QUALITY WHEAT CRC REPORT

International Cereal Chemistry: The 1998 Meeting of the American Association of Cereal Chemists

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The 1998 Meeting of the American Association of Cereal Chemists

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Overview

The 1998 Meeting of the American Association of Cereal Chemists was held in Minneapolis from Sunday 13th to Thursday 17th September, 1998. It was attended by the authors of this report and by several other Australians. The Annual AACC Meeting has become an international meeting, with representation from many countries. This is the world's major cereal-science event, and on this occasion, over 1700 cereal specialists participated despite an airline strike affecting Northwest Airlines which has its hub in Minneapolis.

This year, there were about 360 scientific papers, about equally split between oral and poster presentation. In addition, there were over 250 "table-top" trade displays. This vast number of trade displays, plus posters and the AACC's registration and information area, were accommodated in the enormous domed space of the Minneapolis Convention Center's main exhibition hall, from the Sunday afternoon to the Tuesday afternoon (for only two of the four days of the Meeting). All poster boards had envelopes for business cards to be left as an request for a copy of the paper. Quarto-sized copies were available at some posters for collection.

Oral presentations were all of 20 minutes duration (except only for Fin MacRitchie's Osborne Address of one hour), thus to allow interchange between the several concurrent sessions (up to six). Also concurrent with the oral presentations were many committee meetings, making it impossible to get an overall impression of the meeting's scope, also creating the difficulty of choosing between simultaneous sessions of interest.

The venue allowed proximity for ease of movement between sessions, but this also facilitated the embarrassment of mass walk-outs and invasions for particular talks. One such occasion was the great influx of audience to a talk on the use of the RVA for gluten-quality testing by Mark Bason (Paper 266), and the subsequent exodus after his talk.

A full set of abstracts and program detail is provided in the conference booklet (a spare copy is available), as well as in the July issue of *Cereal Foods World* (**43**, No.7, 1998). The AACC does not publish full versions of papers, but they encourage authors to prepare their papers for submission to AACC journals.

The oral program was a mix of Symposia, with invited speakers chosen to focus on a specific topic, and an accumulation of papers offered for presentation, with the subsequent difficulty of combining the latter papers into any coherence.

As usual, the conference included some highly relevant sessions for the Australian wheat industry. These included an excellent session on the impact of biotechnology on cereal production and utilisation. This highlighted the different approaches and levels of acceptance of genetically modified foods in Europe and the USA. In the US, it has been demonstrated that there is very little opposition to the introduction of GMO products, provided you clearly label them, explain their benefits, and provide consumers with the choice between normal and GMO-derived foods. It was also stated that there are soyabean-derived ingredients in 60% of ALL processed foods sold in the USA. So given the area sown to Round-up Ready Soyabeans in the States, and the lack of identity preservation in the grain-marketing system, like it or not, the average American is already consuming GMO's in reasonable quantities!

AACC Awards

Several "friends" of the Australian cereal-chemistry community were honoured at the meeting:-

Osborne Award. It was especially exciting to see Finlay MacRitchie receive the AACC's research award. In his Osborne Address, he referred mainly to research carried out whilst he was at CSIRO, North Ryde. A preliminary copy of the Address is appended. Fin has been very happy during the past year in his position as Professor in the Department of Grain Science at Kansas State University, Manhattan, KS.

Young Investigator's Award. This award, organised by the Biotechnology Division of the AACC), was made to Renato D'Ovidio (Tuscia University, Viterbo, Italy). We have interacted with Renato in the past, and he spent some time in the CSIRO labs in 1996. The topic of his Award Address was "Molecular analysis of glutenin subunit genes in relation to wheat quality", in which he described their molecular engineering studies to produce modified HMW subunits, namely, Dx5 22% longer, and two versions 70% and 54% shorter than normal, the longer versions contributing better to dough strength. Studies with the LMW subunits of durum wheats have shown that LMW 1 (weakness associated) and LMW2 (strength associated) are similar in amino-acid sequence, but that the LMW 2 subunit is longer than LMW 1; this characteristic was postulated to account for the difference in their contributions to dough properties.

Scott-Blair Award. Ann-Charlotte (Lotta) Eliasson received this award, made for contributions to rheology. Lotta has hosted a few Australian cereal chemists on extended visits to the University of Lund, Sweden. In addition to her Award Address on dough rheology, she had a presentation (Paper 312) on starch-lipid interactions in various maize starches using the DSC (common maize, waxy, *ae*, *du*, and *ae su2* genotypes).

