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Preamble

Wheat is one of the most consumed, produced and stored food crops worldwide. The paramount importance of wheat to human populations can be attributed to the remarkable work done by wheat breeders, who have improved wheat varieties keeping them in the spotlight of global agriculture. The recently completed annotation of the entire genome of bread wheat ended 13 years of collective effort to crack the wheat genetic code. The genome sequence can be used to study gene expression at any point in the life cycle of the plant, and to define which genes to target to improve yield and stress resistance. This massive advance not only allowed better understanding of relevant genes for agricultural applications but also for end-use quality traits.

During the last four years, wheat quality scientists from different countries have worked to develop the Expert Working Group (EWG) on Improving Wheat Quality for Processing and Health under the Wheat Initiative umbrella. This joint effort provides a framework to establish strategic research and organisation priorities for wheat improvement at the international level in both developed and developing countries. This EWG aims to maintain and improve wheat quality for processing and health under varying environmental conditions. The EWG has been focused on wheat quality in the broad sense, including seed proteins, carbohydrates, nutritional quality, grain processing and food safety. Bioactive compounds are also being considered, both those with negative effects, such as allergens and mycotoxins, that cause serious problems that need to be resolved, and those with positive effects, such as antioxidants or fibers, that can potentially be exploited. The EWG also works in the development of germplasm sets and other tools that can be deployed in wheat quality research.

The preparation of this book covering the whole range of grain quality topics is one of the important activities that the EWG is doing nowadays. The book should serve to identify possible gaps in important areas of wheat quality research and to position the EWG as an initial point of reference for the global wheat community regarding the different topics covered in depth here. Forty EWG members worked on 21 chapters of the book. This book adheres to the same policies that the EWG promotes such as using unified nomenclature to name the different alleles and

providing correct information about materials (accession name, Germplasm Bank of origin, etc.) so other researchers know exactly what is being described and how to obtain the same materials or information.

The present book brings together a group of leading researchers from all over the world who describe different aspects of wheat quality for processing and health. During the meetings of the EWG different topics have been identified in recent years that need close attention or updating so more oriented and ordered research can be carried out in the years to come. The chapters on this topic seek to address this question while capitalizing on outputs of other international initiatives, wheat organizations and other EWGs, namely:

1. The importance of wheat
2. Wheat gluten protein structure and function: is there anything new under the sun?
3. Starch and starch-associated proteins: impacts on wheat grain quality
4. Contribution of genetic resources to grain storage protein composition and wheat quality
5. Durum wheat storage protein composition and the role of LMW-GS in quality
6. Gluten analysis
7. Proteomics as a tool in gluten protein research
8. Genotypic and environmental effects on wheat technological and nutritional quality
9. Improving wheat nutritional quality through biofortification
10. Phenolic compounds in wheat kernels: genetic and genomic studies of biosynthesis and regulations
11. Wheat cell wall polysaccharides (Dietary Fibre)
12. Grain quality in breeding
13. High throughput testing of key wheat quality traits in hard red spring wheat breeding programs
14. Molecular marker development and application for improving qualities in bread wheat
15. Durum wheat products, couscous
16. Understanding the mechanics of wheat grain fractionation and the impact of puroindolines on milling and product quality
17. The impact of processing on potentially beneficial wheat grain components for human health
18. *Fusarium* species infection in wheat: impact on quality and mycotoxin accumulation
19. Effects of environmental changes on the allergen content of wheat grain
20. Health hazards associated with wheat and gluten consumption in susceptible individuals and status of research on dietary therapies
21. FODMAPs in wheat
22. Epilogue: The main activities of the International collaboration on wheat quality and safety

In conceiving and compiling this book, we intend to make all these data and recent findings related to the advances on research of wheat quality genomics, proteomics, and other topics accessible to the general scientific community. Considering the importance of this crop in the human diet and its potential to promote health, all the wheat quality research and breeding community will be interested in the topics addressed by the book. Professionals working on the wheat value chain (millers, food manufacturers) or in nutrition and healthcare may also find this book a useful resource to increase and update their knowledge about wheat quality, nutrition and health issues.

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Grain Quality in Breeding

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Abstract Grain characteristics (hardness, protein content/quality, starch properties, enzymatic activity, etc.) play an important role in the definition of end use quality for wheat-based products. Among them, gluten strength and extensibility, mostly determined by glutenin and gliadin composition, are two of the main factors that determine gluten quality. The complex inheritance of most quality traits has led to the development of indirect tests used in breeding for early and advanced generation selection. The main focus of breeders is adding resistance to biotic stress (fungi, insects, nematodes, etc.) and increasing grain yield while selection for quality often occurs in later generations. This often results in the propagation of poor quality lines that must be later discarded. Evaluation of quality in early generations requires suitable tests, preferably non-destructive. Increasing knowledge of the genes involved in quality will facilitate more precise and effective selection. Recent advances in wheat genome sequencing and the extensive genotyping of mapping populations has led to a precise molecular characterization of high molecular weight (HMW) and low molecular weight (LMW) glutenins, as well as the discovery of genes associated with quality traits like grain

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hardness, starch composition (e.g., waxy genes), etc. Massive genomic data will impact in breeding programs allowing quality fine tuning by precise selection of glutenins, starch, hardness and other traits, for specific end uses through marker assisted selection, genomic selection, etc. This chapter will describe different methods used for quality selection in breeding programs and research, and some examples of integration of local breeding programs with the extremely diverse end-uses of wheat based on a series of case-studies. Current and potential approaches to quality evaluation in durum wheat, wild relatives and synthetic wheat breeding programs will be also presented.

1 Methods for Quality Selection and Evaluation

In this section we discuss the efficiency of a diverse subset of methods used for predicting different aspects of wheat quality and its potential use in breeding programs. This subset includes commonly used near-infrared (NIR) spectroscopy and Payne Score, and more sophisticated methods such as the Wheat Simulator and Protein Quality Index (PQI). More specific and quality research methods are also discussed including, the Protein Scoring System (PSS), the High Performance Liquid Chromatography (HPLC) and variants SE-HPLC and RP-HPLC for %UPP determination, and the LC-MS/MS analytical method for screening of water-soluble vitamins. It should be mentioned that there are many methods extensively used for quality selection in breeding programs not included here (such as SDS-sedimentation, SRC, SKCS, among others) that have been already reviewed in other publications. The choice of methods discussed in this section is related with the expertise of co-authors taking part of this book chapter.

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1.1 Applying NIR Techniques in Quality Related Selection in Wheat Breeding

Since the early work of Rubenthaler and Pomeranz (1987), achieving good correlations of water absorption, mixing time, and loaf volume of hard red winter (HRW) wheat to flour NIR spectra, near-infrared (NIR) spectroscopy has been used as a rapid, accurate, and non-destructive technique for measuring many wheat quality parameters. Screening large numbers of lines for several parameters shows that NIR methods are practicable. Based on previous results (Dowell et al. 2006) NIR shows the potential for predicting protein content, moisture content, and flour color b∗ values with accuracies suitable for process control ($r^2 > 0.97$). Many other parameters were predicted with accuracies suitable for rough screening including test weight, average single kernel diameter and moisture content, SDS sedimentation volume, color a∗ values, total gluten content, Mixograph, Farinograph, and Alveograph parameters, loaf volume, specific loaf volume, baking water absorption and mixing time, gliadin and glutenin content, flour particle size, and the percentage of dark hard and vitreous kernels. However when the influence of protein content was removed from the analyses, the only factors that could be predicted by NIR with $r^2 > 0.70$ were moisture content, test weight, flour color, free lipids, flour particle size, and the percentage of dark hard and vitreous kernels. Nowadays, NIR is widely applied to the measurement of cereal quality and cereal product composition. The technique enables the rapid assessment of protein, wet gluten, moisture, and ash, and with lower reliability also determination of Zeleny sedimentation and water absorption. In general, NIR can predict these parameters with a high degree of accuracy, as the relevant spectral regions show reasonably clear differences with changing sample composition. Some success was achieved even when modeling some rheological parameters, especially those being measured by the Farinograph (Hrušková et al. 2001), Extensograph (Delwiche et al. 1998) and Alveograph (Czuchajowska and Pomeranz 1991; Jirsa et al. 2008). Prediction of dough properties by NIR spectra analysis, however, is influenced by many factors, especially errors of reference methods and results dependent on the protein content of tested flours. Reliability of computed characteristics of dough varies according to the calibration sample set, and the extent and quality range of flour parameters (Hrušková and Šmejda 2003). Thus, NIRS can be used to predict many grain quality and functionality traits, but mainly because of the high correlations of these traits to protein content. Another way of utilising the NIR technique in quality related screening is to look for quantitative estimations of protein data related to quality attributes. Wesley et al. (2001) reported successful prediction of gliadin and glutenin content from NIRS. In another study (Scholz et al. 2007), partial least squares regression gave high r^2 values between many protein parameters and NIR/NIT (near-infrared transmittance) spectra of flours, while no such relationship was found for whole wheat grains. The highest correlations were found for the total amount of extractable and unextractable proteins and the monomer/polymer protein ratio. Some positive relationships were also found between the NIR/NIT spectra and the percentage

