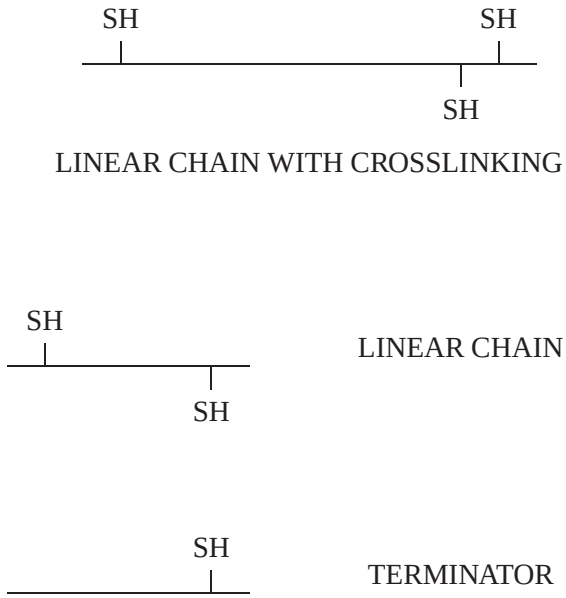

Fig. 2. Different types of polypeptides forming intermolecular disulfide bonds

Recent studies [18], [29] using partial reduction of polymeric glutenin revealed the presence of different types of disulfide bonds and based on these observations different models of glutenin structure were proposed. Determination of exact positions of bonds and details of structure needs extensive further research. However, from practical point of view first of all the amount of GMP and its stability is important.

In the framework of experiments conducted in our laboratory the effect of some factors (reduction, reoxidation, mixing) on GMP quantity was studied. Five Hungarian-grown winter wheat cultivars: Öthalom (HMW-GS composition: 1A2, 1B 7 + 9, 1D 5 + 10, Glu-1 score: 9), Jubilejnaja 50 (HMW-GS composition: 1A 2*, 1B 7 + 9, 1D 5 + 10, Glu-1 score: 9), GK Szőke (HMW composition: 1B 7 + 9, 1D 5 + 10, Glu-1 score:7), MV 14 (HMW-GS composition: 1A1, 1B 7, 1D 2+12, Glu-1 score: 6) and GK Zombor (HMW composition: 1B 7, 1D 2+12, Glu-1 score: 4) were used in experiments. Hand-washed glutens were freeze-dried and used for further experiments. Glutenin was obtained as a residue after extraction of freeze-dried gluten with 70% ethanol. Glutenin macropolymer was separated and measured by a procedure published by SKERRIT et al. [30], HMW-GS composition of wheats was determined by KÁRPÁTI et al. [31].

Reduction and reoxidation of glutenin and measuring the physical properties was carried out using methods described by LÁSZTITY [28].

6. Conclusions

Some conclusions based on the results may be summarized as follows:

- GMP (glutenin macro polymer) content of gluten of varieties with 1D 5 + 10 HMW-GS was higher than that of wheats with 1D 2 + 12 HMW-GS.
- Conditions of the *in vitro* reoxidation of glutenin polypeptides obtained by reduction with beta-mercaptoethanol significantly influence the properties of the reoxidized product.
- The reoxidized material with lower degree of polymerization contains higher ratio of HMW-GS. This suggests that in the initial phase of polymerization the role of HMW-GS is more important than that of LMW-GS.
- Experiments of several researchers confirmed the degradation of GMP during mixing. Parallelly a decrease of ratio of HMW-GS in degradation products has been observed.
- The explanation of results mentioned above needs a more detailed knowledge of glutenin polymer.

As a summary of the results it can be concluded that: Although a significant progress was made in prediction of wheat quality and the prediction based on HMW glutenin subunits has been successfully applied in several countries in the breeding process, further studies are needed to improve the reliability of prediction. Among potential ways of further research consideration of the role of other proteins and non-protein constituents of wheat may be mentioned. From methodological point of view the use of non-destructive methods seems to be very promising

Acknowledgements

Funding for the work by OTKA Res. Project T 029712 is gratefully acknowledged.

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