AACC Fellow. Vladimir Rasper was awarded the honour of AACC Fellow. Vlad has been Editor-in-Chief of *Cereal Chemistry* for the past few years, and worked for an extended period in BRI some years ago.

Symposia of special interest

Symposium: Glutenin-Protein Formation: *in vivo*, *in vitro*, and in practice (Papers 344-348)

This topic arose from the invitation two years ago for Colin Wrigley to arrange a symposium for the 1998 AACC Meeting. Co-chairs were Colin Wrigley and Frank Bekes, but last-minute sickness prevented Frank from attending. Despite it being scheduled for the last session of the meeting, it was well attended, and there was useful discussion. The five papers are to be published in a special proteins issue of *Cereal Foods World* (probably in July, 1999). Draft versions of the papers are available from Colin Wrigley. Unfortunately, the restriction of twenty minutes on each talk did not allow justice to be done to the topics.

Peter Shewry documented his description of storage-protein synthesis with electron micrographs of the organelles involved. He described two alternative pathways of protein synthesis and deposition, speculating about the classes of protein taking each, and the final location of these protein types in the cell. Coalescing of the resulting protein bodies clouds the issue of localisation for the mature endosperm, but distinct localisation of the triticins was claimed. He maintained that the extent of glycosylation (if any) is sufficiently low as to be insignificant for functional purposes. Hydrogen bonding, he claimed, is probably more important for dough formation than has been previously acknowledged, and he suggested it as a profitable focus for study in relation to the effects of growth environment on molecular structure.

In Frank Bekes' absence due to illness, Laszlo Tamas read the second paper (on *in vitro* formation studies), but he also ran into the difficulty of the time restriction. Nevertheless, the content of this paper fitted well into the theme of the Symposium.

Don Kasarda presented the scientific "detective story" of elucidating the unexpected functional properties of wheat transformed with a synthetic glutenin gene, finding that it formed a "circular" molecule. His final slide indicated his view that intramolecular SS bonds form straight after polypeptide synthesis, to be followed at a later stage by intermolecular SS bond formation, the latter process being limited by diffusion processes within the cell. In questioning, he admitted that this diffusion process may be a point at which temperature of growth could be important.

"In practice" aspects were described by Domenico Lafiandra, in collaboration with Blumenthal and Wrigley for some of the content. The first "in practice" section described the design of genotypes to study glutenin formation, especially the recent development of further multi-null lines, as well as more recent lines with augmented glutenin genes. Lines with the full complement of six HMW glutenin polypeptide provide a higher molecular-weight distribution in their hands. The environmental aspect he considered most important in their situation was heat stress. He described the ongoing search for genotypes that would provide tolerance to the effects of heat stress on dough properties. There was some discussion about contrasting results (especially from USDA, Albany) where many results in glasshouse grown / shocked wheat had not shown dough weakening (Papers 134 and 136).

Symposium: Impact of Biotechnology on Cereal Production and Utilization (Papers 305-310)

Chaired by Shewry and van der Kamp, this symposium was arranged by the AACC's Biotechnology Division. It covered baking, brewing, nutrition, grain production and breeding, plus the presentation of the Biotechnology Division's "Young Investigator Award". This was made to Renato D'Ovidio (University of Tuscia, Italy).

Dr van der Kamp set the scene initially by describing the reaction against genetically modified organisms (GMO) in European foods, indicated by manufacturers re-formulating bakery-ingredients to eliminate soy because of the likelihood of soy being transgenic. Despite this, he described progress with the genetic modification of micro-organisms for the production of food enzymes, including transglutaminase (for the cross-linking of proteins to strengthen dough and to act as a binder in meat. He quoted from the May, 1998, ICC Symposium to predict the commercial release of transgenic wheat in Europe in 2003.

In contrast to van der Kamp's picture of opposition to GMOs in Europe, subsequent speakers described public acceptance of genetically modified foods in the USA and England. For example, Bill Atwell, presenting the Pillsbury contribution for Susan Harlander (Paper 308), described the (now) generally relaxed attitude of Americans to genetically modified foods. A planned picketing of one of their factories as a demonstration against genetically modified foods fizzled into only a few people recently. Pillsburys have tested some 48 genetically modified species, and have released 39 engineered products. The company's attitude is presumably indicated by the title of this talk: "Biotechnology as the driving force in restructuring the grain-producing and utilizing industries".