of total unextractable polymeric protein in the total polymeric protein and the percentage of large unextractable polymeric protein in the total large polymeric protein. Predictive methods for high value traits are crucial to facilitate increased genetic gain in plant breeding programs. With small quantities of grain required (<200 g) and rapid turnaround time (<1 min of scanning time), high-end NIR predictions of grain quality traits provide informative data for weighed index selection decisions in a similar time frame to grain yield. Of course, appropriate NIR instrumentation does not come cheap, and the prediction calibrations are only of value when the training population is built from a large and robust dataset drawn from environments representative of the target breeding program and encompasses relevant genetic diversity. In Australian wheat breeding, elite grain quality is central for cultivar adoption, and thus, overall genetic merit. Calibration equations to predict compositional grain attributes (e.g. grain protein) have been common place for several years, even on relatively low cost NIR instruments. However, recent improvements in NIR instrumentation and dynamic biometrics to build dynamic calibration equations for high value flour traits (e.g. milling yield, extensibility, Rmax, colour and ash content) and more recently for derived end product testing (e.g. baking loaf volume) has increased the importance and relevance of NIR in wheat breeding programs that place a strong importance on high grain quality. At Dow Seeds, these robust NIR predictive correlations of physical grain attributes ($r^2 > 0.8$), flour traits ($r^2 = 0.4–0.8$), and end product testing ($r^2 > 0.7$) enable effective discrimination decisions within early stage breeding populations and fixed lines tested within standard crop-season. As the scanned seed is still viable with a rapid turn-around time of NIR, early stage selections can be made in time to allow contra season nursery for enriched germplasm to further increase the rate of overall genetic gain.

1.2 Predicting Dough Properties from Genetic and Biochemical Data

1.2.1 Dough Strength and Extensibility

Relating the protein composition to certain quality traits by statistical means is a frequently used methodology to relate structure/composition to functionality in cereal science. The classic tool used by breeders is the Payne score (Payne et al. 1987) providing a single number to estimate dough strength from the HMW glutenin allelic composition. Despite the large success in using the Payne protein marker score in breeding programs over the years, significant limitations of this the method have been realized. The Payne score in original form and even all later alternative calculation methods are not applicable to fully describe breadmaking quality: they are simple and very useful tools to estimate the most important rheology characteristics of the dough, namely dough strength and – in cases of the more up-to-date formulas – extensibility. These essential characteristics of the dough are directly related to the structure of proteins comprising gluten, and therefore it is meaningful

to relate the composition of these proteins and the characteristics of the dough. Therefore it is not surprising that attempts to predict breadmaking quality simply with Payne score type models have failed (Hamer et al. 1992). Another type of limitation of the original Payne score is that it takes into account only the contribution of the HMW glutenin subunits in relation to dough strength. Several attempts have been introduced to involve the LMW glutenin alleles in similar mathematical formulas (Békés et al. 2006; Cornish et al. 2006; Gupta et al. 1991, 1994; Oury et al. 2010). By the application of sophisticated statistical approaches, the Wheat Simulator (Eagles et al. 2002), and the Protein Quality Index (PQI) (Békés et al. 2006) went one step further: they are capable of describing the effects of both HMW and LMW-GS alleles on dough strength and extensibility, individually and the pair-wise interactions among the alleles (Fig. 1). As it is shown in Fig. 1, besides estimating not only Rmax but also Ext, and besides considering the individual and interactive contributions of both HMW and LMW-GS alleles, the number of HMW-GS alleles have been increased in PQI from 13 to 17 with some very important alleles such as the *Glu-B1al* allele (with the overexpressing subunit 7). The quite large (33×33) matrixes for the estimation of both Rmax and Ext are available on the official web-site of AACCI where the allelic composition, Payne score and PQI of more 8500 wheat varieties, cultivated in more than 80 countries are tabulated (Békés and Wrigley 2013, 2017). The approach of relating allelic composition to quality attributes is possible with careful data selection and applying robust mathematical tools: the genetic potential of a line, with a certain combination of alleles on the six glutenin coding loci, can meaningfully be predicted where both the contribution of the individual alleles and their pair-wise interactions play equally important role (Baracskai et al. 2011; Békés et al. 2006; Cornish et al. 2006). The original version of the PQI model is to predict the genetic potential of dough strength and extensional properties of dough of a wheat flour with 12% protein content and with the ratios of glutenin to gliadin and HMW to LMW GS of 1.0 and 0.2, respectively. The further developed version of the model (Békés et al. 2006) is capable of considering the effects of the expression levels of the different storage proteins genes, so that the actual dough parameters can be predicted. The input to this model is the allelic composition and the quantitative protein composition (including UPP%) (Gupta et al. 1993), while the output provides a good estimate of the actual dough strength and extensibility of a given sample ($r^2 > 0.85$ and $r^2 > 0.75$, respectively). The application of the PQI model on different sample populations (Baracskai et al.

$$Q = \sum_{13}^{i=1} \alpha_i^*(q_H)_i \qquad Q = \sum_{17}^{i=1} \alpha_i^*(q_H)_i + \sum_{16}^{j=1} \alpha_i^*(q_L)_j + \sum_{17\,16}^{i=1\,j=1} \beta_{i,j}^*(q_H)_j^*(q_L)_j \qquad (1)$$

Payne score Payne, 1987

Protein Quality Index (PQI) Békés et al, 2006

Fig. 1 The mathematical formula of Payne score and PQI. The presence or absence of an individual HMW-GS $(\mathbf{q_H})_i$ or LMW-GS $(\mathbf{q_L})_j$ allele in the sample is coded by **1** or **0**, respectively. The $(\boldsymbol{\alpha_i})$ and $(\boldsymbol{\beta_i})$ weighting factors describe the individual and interactive contribution of an allele, respectively, determining **Q** quality attribute (Rmax in Payne score, Rmax or Extensibility in PQI)

2011; Békés et al. 2006) importantly point to the impact of allelic interactions as a major variable determining dough properties (Fig. 2). The realisation of the existence of the large contribution of allele–allele interactions in wheat flour doughs may alter our way of utilising our knowledge of relating genetic/chemical information to quality attributes in wheat breeding, in the grain industry and in basic research. In breeding, the real value of a certain allele has to be investigated in several backgrounds to be able to realize its interaction potential. Consequently, different allelic combinations, rather than certain individual glutenin alleles should be targeted in breeding situations to develop new lines with certain quality attributes, especially to improve extensibility.

The interactive effects of the alleles present in commercial wheat flour blends are responsible for the well-known problem in the grain industry: dough properties, such as dough strength and extensibility are not simply additive characteristics. They usually show non-linear relationships with the blend formulation (Békés et al. 1998, 2001): applying the Fig. 1 equation to describe the dough strength (Rmax) of a blend with two (u and v) components, it is clear that the difference between the non-linear and linear model does contain only interactive elements (non-linearity is the function of the interactions of alleles of component u with those of component v). If the allelic composition of the components as well as the $\alpha_{i,j}$ and $\beta_{i,j}$ parameters are known, the quality attributes of blends can be estimated, providing an efficient way of developing non-linear optimization models for blend formulation. It is important to note that the quality attributes of hybrid wheats with their complex genetic makeup can be estimated using the quality attributes of sibling lines and the above mathematical model.

At the time of the introduction of Payne score only the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) based methodology was available to identify the glutenin alleles in a sample. While in case of HMW-GS alleles it is a simple, routine task, the identification of LMW-GS alleles is rather complicated and requires special skills. Since the routine application of the above prediction methods requires knowledge of both HMW and LMW-GS alleles, sensitive, reproducible

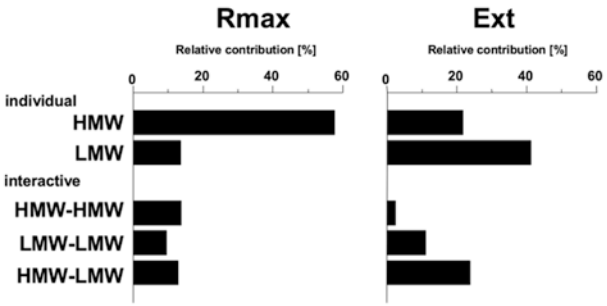

Fig. 2 Typical relative contributions of the individual and interactive effects of HMW and LMW glutenin alleles on dough strength (Rmax) and extensibility (Ext) in a set of wheat varieties (n = 107) using the data of Baracskai et al. (2011)

and high throughput methodologies had to be developed. The molecular marker (Abdel-Mawgood 2008; Howitt 2010; Howitt et al. 2006; Liu et al. 2012), and MALDI-TOF (Gao et al. 2010; Liu et al. 2009, 2010; Ma et al. 2009; Wang et al. 2015) technology developed for both HMW and LMW-GS alleles nowadays provide a solution for this task. In Australia, service companies, specialised for the identification of glutenin alleles, even for the application of Wheat Simulator (Eagles et al. 2002), or PQI (Békés et al. 2006) are available to wheat breeders. Illustrating the capabilities of NIR-based and PQI estimation of Rmax and Ext, the relationships between predicted and measured values of a small routine sample set is shown in Fig. 3. These levels of relationships provide a reliable, cost-effective solution in breeding situation to rank and select/omit breeder's lines with high throughput.

1.2.2 Estimation of Water Absorption

The above models however are only suitable for the estimation of certain dough properties. Other parameters such as water absorption (WA) cannot be predicted on the basis of gluten proteins only. Water absorption – the amount of water needed to hydrate flour components to produce a flour with optimum consistency – can be described as a function of protein content, the amounts of pentosans and the level of starch damage in the sample (Bushuk and Békés 2002). Experiments carried out with flours supplemented with different protein classes resulted in the observation that while mixing requirement, dough strength and extensibility significantly depends on the glutenin to gliadin ratio, WA is not sensitive to this ratio (Uthayakumaran et al. 1999). However, the ratio of the amount of gluten proteins to soluble proteins can significantly alter WA (Tömösközi et al. 2004). Supplementing

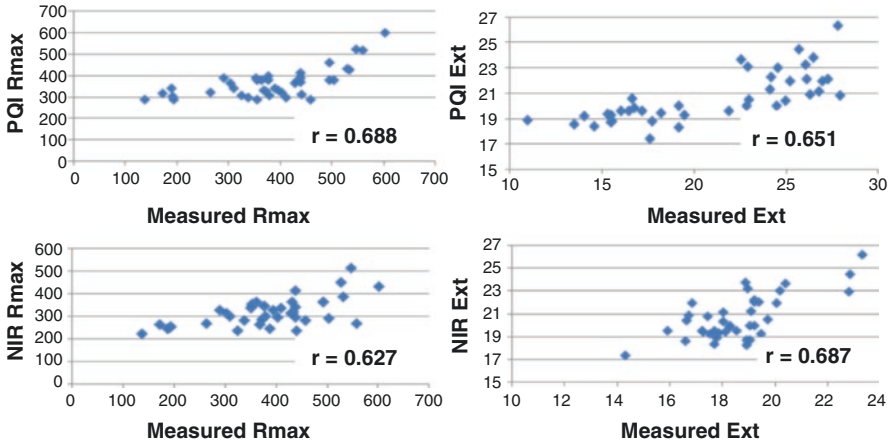

Fig. 3 Comparison of measured Rmax and extensibility data with predicted values derived from NIR calibration or PQI method

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). A significantly high positive correlation ($r^2 > 0.8$) was obtained in 63 straight run flours between WA and damaged starch content compared to a value of $r^2 = 0.5$ for protein content (Tara et al. 1972). Prediction equations using multiple linear models with protein content and starch damage have been developed and it was found that more than 90% of the variation in WA is covered by these two parameters. It was also concluded that the remaining variation is mostly related to the pentosan content of the flours. The relationships between the pentosan composition and water absorption of flours indicated that arabinoxylans (AX) play the major role and the largest effect is caused by the soluble – small and medium sized – arabinoxylans, while the large polymers do not have marked effects on WA (Primo-Martin and Martinez-Anaya 2003). 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 a reasonable 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, 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 as well as their significant contribution to determine WA it was concluded that they can be target traits to alter during wheat breeding programs to improve WA. This predictive equation provides explanations for the relative importance of several components of the flour in relation to water absorption, however the direct measurement of water absorption using small scale equipment such as the DoughLab seems to be a simpler and less time-consuming option to determine this important parameter.

1.2.3 Predicting End-Product Quality

In the last 20 years numerous researchers have attempted to predict bread quality by combining measurements made from grain, flour, or dough and combining them into prediction models (Békés 2012a, b). Protein content and composition, falling number, ash content, water absorption, mixing and rheological parameters have been applied in these studies in different combination using different linear mathematical models. The published predictions showed a wide range of correlations with loaf volume ($r^2 = 0.39–0.78$). A new generation of predictive models have been developed (Békés 2012b). All of the previously mentioned models do not deal with the fact that most relationships between grain or flour parameters and loaf volume are not linear: there is an optimum level of energy needed to produce the largest loaf; below the level the dough is under-mixed, and above that it is over-mixed, resulting in smaller loafs. So, for a set of technological parameters and ingredients, there is an optimal dough strength and extensibility. The Protein Scoring System (PSS) (Békés 2012b) uses the Morup- Olesen transformation (Møup and Olesen

1976) (developed originally for describing the effects of individual essential amino acid levels in food or feed stuffs on the biological value) on certain dough parameters prior to multiple regression. Using only four parameters –protein content, water absorption, dough strength (Rmax), extensibility– a $r^2 > 0.85$ can be achieved with low standard error of prediction on loafs produced with commercial bread-making formulations. The model is in use in both breeding and quality control situations applying dough property parameters predicted based on NIR spectra, making the end-product quality estimation incomparably cheaper than carrying out baking tests, with stronger relationships between predicted and measured data than the direct loaf volume prediction from NIR spectra.

1.3 HPLC Methods to Determine Quality in Wheat

High performance liquid chromatography (HPLC) has been applied within research during the last 30 years for determining protein quality in wheat. One of the major steps towards developing HPLC methods for quality determination occurred in the lab of Prof. Finlay MacRitchie in Australia, from which the first publications about the topic came around 1990 (Singh et al. 1990). The work in this lab resulted in the current world-wide use of size exclusion (SE)-HPLC technique for determination of %UPP (Gupta et al. 1993). Simultaneously as the Australian group developed the SE-HPLC method, a reversed phase (RP) based HPLC method was developed in Canada for prediction of wheat quality (Marchylo et al. 1989). The RP-HPLC method was later refined in order to quantify the amount of certain protein groups and subunits (Wieser and Seilmeier 1998), and by applying various extraction buffers to understand how proteins in the polymer are bound (Kuktaite et al. 2004; Rasheed et al. 2016). Since the development of the SE-HPLC method to determine %UPP, this method has been used widely in research laboratories around the world. The method is based on a two step extraction (Gupta et al. 1993), where the first step extracts proteins soluble in SDS, while the second step extracts proteins soluble by sonication. In principle, the first step extracts polymers and monomers not bound to large polymeric proteins structures through disulphide bonds. In the second step, the sonication is primarily breaking the disulphide bonds, although sonication in too tough conditions is also able to break peptide bonds, while a too weak sonication does not break enough disulphide bonds to solubilize all the proteins in the wheat grain (Gupta et al. 1993). The %UPP is calculated combining information from both the chromatograms as %UPP = amount (area under the chromatogram) of unextractable (chromatogram from sonication) polymeric protein (PP)/(amount unextractable + extractable (chromatogram from SDS) PP) ×100. Thus, %UPP is describing how large part of the polymeric proteins, being extracted by the two-step extraction procedure adopted, that is not easily extractable (sonication is needed instead of SDS) and not bound with disulphide bonds. The method as described above and as developed by MacRitchie's lab has been applied as such in many labs; e.g. in Sweden (Johansson et al. 2001; Kuktaite et al. 2004; Rasheed et al. 2016), Italy (Pirozi et al. 2008), Norway (Tronsmo et al. 2003), the USA (Naeem

et al. 2012), Canada (Edwards et al. 2007) and China (He et al. 2005). In the majority of these studies, the %UPP has been correlated to wheat quality, and primarily a positive correlation has been stressed towards other quality evaluations, measuring gluten strength. A number of studies have also used the total amount of SDS extractable proteins (proteins from extraction in step one) measured with SE-HPLC as a measurement of grain protein concentration as a close correlation prevails (Malik et al. 2011, 2013). Furthermore, some studies have divided the SE-HPLC chromatograms into different parts where the majority of the glutenins and gliadins are present, extrapolating that the glutenins are forming the PP while the gliadins are forming the monomeric proteins (MP). Thereafter the glutenin to gliadin ratio from the SE-HPLC chromatogram has been correlated to gluten quality (Park et al. 2006; Wang et al. 2014). In a recent study, alternative calculation methods were used for %UPP (Hu et al. 2017). Although a definition is presented in this publication, such alternative definitions of already existing abbreviations is a bit messy. The RP-HPLC methods have been used in a range of studies both to quantify different subunits, as e.g. HMW-GS can be distinguished as separate peaks with the appropriate running conditions and column for the analyses (Wieser and Seilmeier 1998; Wieser et al. 1998). However, RP-HPLC methods have also been used to quantify amounts of proteins extractable with a range of different extraction buffers (Johansson et al. 2001, 2002, 2003; Kuktaite et al. 2004, 2011; Rasheed et al. 2016). Thus, such analyses can contribute to an understanding of the relations of proteins that are cross-linked through different types of bonds including disulphide, sulphur and peptide bonds (Ceresino et al. 2019; Rasheed et al. 2018). Cross-links between storage proteins of wheat is a major contributor to the quality of wheat (Johansson et al. 2013) and their existence in the wheat grain and their interchange during the mixing and bread-making process are affecting the quality of the wheat dough (Hussain et al. 2012; Johansson et al. 2013). In general, the HPLC methods have contributed largely to an increased understanding of protein behaviour in the wheat grain and in flour during mixing as well as in dough during bread-making (Johansson et al. 2013). Still, the HPLC methods are not used to a large extent in plant breeding for improving bread-making quality. Several of the methods, especially SE-HPLC to determine %UPP, are high through-put, need only a small amount of material, and are relatively cheap. Consequently, at least SE-HPLC for %UPP determination should be of interest to implement as a selection method for plant breeders to select for wheat quality. However, despite the many positive characteristics of the method, it has also some clear drawbacks. The major drawback is the lack of consistency of the method over different labs using the method. This is due to differences in set up and running conditions but also to the fact that the type of equipment, column and even temperature during running are affecting the results. Also columns from different suppliers result in differences in chromatograms and even columns from the same supplier may not be consistent. Thus, to get comparable results, samples always need to be run on the same column, in the same batch and in the same lab. For comparisons over various columns, batches and even more so over different labs, standard samples need to be used and results recalculated according to these standards. Besides issues with repeatability, a correct interpretation of the HPLC results often require experience running this kind of analyses.