Prominent examples in US foods at present are maize (Bt resistance to European corn borer increasing yields by 18%, Roundup resistance), potato (Bt gene), soy (Roundup-ready soy). "GMO permeates the entire food chain." Soy ingredients feature in 60% of US food products, with maize in many also. The result is that it would now be impossible to track down the presence of GMO ingredients. The next GMO wave for agriculture will be value-added raw materials with better functionality, including neutraceuticals, especially for oilseeds, cereals and legumes. Bill pointed out the consolidation of ag-biotech companies who are now concerned to recoup their enormous investment, claimed to exceed $8b. But, there are many unresolved matters, making a field day for lawyers, as well as the problem on inadequate provisions in the US grain industry for identity preservation of value-added (GMO) grain shipments. He sees that there will be contract growing of GM wheats, but there is the pressing need is to have rapid test systems to identify these new grain varieties.

Dr Schuch of Zeneca, UK, told the story of the introduction of canned GM-tomato puree into the UK, in which Zeneca worked closely with the major supermarket chains of Safeways and Sainsburys. They teamed up to distribute booklets explaining the advantages of GM tomatoes. The cans with genetically modified tomatoes were clearly labelled as such, with explanation of the meaning of this difference, and these cans were slightly larger than the non-GMO tomatoes (both the same price).

Dr Krebbers (Paper 307, Dupont Agricultural Biotechnology, Wilmington) described progress to insert genes to increase the lysine (to 0.5%) (and methionine) content of soy and maize. Maize genotypes with these improvements will be combined with those already

available for higher oil content (9%) for commercial release in 2001. Further plans for soy involve improvements in the functionality of the protein fractions, and suppression of the synthesis of inhibitory proteins such as the Bowman-Burke protease inhibitor.

Rudi Appels described prospects for biotechnology to accelerate breeding processes via improved selection for quality as well as via transgenic methodology. He emphasised the need for us to understand the aspects of phenotype that should be modified by the new advances in biotechnology. He instanced Plant Industry research on very-small scale dough experiments and the expansion of the Mixograph trace to follow interactions as each pin passes another as a novel means of understanding dough properties, offering models to describe dough structure. QTL maps were described as means of identifying molecular markers to provide better approaches to breeding for specific quality traits.

General talks and posters

- There were many talks and posters that included results from the RVA, indicating that it is becoming the method of choice for routine and research characterisation of starch.

- The image analysis system developed by Harry Saperstein, Uni of Manitoba, has become a very effective system and shows promise for assessment of bread-crumb changes with different formulations. The key has been an allowance for the effect of cell structure on brightness.

- Peter Shewry gave a rather rushed summary of the EUROWHEAT project (Paper 265), in which CSIRO Plant Industry has been peripherally involved. It involves ten labs in six countries, with a budget of 1.2 m ECUs plus "in-kind". It is aimed at determining the structure of HMW glutenin subunits (concentrating on Dx5), using a diversity of technologies. These include production of the gene-expression product (one gram available), crystallography (no pattern yet), chemical characterisation (insufficient glycosylation found to have any functional significance), characterisation by urea-gradient electrophoresis and MALDI-MS, of protease digests, and transformation studies (leading to the production of lines with longer and shorter versions of the genes, and over-expressions of them. The project has still a year or so for the funding to run, so this was an interim report. There will be a good story to tell in another year or so.

- The selection of Hard White wheats in Montana for both noodles and bread which showed that they were still off the track.

- Laboratory testing protocols for Chinese steamed breads being developed at the Wheat Marketing Centre, Portland Oregon, which indicated that they are coming to grips with Asian Foods and still have a way to go, but will get there.

- Food texture work presented from KSU by Susan Sun using electromyography to measure the response of chewing muscles to food was very interesting but probably not practical.

- David Hatcher from GRL, Canada is doing a lot of noodle work that is quite similar to that happening in Australia. For instance he has just looked at changes to proteins through the process using RP HPLC. And has initiated noodle speckiness studies using an in-house system.

- The use of the Perten Single Kernel Characterisation System (SKCS) by the USDA Grain Marketing Research Lab. to predict end-use properties of hard red winter wheats, particularly milling yield and loaf volume (Paper 178). The prediction of milling quality involved only modest correlation coefficients.