1.4 LC-MS/MS Method and Perspectives to Improve the Evaluation of Vitamins in Wheat

Wheat as one of the major world agricultural products, has so far received great attention regarding its technological quality attributes but little work focuses on its nutritional quality. Water-soluble vitamins are an important class of compounds that require quantification from food sources to monitor nutritional value. Measurement of vitamins in food is complicated and represents a complex analytical problem for several reasons. Firstly, vitamins are compounds having diverse chemical structures and properties. As a consequence, it is very difficult to develop a single method for their simultaneous determination. Secondly, wheat vitamins are present at relatively low concentrations that require sensitive methods for their analyses (Fardet 2010). Finally the susceptibility of vitamins to degradation by exposure to light, air, heat, alkaline pH and their diverse forms make their extraction from food matrices very challenging. An important amount of studies have been devoted to the development of analytical methods which could explore and monitor the nutrient composition of whole-grain wheat or end-use products. Nevertheless, in the objective of a large scale varietal screening, none of the reported methods were suitable for the analysis of wheat flour and wheat food products. The present study briefly describes the LC-MS/MS method which was recently developed. In addition some perspectives to improve the nutritional evaluation of vitamins in wheat will be discussed.

1.4.1 Evaluation of the LC-MS/MS Analytical Method for the Simultaneous Screening of Seven Water-Soluble Vitamins

Taking advantage of high sensitivity and selectivity of the MS/MS detection the extraction procedure was considerably simplified. Effectively one of the main goal was to find a procedure allowing the analysis of all vitamins present in food products in a single chromatographic run and to simplify as much as possible the extraction procedure in order to apply this method at high throughput. The analytical procedure was optimized by taking into account both the nature of the analyses and the nature of the matrix. An enzymatic mixture (Ndaw et al. 2000) was selected and optimized for the analysis of the free and some chemically bound forms of vitamins in foodstuffs. This extraction is simple, fast, accurate and can be extended to different wheat food sample (wholemeal grain, flour, dough, bread, toasted bread, biscuit). Due to the heterogeneous nature of the materials being studied, the important issue of the matrix effect was investigated to avoid any interferences which could induce bias during the analysis. This difficulty was overcome by the use of one isotope labelled internal standard for each class of compounds (the seven analyzed vitamins were classified into three classes of homologous compounds). The excellent precision and bias (Nurit et al. 2015) of the method within the different materials validated the choice of using three internal standards. The choice of a reverse column coupled with accurate chromatographic conditions have allowed the separa-

tion of the seven water-soluble vitamins in 15 min with excellent performances in terms of peak shape and peak intensity for most of the vitamins. The method was applied to the determination of water-soluble vitamins in manufacturing wheat-based food products (Nurit et al. 2016) and in a large set of 195 genetically diverse cultivars (Nurit 2015). The 195 accessions were chosen from the INRA worldwide bread wheat core collection of 372 accessions curated by the Clermont Ferrand genetic resources Center (Balfourier et al. 2007). This core collection, representative of the world's wheat diversity, was also studied for agronomic performances and quality traits (Bordes et al. 2008). The 195 accessions brought a huge phenotypic diversity as revealed particularly on the seven water soluble vitamins (see chapter "Environmental effects on wheat technological and nutritional quality" in this book). The simplicity of the extraction procedure, as well as the direct injections of the extract in the LC- MS/MS system make this method rapid and potentially high-throughput. As a consequence, this procedure is suitable for a fast screening of vitamin contents in wheat flour and wheat-based food products with the objective of a large scale varietal screening.

1.4.2 Perspectives about Improving Techniques

Water-soluble vitamins are an important class of compounds that require quantification from food to monitor nutritional value. Nevertheless, most of the vitamins exits as groups of chemically related compounds having similar biological activity capable of meeting a nutritional requirement (frequently called "vitamers"). For the case of water-soluble vitamins, niacin (nicotinic acid + nicotinamide) and vitamin B6 (pyridoxine, pyridoxal and pyridoxamine) constitute an interesting case, in which, the glycosylated forms of pyridoxine and nicotinic acid are prevalent in plant-derived foods (Gregory et al. 1991). In addition, it has been shown that in the milky kernel of maize, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) were the predominant form in which niacin occurs (Wall and Carpenter 1988). Common extraction processes for these vitamers involve alkali or acid hydrolysis under heating condition (either in a boiling water bath or in an autoclave). Such extraction releases free vitamins from its bound forms. In the presented LC-MS/MS method such extraction which was destructive for an important part of vitamins led us to achieve a gentler extraction method which did not allow the complete release of the vitamins present in the sample as NAD, NADP, nicotinic acid glucoside and pyridoxine glucoside. In data previously reported (Sampson et al. 1996), there was a significant fraction (average of 68%) of vitamin B6 in wheat present as pyridoxine glucoside. An important additional finding was the changes in the distribution of niacin compounds in corn during its development. Immature sweet corn is an effective source of NAD and NADP, whereas niacin in mature field corn is largely present as glycosylated forms of niacin (Wall and Carpenter 1988). These glycosylated forms were reported as nutritionally unavailable (Gregory et al. 1991; Wall and Carpenter 1988). Thus, it will be of interest to develop a simple, reliable and high throughput analytical method

which allows the measurement of all biologically active water–soluble vitamers. Therefore, it will be interesting to take full advantage of the high sensitivity and selectivity of the MS/MS detection. The challenge will be to optimize the MS/MS parameters for the identification and quantitation of the different binding forms of water-soluble vitamins and to find chromatographic conditions allowing the separation of the bound and free forms of vitamins in a short run time with a good performance in terms of peak shape and peak intensity. Such methodology should help to develop generalized approaches to account for differences in bioavailability of vitamers and to thoroughly enlighten the consumer about the nutritional labeling of cereal products. However, the current high costs of chemically analyzing large populations by LC-MS/MS may limit its use for breeding (particularly for early breeding generations which implies a large number of samples to be screened) and, consequently, LC-MS/MS will probably not become a standard selection tool, unless effort in developing analytical technique such as infrared spectroscopy are taken, then development of the calibration models for quantifying the wheat flour vitamins could be achieved using primary reference data obtained from LC-MS/MS method.

2 Quality Selection in Breeding Programs, Case Studies

In this section we review quality selection strategies considering bread wheat breeding in Germany, Uruguay, Argentina and USA (bread and soft wheats). Durum wheat quality selection strategies are also reviewed. The last part of this chapter covers quality evaluation in wheat wild relatives considering the experience of Kazakhstan.

2.1 Wheat Breeding for Improved Baking Quality in Germany

2.1.1 Introduction

Today, the German wheat classification system categorizes varieties according to their baking quality as part of the registration process. E- (elite) wheats have the highest quality, followed by A- (blending), B- (bread making) and C- (not usable for baking) wheats, the latter having the lowest quality. Assignment to a certain quality group is dependent on particular minimum requirements with respect to individual quality traits and on a comparison with a defined reference variety. New varieties are registered and protected after successfully passing 3 years of official testing in several environments for agronomic value, homogeneity and novelty. Direct and indirect quality parameters (loaf volume, dough elasticity and surface, crude protein content, falling number, sedimentation value, water absorption and milling yield) for baking quality are part of the official variety approval and registration process in

Germany (BSA 2017). Selection decisions for quality in early generations with limited grain and large number of samples are performed by markers linked to the known *Glu-1* and *Glu-3* glutenin loci, followed by indirect phenotypic selection based on correlated quality traits like crude protein content, sedimentation value, grain hardness and falling number. The development of alternative breeding strategies to increase the selection gain per year is a continuous challenge for plant breeders; as a consequence, genomic selection is already being applied as an efficient approach to support wheat quality improvement in German breeding programs.

2.1.2 Breeding Progress

From the historical perspective breeding for improved baking quality of winter wheat was very successful in Germany after World War II. The introduction of shorter varieties allowed higher levels of nitrogen application as well as late top dressing, and together with the release of varieties with better protein quality it was possible to produce winter wheat with acceptable baking quality. After returning to self-sufficiency after World War II, Germany still had to import about two million tonnes of high quality baking wheat from Canada every year until the 1970s. In the course of the 1970s, however, winter wheat production in Germany was able to cover the domestic demand of wheat with sufficient baking quality (Laidig et al. 2017). The aim of obtaining wheat of better baking quality has been accomplished over the decades by both the elimination of inferior quality varieties and the development of new varieties with superior quality. This was confirmed by trends in yield and baking quality of winter wheat varieties released during 1961–2008 and 1983–2014 in Germany (Hartl et al. 2010; Laidig et al. 2017). Hartl et al. (2010) observed a clear increase of grain and protein yield, sedimentation value and loaf volume based on genetic improvements, whereas protein content was only raised in magnitudes obligatory for reaching the criteria for high baking quality wheat. They assumed that selection decisions in early generations were mainly carried out on the basis of sedimentation value in German wheat breeding programs. As a side effect, application of this single selection criterion might have led to the increased occurrence of so-called correlation breakers, i.e. varieties that achieved, despite a high sedimentation value, only medium loaf volumes. However, on the positive side, Hartl et al. (2010) concluded that breeding progress in Germany has improved the specific loaf volume (loaf volume/% crude protein) progressively and that an increasing number of registered varieties reached high to very high loaf volumes with a medium protein content. Laidig et al. (2017) found a large significant gain in grain yield (24%), but a strong decline in protein concentration (−8.0%) between 1983 and 2014. They showed that both traits are strongly negatively related, which was consistent over all varieties and also within quality groups. However, the study indicated that losses in baking quality were mitigated in Germany between 1983 and 2014 or even partially improved. Improvement of indirect baking quality parameters were achieved for falling number (5.8%), sedimentation value (7.9%), hardness (13.4%), water absorption (1.2%) and milling yield (2.4%). The apparent gain in sedimentation value provided

evidence that progress in baking quality was mainly due to improved protein quality as well as their interaction with other fractions like the puroindolines that correspond to grain hardness. The authors further reported that grain yield, falling number and protein concentration were highly influenced by environment, whereas for sedimentation value, hardness, water absorption and loaf volume it was stated that genotypes accounted for more than 60% of total variation. They concluded that the strong negative relation between grain yield and protein concentration on the one side and the strong association between protein concentration, sedimentation value and loaf volume on the other side makes it difficult to achieve breeding progress in traits related to end-use quality.

2.1.3 Conclusions

In summary, breeding for improved baking quality in wheat is determined largely by the common negative correlation between yield and crude protein content. Over recent decades, wheat breeding in Germany has concentrated on yield, so that newer varieties, generally, have higher yields and lower crude protein content. As compensation for lower crude protein content, there has been an efficient selection for higher gluten quality. For the future, selection of wheat varieties that combine high yield and sufficient baking quality in lower nitrogen-input and higher climate-variability cropping systems may require an advanced breeding approach. There is a need for varieties with a maximum ability for nitrogen uptake, high nitrogen remobilization and reallocation efficiency during grain filling and an efficient conversion of nitrogen into high-quality proteins. What is sought-after are not necessarily wheat varieties that consistently achieve 13% protein, but varieties that will be suitable for bread-making at 11–12% crude protein with stable quality under increasingly fluctuating environmental conditions.

2.2 Wheat Breeding for Improved Bread-Making Quality in Uruguay

Quality screening in Uruguay has been reported since 1925 (Boerger 1928), when test weight, flour extraction and baking performance were evaluated for advanced lines. Although Farinograph was applied since 1929, during the subsequent decades, several faster tests such as Pelshenke and Zeleny sedimentation proved to be useful. This was the practice until the early 90s, when the SDS sedimentation test (Amaya et al. 1991) was incorporated as a key element for early generation screenings. Due to markets requirements (Peña 2007), a more sophisticated approach was adopted since the 1990s. While gluten strength was still evaluated by the same SDS sedimentation test in early generation screening, selection for rheological properties in later steps became essential. The Mixograph proved to be more effective than the Farinograph to characterize mixing properties since it is faster and a smaller sample

is required. Extensional properties were proven to be key for breadmaking properties, so the Alveograph was included in the protocols (Vázquez and Watts 2004). Protein/gluten content was evaluated by two independent methodologies. Wet gluten was included as a characterization parameter due to requirements from the milling industry. Traditional Kjeldahl analyses were used until near infrared spectrometry (NIRS) technology was developed specifically for the breeding program (Vázquez et al. 2007). Although protein and rheological properties are considered the most relevant characteristics, several independent parameters should be considered as well, such as milling behaviour and proper seed dormancy and resistance to preharvest sprouting through Falling Number. Grain hardness was traditionally evaluated by the pearling index method. More recently, Particle Size Index was used to develop a NIRS equation (Vázquez et al. 2007), which is extensively used. Once selected genotypes are almost ready to be released, breadmaking tests are performed, including both the standard AACC 10–10 and one specifically designed for local breads (Paulley et al. 2004). Further tests have been evaluated. For example, Mixolab proved to generate information that is complementary with that which is traditionally used (Vázquez and Veira 2015). Pentosans were also evaluated and proved to significantly affect rheological properties of the dough (Garófalo et al. 2011). However, these parameters have not been included in the routine characterization of the genotypes because of the cost-benefit ratio. All genotypes are evaluated in several environments, considering the importance of the genotype by environment interactions (Vázquez et al. 2012) and stability (Vázquez and Castro 2018). Several technologies are being considered for the near future. New NIRS calibrations are on the agenda, including equations for gluten strength (Vázquez et al. 2007). Genomic selection proved to be useful, mostly when combined with other tools (Lado et al. 2018).

2.3 Wheat Breeding for Improved Bread-Making Quality in Argentina

Records of wheat introduction in Argentina appeared in the early XVI century by Spanish conquerors, and for more than three centuries wheat was cultivated on a low scale, at a level close to mining communities in the central-east part of the country. We have to wait until the end of the XIX century to witness a rapid expansion of the crop boosted by the European immigration wave, the development of land (railway) and sea transportation systems and the increase of food demand by Europe. Wheat breeding started at the beginning of the XX century with efforts concentrated on improving disease resistance, bread-making quality, tolerance to the main abiotic stresses, and grain yield, mostly as a response to disease tolerance, lodging resistance, and management practices (Slafer and Andrade 1989). National and international markets demanded wheats with strong gluten pushing breeding programs to release high breadmaking quality wheats. However, the lack of a premium price

prompted by quality segregation pressed farmers to sacrifice quality for yield, as wheat was exported as a commodity. This situation has changed over the last 10 years as more than 70% of the crop area are planted to good quality high yielding cultivars. In 2018 the total grain production was 18.4 million tonnes (average yield 3.2 t/ha) (trigoargentino 2018). In 2017, 12.6 million tonnes were exported to 47 different destinations; Brazil was the main buyer with 40% of total wheat exported (Indec 2018). Argentina is the main wheat producing and exporting country in Latin America. Wheat trading is based on Standard of Commercial Grades that includes in the hard wheat price a bonus of 2% by protein content when above 11% (13.5% moisture basis) if the test weight is greater than 75 kg/hl. When protein content is under 10.9% gradual and cumulative discounts are applied: 10.9–10% a discount of 2%, 9.9–9.0% a discount of 3% and less than 9% a discount of 4% (norma 2018). In Argentina grain and flour quality characterization have shown some logical modifications over time. For example, a standard quality characterization for varieties released during the 1950–70's considered (i) physical and chemical tests on grain including test weight, weight per 1000 kernels, flour yield, protein content and ash content; (ii) flour tests including ash content, protein content, wet gluten content and gas production (Elion 1933); (iii) flour baking tests, baking water absorption, baking mix time, baking loaf volume, crumb grain (0–100) and crumb whiteness (0–100); and (iv), the Alveograph (Chopin) dough testing device parameters P, L, G, W and P/L. During the 1970–80s with the support of CIMMYT, local breeding programs introduced Mexican germplasm with a positive impact on yield genetic gain (Lo Valvo et al. 2018). Also, Pelshenke test (early generation), flour (dough) testing using the Zeleny test, and the Farinograph (Brabender) dough testing device with parameters water absortion, development time and stability time, were included for grain and flour quality characterization, and during the 1980–90's falling number was included as an additional flour test. We have to wait until 1998 to find a significant contribution to grain and flour quality characterization for variety release: the creation of an internal quality classification system. The proposal defined three wheat Quality Groups (QG): QG 1 involved cultivars with extra strong gluten suitable for blending; QG 2, cultivars adapted to traditional baking in Argentina (fermentation time longer than 8 h); and QG 3, cultivars suitable for direct baking methods (fermentation time less than 8 h). The classification system was based on the information obtained from a set of quality tests including test weight, protein content, wet gluten content, flour yield/ash content ratio, Alveograph W, Farinograph stability, and baking loaf volume (Cuniberti and Otamendi 2004). The next relevant contribution to grain and flour quality characterization was the creation of a quality index based on weighted contributions of the same set of quality tests used in the above quality classification system, with 45% of the variation of the quality index accounted by the variation of W value from the Alveograph and baking loaf volume variables (Salomon and Miranda 2003). In 2007 grain and flour quality characterization for cultivar release included for first time grain color (Minolta, parameters L∗, a∗ and b∗), HMW-GS, 1BL/1RS and 1AL/1RS wheat-rye translocations, determined by SDS-PAGE and A-PAGE, respectively (Bainotti et al. 2009). Lastly, in 2011, grain hardness (SKCS score) was included with no additional modifications

in grain and flour quality characterization for cultivar release to the present day. In Argentina more than 98% of wheat production is bread wheat, mostly hard red spring with 120 cultivars in the market; and remaining 2% are durum, soft and waxy wheats. In general terms breeding programs select bread-making quality at different stages of the process as it follows: various technological micro-tests like Zeleny and SDS sedimentation are used to select strong gluten in segregating materials (individual plants) at early generations. Selected lines advanced to preliminary and regional yield trials are evaluated and selected based on grain properties using test weight, weight of 1000 kernels and protein content. Milling, flour, and dough properties of lines advanced to preliminary trials are evaluated considering flour extraction (>70%), wet gluten content and Mixograph (National Manufacturing) parameter mixing time. After that, lines advanced to regional yield trials are evaluated based on rheology properties including Alveograph (Chopin) parameters W and P/L, Farinograph (Brabender) parameter stability and baking loaf volume. Table 1 summarise typical quality test for bread wheat selection in a breeding program in Argentina. A putative new variety is evaluated based on official regional yield trial information considering 2 years of trials in three locations or 3 years in one location and QG is defined by comparison with three reference varieties (Bainotti et al. 2009, 2017).