- The SKCS received a lot of coverage as a tool for assisting milling.

- Paper 161, "Prediction of vomitoxin in single wheat kernels using NIR" by Dowell *et al.* (USDA ARS Grain Marketing and Product Research Centre, Manhattan, Kansas), showed that the SKCS could accurately determine the presence of "scab" infected kernels, but not the resultant levels of deoxynivalenol (DON).

- There were some excellent posters from the Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg.
    - For example Paper 87 described comparisons between protein- and DNA-based use of marker assistance to select for gluten strength, using doubled-haploid populations.
    - Paper 80 described several small-scale tests for wheat quality, including test results that correlated ($r > 0.90$) with frozen-dough baking quality.
    - A small-scale noodle machine was described in Paper 99 (Kovacs *et al.*). It requires 10-50 grams of flour to produce "commercial-quality noodles" which correlate well with results from a normal Ohtake laboratory noodle machine.
    - In Paper 115, Kovacs *et al.* demonstrated that semolina from Canada Western Extra Strong hexaploid wheat (Glenlea) has comparable pasta-cooking quality to the high-quality durum wheats, suggesting that in times of high durum prices, an alternative is available.

- Paper 296 described the use of multi-stacking SDS gel electrophoresis to characterise the size distribution of storage proteins of immature grain. Polymeric glutenin was present at the early stages of grain development; its size increased rapidly in the early phase of grain filling, and remained stable in the later stages.

- Paper 67, entitled "Distribution of PPO activity among a large number of hexaploid wheat genotypes using an improved L-DOPA assay" (by Craig Morris *et al.*, USDA-ARS Western Wheat Quality Lab. Pullman, Washington), demonstrated a useful method for polyphenol oxidase determination. However, the interesting point was that in ranking a large number of wheats from around the World for PPO activity, the NSW variety Swift was one of the very lowest; this may be news to Australian chemists. There were also a lot of varieties from Greece and South Africa which were also very low in PPO activity; these may well have some application in Australian programs.

- There were several presentations from the Albany USDA labs on the effects of heat stress on grain composition and quality. Paper 86 described RT-PCR studies on heat stressed plants, indicating that the levels of glutenin subunit transcripts remain remarkably stable during episodes of heat stress. Heat stress was reported to cause changes in (unidentified) non-storage proteins rather than in storage proteins (Paper 136), and changes in the dough quality of Cheyenne wheat, but not of varieties Butte and Arapahoe (Paper 134).

- Puroindolines a and b were studied for their possible relationship to grain hardness (Papers 138 and 167). The content of these proteins did not provide distinction between hard and soft wheats. Apparently the genes for them are linked to the hardness genes, but are not the actual genes for grain hardness (*Ha*). Craig Morris's studies have produced a series of genetic mutants for hardness.

- Harry Sapirstein described a new test showing a relationship to dough strength (Paper 270). It involves the spectrophotometric determination of insoluble glutenin.

Table-top trade displays

A great majority of the trade displays related to ingredient suppliers to the US baking industry. Displays of interest to us included ...

- Matt Hesser of HRA Associates, previously Executive Director of the International Wheat Gluten Association. The IWGA is now almost defunct; at least, Matt is no longer involved, but he is continuing his association with the wheat industry by producing a newsletter to provide for the commercial needs of industry worldwide.

- The Utah-based company that manufactures equipment for field-flow fractionation (FFF) for the size-based fractionation of proteins, hoping to provide equipment for this purpose to lots of companies in the wheat industry (!?).

- Newport Scientific ((Sydney), manufacturers of the RVA, displayed a much cheaper version of the RVA - the "StarchMaster" - to provide for routine testing requirements, in contrast to the much more versatile RVA itself.

- National Manufacturing - TMCO, Lincoln, NE, with whom we have interacted in the re-design and manufacture of the two-gram Mixograph. John Albers hopes to make a visit to Australia early next year.

Committee activities

Several Australians are active in AACC committees and administration. These activities can make for a very busy meeting, with regular conflicts between committee meetings and talks.

AACC Board

Bob Cracknell attended his first AACC Board meetings as the new International Director. Bob was immediately drafted onto the International and Emerging Issues Committees, and was given the task of formulating a proposal to insulate international members from the escalation of membership costs arising from the various currency crises.