A special mention should be given to the use of molecular markers in breeding for quality traits. First attempts of marker assisted breeding in Argentina come from the late 1990s with the INTA National Wheat Breeding Program and were focused on selection of superior HMW-GS in small segregating populations by SDS-PAGE. More recently, molecular information of breadmaking quality protein/traits HMW-GS (Gianibelli et al. 2002), LMW-GS (Lerner et al. 2009; Demichelis et al. 2018), wheat-rye translocations (Vanzetti et al. 2013), puroindolines (Moiraghi et al. 2013), *Gpc-B1* for grain protein content (Tabbita et al. 2013), and the GBSS I gene (Vanzetti et al. 2009), among others, was exploited by breeding programs for germplasm characterization (introductions, crossing blocks), and selection (RILs) with the aim of fixing valuable alleles (HMW-GS *Glu-B1al* allele, GPC, others) into adapted germplasm. Examples of marker assisted breeding are cultivars Biointa 2004 and MS INTA 416 with introgression of disease resistance genes *Lr47* and *Fhb1* (Bainotti et al. 2009, 2017); the release of commercial cultivars with marker assisted introgression of quality traits is still a pending task.

2.4 Hard Wheat Breeding for Improved Bread-Making Quality in USA

Wheat breeding crosses are made to align attributes of two varieties in order to make progeny which are genetically superior to both parents. Crosses are made and traditional selection occurs or the doubled haploid process is applied to make

Table 1 Quality tests used in Argentina for bread wheat selection in breeding programs

Test	Application	Sample type	Sample Size	Comments/protocol
Zeleny test	Measures the swelling of the proteins in a solution of lactic acid and propanol. Indicates the quality of the gluten	Flour	3.2 g	To select strong gluten in segregating materials at early generations (F_{2-3-4}). Strong correlation with protein content. AACC 56–61
Test weight	Measures the density of a grain and how well the endosperm has filled out	Grain	500 g	For characterization of lines advanced to preliminary trials (F_{6-7}) and regional yield trials (F_{8-9-10}). Values higher than 76 kg/hl
Weight of 1000 kernels	Grain characterization	Grain	1000 kernels	For characterization of lines advanced to preliminary trials (F_{6-7}) and regional yield trials (F_{8-9-10}). Values higher than 35 g
NIR	Predicts protein content	Grain	5 g	For characterization of lines advanced to preliminary trials (F_{6-7}) and regional yield trials (F_{8-9-10}). Values higher than 10.5%, 13.5% moisture basis. AACC 39–21
Wet gluten content	Gluten characterization	Flour	10 g	For characterization of lines advanced to Preliminary Trials (F_{6-7}). Values higher than 25%. IRAM 15864
Mixograph (National Manufacturing)	Indicator of gluten strength	Flour	10 g	For characterization of lines advanced to preliminary trials (F_{6-7}). Parameter mixing time > 3 min. AACC 54–40
Alveograph (Chopin)	Indicator of gluten strength and breadmaking quality	Flour	250 g	For characterization of lines advanced to regional yield trials (F_{8-9-10}). Parameters W > 240 and P/L = > 1. AACC 54–30 A
Farinograph (Brabender)	Measures flour water absorption and dough mixing characteristics; indicator of gluten strength	Flour	50 g	For characterization of lines advanced to Regional Yield Trials (F_{8-9-10}). Parameter stability should be between 10–40 min. IRAM 15855
Baking loaf volume	Indicator of dough suitability for breadmaking quality	Flour	100 g	For characterization of lines advanced to regional yield trials (F_{8-9-10}). AACC 10-10B
SDS-PAGE	HMW-GS characterization	Grain	3–5 grains	For characterization of lines advanced to Regional Yield Trials (F_{8-9-10}). Lawrence and Shepherd (1980)

segregating populations of wheat into fixed, true breeding lines. Through the time of segregation, generally only molecular markers or grain grading are used to make selections for wheat quality. Finally the line is fixed, or true breeding, but at this first stage, usually very little seed is available, and almost all the seed needs to be used in multiplication for the next year. Grain grading for kernel size, color, and vitreousness may occur at this stage. Typically, only enough seed is available for genotyping and planting of first year yield trial. Following the first yield trial, a limited amount of excess seed should be available for wheat quality testing. Wheat grain is assessed for pre-milling characteristics that impact marketing. These tests include kernel weight, test weight per volume, color, hardness, vitreousness of the kernel, and total protein content, and any other tests which may be conducted on NIRS systems. At this stage of heavy seed limitation and high entry numbers, small-scale tests are typically utilized, such as small scale milling (Brabender) along with smaller rheological testing such as Mixograph (National Manufacturing), Zeleny or SDS-sedimentation tests (AACC 2010). Though many international standards require Farinograph or Alveograph testing, the Mixograph is preferred by most US breeding programs. With these data, as well as field phenotypes, and any molecular data or GS prediction, typically one round of breeding selection is made, and entry numbers are highly reduced. After this stage, and into replicated yield trials, enough seed is typically available to conduct milling, Mixograph, and baking tests. Individual breeders determine their breeding pipeline allocations to early generation work, molecular markers, yield trials, disease screening, and quality testing. Many times this leads to wheat quality testing occurring later in the pipeline and on fewer materials which are already highly screened for yield and adaptation traits. The art of breeding is applied in creating a pipeline in which genetic gains are coming rapidly and fewer lines need to be discarded due to mandatory traits. In US breeding programs, no official recommendation system is in place for the release of varieties. Breeders utilize milling, dough rheology, and baking tests, as well as molecular markers, along their release decision making pipeline to insure they individually release high quality wheat varieties. Internal decision making committees which consider all attributes of a wheat variety and vote upon release decision are common. Wheat is segregated by market class in the United States. The predominant types are hard red winter, hard white, hard red spring, soft red winter, and soft white wheat. These marketing classes all have unique end-user standards. The Wheat Quality Council (Wheat Quality Council 2018) has targets approved for each market class in the USA, which can be found in their reports.

2.5 Quality Evaluation in Soft White Wheat

The U.S. Pacific Northwest states of Idaho, Oregon, and Washington produce approximately 5.4 mmt of soft white wheat annually. This production is apportioned about 6% club and 94% soft white ‘common’ (those varieties having a com-

pact and lax spike, respectively). Both winter and spring varieties are grown, but winter types dominate due to the generally higher production potential of winter wheats in general. Soft white wheats are bred and selected to have superior and unique end-use quality. Much of the work involved in evaluating experimental wheat breeding lines and conducting wheat quality research occurs at the U.S.D.A. Western Wheat Quality Laboratory (WWQL). Following, is a brief description of the relevant testing that occurs. The WWQL also evaluates hard red and white 'bread' wheats. Naturally, grain yield is the primary consideration of wheat breeders, followed by yield protection in the form of resistance to abiotic and biotic stresses. From the farmer perspective, high bulk density (test weight), appropriate protein content (usually 8.5–11.0%), and high Falling Numbers are their measure of quality. But from a breeding perspective, milling performance, functionality of starch, components influencing water relations, color, dough strength, and cookie and cake quality are measured and selected for.

Milling Performance Milling performance is evaluated using a 'MicroMill' (30 g samples), a modified Quadrumat Sr. (600 g), Buhler MLU-202 (1500 g), and Miag Multomat (40–300 kg) flour mills. Desirable criteria include soft kernel texture, ease of endosperm-bran separation, mellow friable endosperm, high break flour and straight grade flour yields with low ash, bright white color, and low starch damage. The Quadrumat system performs the bulk of sample testing, usually around 4500 individual breeding lines per year.

Starch The primary selection criterion for starch functionality relates to 'normal' vs. 'partial waxy' genotypes, the latter are targeted for the Northeastern Asian noodle markets of Japan and South Korea. The partial waxy trait is conferred by a single null allele in granule bound starch synthase, and causes a slight decrease in amylose and an increase in starch swelling. This increased swelling creates a softer but resilient noodle texture. The majority of soft white varieties have normal starch. Testing for partial waxy lines is achieved by using the Flour Swelling Volume Test, the RapidVisco Analyzer, and the Amylograph.

Water Relations A number of different factors can contribute to water relations in doughs and batters. In general, soft white wheat has low water absorption. Selection for low water absorption involves low values for water solvent retention capacity (SRC), sodium carbonate SRC, sucrose SRC, and low dough water absorption in the 10-g Mixograph and 50-g Farinograph. These tests are aimed at capturing low starch damage, low arabinoxylan content, and are certainly influenced by protein and bran contamination.

Color As mentioned above, soft white wheat flour should be bright white and free of bran specks. Additionally, breeding lines are screened for polyphenol oxidase activity using the AACC International Approved Method that employs L-DOPA (L-3,4-dihydroxyphenylalanine) as the substrate in a whole-seed assay. Further, raw white salted and alkaline noodle sheets are evaluated for brightness after 24 h using the C.I.E. L^*, a^*, and b^* color space. High L^* values and good color stability (low ΔL^*) are desirable.

Dough Strength Dough strength is the primary trait that separates soft white common and club wheat sub-classes. Whereas soft white common varieties range from moderately weak to moderately strong, club wheats are selected for uniformly weak dough strength. The methods used include flour SDS micro- sedimentation and the 10-g Mixograph. The Farinograph and Alevograph (AlveoLab) are also suitable but cannot produce sufficient sample throughput in early generations.

Cookie and Cake Quality All soft white breeding samples receive a cookie bake. The cookie test captures a number of different flour attributes but is especially influenced by starch damage and arabinoxylans. Large diameter cookies that result from greater oven spread are desirable. More advanced generation soft white common lines and all club wheat lines additionally receive a sponge cake bake test. The sponge cake method involves gently folding the flour into a prepared egg-sugar-water foam. Large volume cakes with a symmetrical shape and soft interior crumb are desirable. The combination of a very low moisture cookie and high moisture cake batter capture a wide range of products typically made from soft white wheat. On an occasional basis, steamed breads and boiled white salted noodles are evaluated. Many of the world's foods are best made using soft wheat flour. For these, U.S. Pacific Northwest soft white wheat is particularly well suited. As in all wheat breeding endeavors, this is not by accident or chance. A considerable amount of resources and a large variety of individual tests are employed to provide breeders a detailed portrait of end-use quality. These data are used in a rigorous selection process to ensure that growers, millers, processors and consumers receive the highest quality varieties, grain, flour and foods.

2.6 Current and Potential Approaches to Quality Evaluation in Durum Wheat Breeding Programs

Durum wheat breeding programs are scattered around the globe and present in many of the durum wheat producing nations (Canada, France, Italy, Spain, USA, Algeria, Morocco, Syria, Mexico, India, Germany, Kazakhstan, Austria, Turkey, Tunisia, Argentina and Australia). The breeding objectives for quality targets and extent of testing depend on the specific countries focus to supply durum wheat for internal use or export. While customers have specific requirements, key quality features should be met to sell in the market to meet the requirements for good pasta, couscous or other durum derived product quality. Nevertheless, many breeding objectives for quality are common across programs with differences related to specific issues for the country, for example screening for low grain cadmium content is important in Canada due to its higher soil content.

The breeder and quality chemist must try to satisfy the requirements of the entire production chain consisting of grower, grain trader, miller, pasta maker and consumer with each having different requirements (Troccoli et al. 2000). The typical process in a breeding program is to decrease the large number of lines (hundreds to

thousands) arising from a cross, selecting the best lines for yield, adaptation, quality performance and disease resistance. Lines are assessed against check varieties which are commercial varieties grown under identical conditions to the test lines. If a line is to be advanced it should perform equally or better than the checks. Characteristics may correlate but in opposition, for example the inverse relationship between yield and protein makes it a challenge to select for both and the breeding team needs to make decisions about what criteria take the highest priority in advancing a line. However, a Canadian variety Strongfield achieved a 13.5% yield increase over the check, Kyle, but also a 0.3% units higher protein content showing both targets can be achieved (Li et al. 2018). For quality evaluation, the test depends on the amount of sample and resources and methods available. In general, the earlier the stage of the breeding program, the less seed available, the larger number of samples to test and the more limited the testing possible due to resources available. Quite often predictive tests are used in early generation testing which results in less accurate prediction of quality. Table 2 summarises typical durum quality tests for pasta derived products made from durum wheat [the reader is referred to other texts for more details on these tests and those applicable to other durum derived products in the reference list (Abecassis et al. 2012; Sissons et al. 2012)] used in breeding programs. These tests are designed to ensure released varieties meet the various grading standards in each country and are related to uniform kernel size, high test weight, a high proportion of vitreous kernels, a low percentage of sprouted kernels, meeting protein, moisture, semolina, dough and pasta quality and consumer/buyer requirements of the durum production chain. While the above tests are commonly used in later generation testing of durum wheat when sufficient sample and resources are available, the choice of test for early generation testing is different. Some of the potential early generation tests are summarised in Table 3 and have been applied to varying extents in breeding for quality. There are several tests to measure dough properties directly (Mixograph, Glutomatic, Farinograph, SDS Sedimentation (SDSS)) that have found routine application in breeding programs while the Glutopeak is showing potential to replace some of these methods being faster and easier to operate and better at discriminating poor from medium-strong dough strength. Other instruments like the Kieffer rig and CSIRO extension tester have only been used for research. To create the semolina (flour) needed to conduct dough tests from limited grain quantities, small-scale mills can be used such as the falling number and Udy Cyclone mills (which produce wholemeal) or similar, Brabender Quadrumat Junior mill and Chopin mill are capable of producing semolina from as little as 20 g of grain.

Many samples can be milled to produce samples for further testing like SDSS. Where very limited seed is available (<5 g) specialised micro-mill such as FQC 2000 can be used to make a “semolina” or when many samples are to be evaluated, biochemical based tests have found some favour for prediction of dough strength such as SE-HPLC to determine %UPP, 96 well microplate reader to measure % insoluble glutenin (IP%) and the swelling index of glutenin (SIG). The latter has found application in durum breeding (Li et al. 2013). The most useful and commonly used instrument for assessment of grain and semolina is NIR. Using scan-

Table 2 Typical durum quality tests for pasta derived products

Test	Application	Industry sector	Sample type	Sample size	Desirable range	Reference
Test weight	A measure of the density of a grain and how well the endosperm has filled out	Grain trader, miller	Grain	500 g	>74 kh/hl	AACC 55–10.01
Falling number	Used to assess weather damaged grain, which lowers starch viscosity	Miller, pasta maker	Wholemeal flour	100 g grain	>250 s	AACCI 56–81.03
Vitreous kernel	Kernels with translucent, vitreous appearance	Trader, miller pasta maker	Grain	300 kernels	>70%	ICC method 129
Screenings	Grain with high percentage of undersized grains reduces semolina yield	Grain trader, miller	Grain	>150 g	<5%	Particles below 2.0 mm
Protein	Dry pasta made from high-protein semolina (12%) is physically stronger and more elastic than pasta from lower protein semolina	Grain trader, miller, pasta maker	Grain	>50 g	>13%	AACC 46–30.01; ICC method (NIR) 159
Yellow pigment	The main pigments in durum wheat responsible for the yellow color are carotenoids	Pasta maker	Grain, semolina	2–3 g	Exceed check varieties	AACC 14–50.01
Yellowness	A bright, yellow color in semolina ensures a good color in the pasta	Pasta maker	Semolina, pasta	>20 g	Minolta b∗ >26	AACC 14–22.01
Moisture	Post harvest low moisture content is expected, which is necessary for the storage life of the grain	Grain trader, miller	Grain	>50 g	Below 12%	AACC method 44–15.02
Ash	Mineral content remaining when all the organic content has been removed by combustion at very high temperatures	Miller	Grain, semolina	2–3 g	<0.9%, dmb	AACC 08–01.01 ash- basic method
Semolina yield	Millers aim to produce as much semolina from a given amount of grain with minimal flour	Miller	Grain	>20 g	>65%	AACC 26–41.01
Granularity	Affects the amount and uniformity of water absorption during mixing	Pasta maker	Semolina	100 g		AACC 66–20.01
Gluten quality	Gluten strength	Pasta maker	Semolina	>2-100 g	Medium- strong dough	AACC 54–40.02, 54–22.01, 54–30.02, 54–10.01; ICC 158
Pasta quality	Capacity of the product to maintain good texture after cooking	Consumer	Pasta	>5 g	Firm, good colour, taste, aroma	AACC 14–22.01

ning instruments internal calibrations can be set up in the laboratory to predict a range of characteristics. Machines that can handle grain are desirable as they are non-destructive and faster to process samples than semolina/flour. Examples of some uses are listed in Table 3. The most accurate measures are those with a high RPD such as protein and moisture while many of the other predictors are better suited to rough screening (Sissons et al. 2006) which is still useful to make changes to the direction of a population of plants in the desired way. More recent technology such as image analysis is more accurate at assessing grain defects such as hard vitreous kernels, black point percentage, identifying insect infestation than NIR and grain grading is being used in some countries at grain silo receival stands with potential for future applications (Saini et al. 2014). Starch properties can be readily measured using small samples of semolina or ground pasta using an RVA (Table 3). However, this has mainly been used for research as the role of starch in durum wheat quality evaluation has not been considered important enough to measure in a breeding program unless the RVA is being used to predict falling number. The RVA is particularly useful to discriminate waxy from high amylose durum where each has distinct RVA profiles (Lafiandra et al. 2012). Some equipment like the GRL extension tester and Viscoelastograph to measure pasta viscoelasticity are unique to a laboratory (Grain Research Laboratory) or no longer in production leaving texture analysers and cooking tests (cooking time, cooking loss, water absorption, total solids) as the main tools to assess pasta quality for breeding lines. Even small-scale pasta extruders can be used to prepare pasta for assessment (Table 3) but there are limitations with sufficient sample available for pasta analysis together with loss of appearance and colour leaving this approach more to research.