Although the AACC has a reputation for being an inwardly focussed organisation concentrating on US-based activities, in recent times efforts have been made to change this, with the establishment of an office in Europe and the promotion of Pacific Rim Meetings and an associated Newsletter. Over 25% of the Association's members reside outside the US, and remarkably, a recent member survey showed strong support for globalisation and the promotion of international activities.

The new President of the AACC, together with the Board, has decided to have a series of re-evaluations of all AACC activities, this task being given to some review committees.

The AACC Board requested that Lindsay O'Brien and Robert Henry convene an AACC Symposium at the Cereals and Bread Congress in 2000 on the Gold Coast, on the topic "Transgenic Cereals". All speakers will be invited and the proceedings will form a book to be published by the Symposium.

Nominations Committee

Tony Blakeney and Colin Wrigley attended a meeting of the Nominations Committee, the body that suggests names to the Board for future positions in the AACC.

Program Committee

Colin Wrigley was asked to attend a meeting of the Program Committee, for discussions of ideas for the next AACC Meeting, to be held in Seattle, October 31 to November 3, 1999.

Technical Committee Meetings

The Soft-Wheat Technical Committee Meeting was attended by Lindsay O'Brien. A "Solvent-Retention Capacity Test" was proposed for preliminary collaborative testing.

The Asian-Food Products Technical Committee Meeting, also attended by Lindsay O'Brien ...

- Proposed further collaborative work on alkaline-noodle colour measurement.
- A survey is in progress on current methods being used worldwide for alkaline-noodle colour measurement.

The Test-baking Technical Committee Meeting was attended by Ken Quail. Bromate is still a major issue, where they expect the FDA to introduce a residue maximum of 20 ppb. This will cause an explosion in testing for compliance. Yamazaki from Japan have a HPLC method good to ±3 ppb. AIB is looking for a quick colour test as are others.

***Cereal Chemistry* Editorial Board Meetings**

Colin Wrigley attended meetings of the Editorial Board of the AACC's research journal *Cereal Chemistry* (as one of three Senior Editors). There was a unique opportunity for the AACC staff to meet the reviewing scientists, as on this occasion the meeting was held in the AACC's headquarters city.

The following table of statistics were presented by the Editor-in-Chief, Vladimir Rasper. The increasing popularity of the journal is shown, based on the numbers of papers offered and published. The falling rejection rate was attributed to improving quality of submissions.

Issues discussed included ...

- the speed of the refereeing process (three months was the best time from receipt to print, but a few poor times, too)
- electronic refereeing (to hasten the review process)
- electronic publishing and electronic access to past papers, and
- many procedural matters in the review process.

Recent Statistics for *Cereal Chemistry* journal

	1994	1995	1996	1997
Numbers of papers published	130	138	142	162
Total pages per year	654	630	782	862
Avcrage length of each paper	5.0	4.6	5.5	5.3
Papers rejected (as % of papers submitted)	34%	35%	29%	23%
ORIGINS ON PAPERS	(as % ages)			
USA		53	50	54
Canada		11	7	8
International		27	37	28
International cooperation		9	6	10

Presentations by Wheat CRC staff (papers with CRC as an address)

The Protein Division's Symposium, chaired by C.W. Wrigley and F.Bekes, entitled "Glutenin-Protein Formation: *in vivo*, *in vitro*, and in practice", included the following talks:-

Bekes, F., and Gras, P.W.
In vitro studies on gluten protein functionality (Paper 345).

Lafiandra, D., Masci, S., Blumenthal, C., and Wrigley, C.W.
The formation of glutenin protein in practice (Paper 347).

Wrigley, C.W., and Bekes, F.
The significance of glutenin-protein formation during the continuum from anthesis to processing (Paper 348).

Appels, R.
Molecular breeding: Exploiting biotechnology in developing improved cereals.(Paper 309)

Quail, K., Bekes, F., Blakeney, A., and Southan, M.
Functional properties of flour components – An overview. A look at protein in baking and starch in noodles and how a crossover of information between products may be useful. (Paper 247).

Turner, N.E., Bason, M.L., Uthayakumaran, S., Bekes, F., and Wrigley, C.W.
Measuring gluten quality in flour - A novel method using the Rapid Visco Analyser (Paper 266).

Rathmell, W., and Wrigley, C.W.
Research achievements to benefit the Australian wheat industry; the work of the Quality Wheat Cooperative Research Centre (Paper 155).

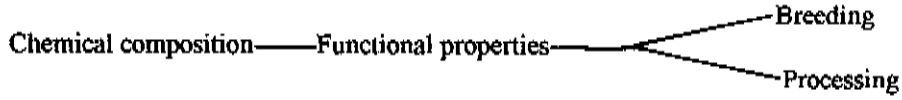
Plaut, Z., Blumenthal, C., Gras, P.W., and Wrigley, C.W.
Variations in dough quality due to the effects of water stress during grain filling (Paper 208).

1998 Osborne Award Address

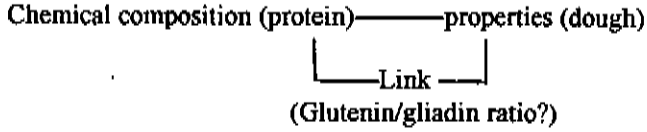
Presented at the 1998 Meeting of the American Association of Cereal Chemists by
Professor Finlay MacRitchie,
Department of Grain Science, Kansas State University, Manhattan, KS, USA

It seems appropriate that the name of Osborne is in the title of this article. Thomas Burr Osborne was a protein chemist and this discussion will be centered on wheat proteins, an area of research that I have been associated with. Osborne was one of those who showed the way in cereal science and I would like to stress this fundamental point that cereal science (and, for that matter, any science) progresses only by building on the efforts of previous workers.

One of the general aims in cereal science has been what is depicted below.



The objective is to relate the chemical composition of the wheat grain to its functional properties. This knowledge can then be applied directly to resolve problems in processing or can be used in breeding to improve the processing quality of varieties. If we focus on dough properties, it is well established that it is the protein component that controls the special dough properties that make wheat flour suitable for leavened products.

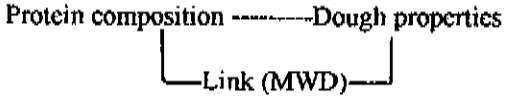


What has been sought is a relationship or a link (1) between protein composition and dough properties and, for a long time, it was thought, quite logically, that this link might be the glutenin/gliadin ratio. Based on the properties of separated glutenin and gliadin, dough strength would be expected to increase as this ratio increased. However, this hypothesis has never been definitively established. Studies by Orth and Bushuk (2), Huebner and Wall (3) and Finney (4), amongst others, have thrown light on differences in proportions of glutenin and differences between glutenins.

Molecular weight distribution of wheat proteins

With the great wisdom that we acquire from hindsight, we see that the molecular weight distribution (MWD) may be the more appropriate parameter and that this can vary in two main ways - first, by a change in the relative proportions of monomeric and polymeric proteins and second, by a change in the MWD of the polymeric protein as in Fig. 1 (5).

For the sake of simplicity, the terms polymeric protein and glutenin will be used interchangeably. Although this is not strictly correct, glutenin is by far the major component of the polymeric protein. The MWD of the gliadins doesn't change much from one variety to another but that of glutenin can. We also need to remember that the MW scale shown in Figure 1 is logarithmic and not linear.



Therefore, a better parameter for the link between protein composition and dough properties may be the MWD. This is the parameter that has been shown to be the main determinant of the physical properties of high polymers. We therefore need to apply the vast knowledge that has been obtained about the relationships between molecular characteristics (particularly the MWD) and physical properties of polymers to help to explain physical dough properties where the gluten proteins form the continuous phase and therefore govern the properties.

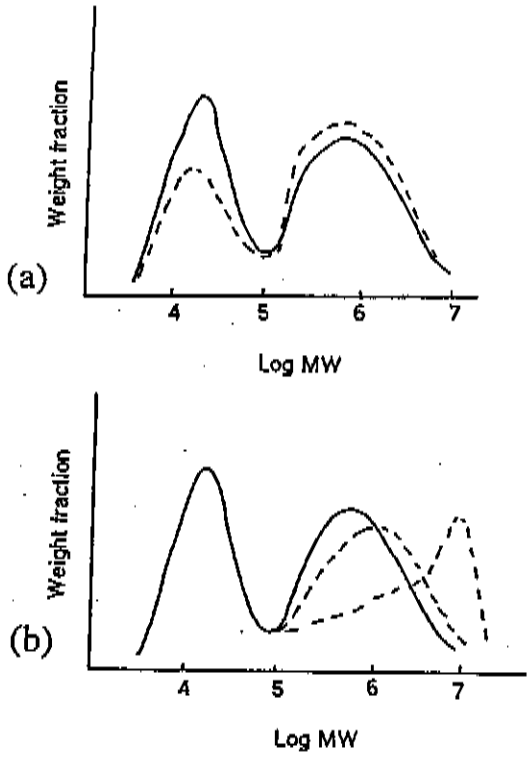


Fig. 1. Schematic molecular weight distribution for wheat protein (5).