2.7 Quality Evaluation in Wheat Wild Relatives, the Case of Kazakhstan

Plant genetic diversity in improving agricultural production is a key factor providing adaptability to unpredictable environmental and climatic changes, maintain resilience in the face of variation of productions systems and meet the needs of the expanding human population (Esquinas-Alcazar 2005). However, in the search of high yield elite varieties, modern crops suffer the narrowing of the genetic base (Tester and Langridge 2010) to the point of virtually eliminating local germplasm generated during centuries of traditional agriculture. In the case of wheat, as a staple crop providing around 20% of human dietary energy, sustainable and steady increases in wheat yields, boosted by genetic diversity are vital for the food security of next generations. Fortunately, wild relatives and progenitors of bread wheat still preserve remarkable genetic diversity in terms of alleles that may contribute to adaptive processes which may be utilized to develop hardy high-yielding varieties combining also genetic variation for quality traits. For more details a recent review of the contribution of wild relatives as source of variation for wheat grain quality improvement has been published (Alvarez and Guzmán 2018).

Table 3 Small scale tests to evaluate durum quality

Test	Application	Sample, quantity	Comments/need, references
Swelling index of glutenin	Predict gluten strength; measures proportion of flour protein that consists of glutenin polymers of very large molecular weight	Semolina, flour, other (35–45 mg)	Samples with different glutenin swelling properties; application in breeding (Wang and Kovacs 2002; Sissons and Smit 2018; Uthayakumaran et al. 2007; Li et al. 2013)
SDS sedimentation volume	Measures the amount of sediment after mixing flour in an SDS–lactic acid solution for a fixed time; indicator of gluten strength	Flour, semolina, other (1 g)	Poor discrimination of moderately strong from strong gluten; most common early generation dough test (Dexter et al. 1980; Dick 1983)
Mixograph or similar	Measures dough mixing characteristics using a pin mixer; indicator of gluten strength	Flour, semolina (2-25 g)	Poor at discriminating moderately strong from strong genotypes; Extensively used in breeding programs (Dick and Youngs 1988)
Glutomatic system	Wet/dry gluten, gluten index; indicator of gluten strength	Flour, semolina (10 g)	Very weak samples or those with poor gluten development giving no results. Extensively used in breeding programs (Cubadda et al. 1992)
Glutopeak	Measures the aggregation behavior of gluten in flour samples; indicator of gluten strength	Flour, semolina, other (8–10 g)	Can predict GI, rapid; Gaining more interest (Sissons 2016; Sissons and Smit 2018; Marti et al. 2014; Wang et al. 2017)
Farinograph-E	Measures flour water absorption and dough mixing characteristics with a Z-arm mixer; indicator of gluten strength	Flour, semolina (10 g)	Slow with poor discrimination of moderately strong from strong types (ICC115/1; AACC54–21.02)
Small scale mills	Mill grain into flour or semolina	Grain (1–100 g)	Brabender Quadramat junior mill; Chopin CD2 mill; Extensively used (Varga et al. 2000; Békés et al. 2003)
NIR	Predict protein, moisture, ash, test weight, semolina yield, HVK, yellow pigment, b∗, hardness, wet gluten	Grain, flour, semolina, pasta, other	Scanning or fixed wavelength NIR; extensively used in breeding programs (Sissons et al. 2006; McCraig et al. 1992; Wang et al. 2002; Osborne 2006; Wesley et al. 2005)
Image analysis	Predicts HVK, specks, semolina yield, blackpoint	Grain, flour, semolina, pasta	Image analyser with calibrations (Cervitec 1625, EyeFoss; Seedcount, Next Instruments). Good use in industry less so in breeding programs (Symons et al. 1996; Novaro et al. 2001; Gorretta et al. 2006; Wang et al. 2003)
RVA	Measures pasting viscosity of starch or semolina/pasta solution, falling number	Flour, starch, other (3–5 g)	Widely used (Batey and Curtin 2000; Grant et al. 2001; Aravind et al. 2013)
Texture analyser	Dry and cooked pasta texture	Dough, pasta	Texture analyser; extensively used AACC 66–50.01 (Sissons et al. 2008; Cubadda et al. 2007)

In Kazakhstan, interspecific crosses including winter bread wheat and wild relatives are being performed as method of creating fundamentally new plants that combine their hereditary basis of the most valuable features and characteristics of cultivated and wild plants (Kozhahmetov and Abugalieva 2014). Wild relatives used for interspecific crosses with bread wheat include tetraploids *Aegilops triaristata* (2n = 28 CUCUMM), *Triticum militinae* (2n = 28 A^tA^tGG), *Triticum timopheevii* (2n = 28 A^bA^bGG), *Aegylops cylindrica* (2n = 28 CCDD) and the hexaploid *Triticum kiharae* (2n = 42 A^bA^bGGDD), among others.

Advanced lines obtained from interspecific crosses are characterized considering agronomic and botanical descriptors, disease resistance and yield as previously described (Kozhahmetov and Abugalieva 2014). Also grain components together with their physico-chemical properties are used to define grain quality characteristics. Table 4 shows Farinograph and Alveograph parameters obtained from advanced synthetic lines selected in interspecific crosses. According to the physical flour and dough properties, synthetics varies according to the dilution test at a level of 80–170 farinograph units, with the best value for both liquefaction and valorimetric evaluation for genotypes 231 and 1712 with 90–42 farinograph units and 80–45 units, respectively (Table 4).

High molecular weight glutenins composition in synthetic forms has been also analyzed and is described in Table 5. Additional infomation including presence of 1BL/1RS rye translocation and Payne Score is also indicated. Eight genotypes showed no segregation for HMW-GS and rye translocation, two of them with a Payne Score of 10. Remaining genotypes were characterized by segregation of HMW-GS and rye translocation (Table 5). HMW-GS identification, and therefore quality prediction in wild relatives derived synthetics is difficult because of the important differences of these cultivars in terms of glutenins and other grain components important to define wheat quality (Abugalieva and Morgounov 2016).

Table 4 Physico-chemical properties obtained from transitional winter wheat forms. Farinograph and Alveograph parameters depending on the year of cultivation are described

Genotype	Cross	Dilution test[a]		Valorimetric test [a]		P/L	W
		2016	2017	2016	2017		
231	(Bezostaya1 × *Ae.triaristata*) × Karlygash	90	–	42	62	0.49	387
1712	Erythr.350 × *T.militinae*	80	100	45	50	0.34	221
1721–6	(Bezostaya 1 ×*T.m.*) × *T.m.*-6	130	100	30	43	0.27	279
1721–9	(Bezostaya 1 ×*T.m.*) × *T.m.*-9	150	70	28	56	0.39	177
1721–4	(Bezostaya 1 ×*T.m.*) × *T.m.*-4	150	120	21	40	0.36	174
1671	Zhetysu × *T.m.*	140	120	33	44	0.29	174
1727	Erythr.350 × *T.kiharae*	160	80	31	50	0.59	240
1676	Stekl.24 × *T.timopheevii*	–	160	–	35	0.21	157
1674	Zhetysu × *T.t.*	–	80	–	53	0.35	279
1718	Bezostaya1 × *Ae.cylindrica*	170	120	27	36	0.33	119
1825	Stekl.24 × *Ae.c.*	–	–	–	56	0.41	205
	Almaly	130	110	38	44	0.48	187

[a]Measured in Farinograph unit

Table 5 Protein marker composition including HMW-GS and wheat-rye translocations in the prediction and grain quality chracterization of winter and synthetic wheat forms

Genotype	Cross	*Glu-A1*	*Glu-B1*	*Glu-D1*	Status 1B/1R	Payne score
231	PEG 231 × Karlygash	2∗	7 + 9	5 + 10	1B/1B	9
1712	Erythr.350 × *T.militinae*	2∗	7 + 9	5 + 10	1B/1B	9
1721–6	PEG ×*T.m.*) (6)	0	7 + 9	2 + 12	1B/1B	5
1721–9	PEG ×*T.m.*) (9)	2∗	7 + 9	5 + 10	1B/1B	9
1721–4	PEG ×*T.m.*) (4)	0	7 + 9	2 + 12	1B/1B	5
1680	Steklovidnaya 24 × *T.m.*	2∗	7 + 8/7 + 9	5 + 10	1B/1B	10/9
1671	Zhetysu × *T.m.*	1	17 + 18/21 + 18	5 + 10	1B/1B/ 1B/1R	10
1671	Zhetysu × *T.m.*	2∗	7 + 9	5 + 10	1B/1B	9
1723	PEG × *T.kiharae*	1	7 + 9/6	5 + 10/4 + 10	1B/1B/ 1B/1R	8/6
1675	Zhetysu × *T.k.*	0	7∗	5 + 10/4 + 10	1B/1B	6
1675	Zhetysu × *T.k.*	2∗	7 + 8	5 + 10	1B/1B	10
1727	Erythrospermum350 × *T.k.*	2∗/0	7 + 9/6 + 8	5 + 10	1B/1B/ 1B/1R	9/6
1676	Steklov.24 × *T.timopheevii*	1	7 + 8	5 + 10	1B/1B	10
1674	Zhetysu × *T.t.*	0/1/2∗	7 + 9/7 + 8	5 + 10	1B/1B	7/10
1718	PEG × 1718	0	7 + 8/7∗	3 + 12	1B/1B	6/5
1825	Steklovidnaya 24 × *Ae. cylindrica*	0	21 + 8/7∗	5 + 10	1B/1B/ 1B/1R	6

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