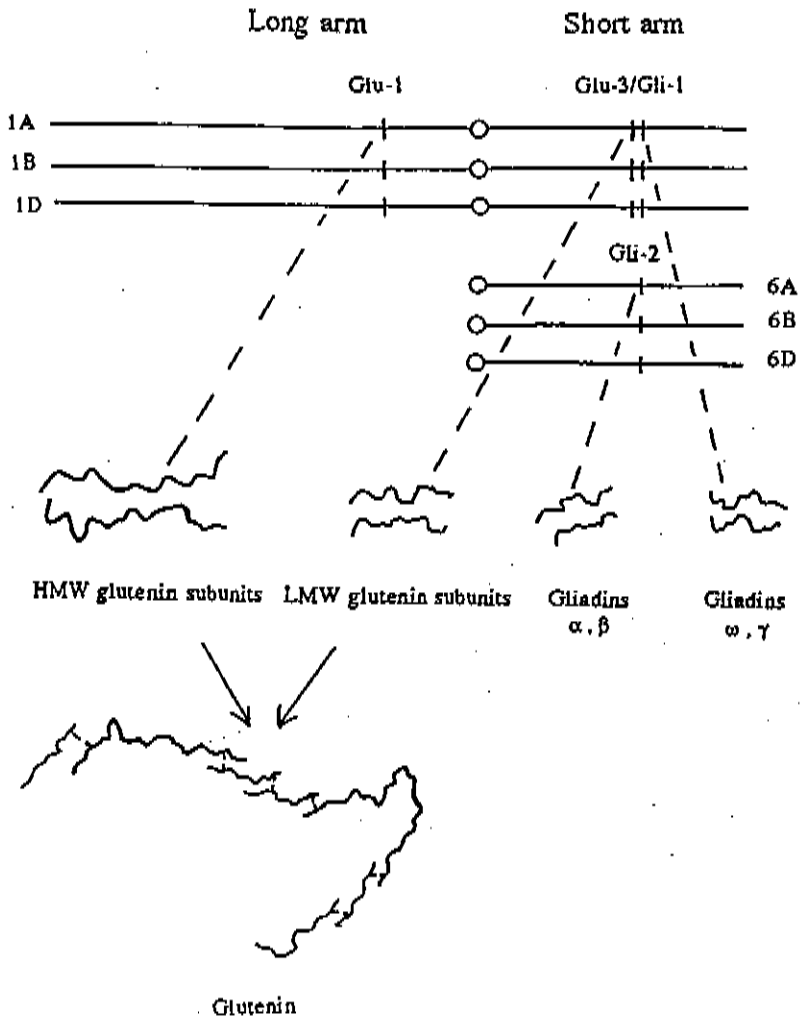


Fig. 2. Chromosomal location of genes coding for the main protein classes (5).

Chromosomal location of genes coding for the main protein classes

To diverge momentarily, one of the most important advances in cereal science in recent times has been establishment of the chromosomal location of genes coding for the main wheat protein classes and this has been pushed ahead by the work of Sears (6), Wrigley and Shepherd (7) and Payne (8) to mention a few.

The rather simplified picture of Figure 2 (5) shows that the glutenin subunits which are the gene products are coded by genes on group 1 chromosomes, the high molecular weight subunits (HMW-GS) on the long arms and the low molecular weight subunits (LMW-GS) on the short arms. Gliadins are coded by genes on the short arms of groups 1 and 6. The genes for gamma and omega gliadins on group 1 short arms are tightly linked to the genes coding for LMW-GS. All of these loci exhibit allelic variation. During grain development, the glutenin subunits polymerize slowly over time through disulfide bonds to form polymers with a range of molecular weights (9).

One of the long term aims in cereal science has been to use the relationships between protein composition and functionality to derive strategies to manipulate protein composition in order to produce varieties giving dough properties that conform to certain specific end use requirements. The link between relating the knowledge that we acquire about protein composition-functionality relationships on the one hand and strategies to be used in breeding on the other is the genetic control of the different proteins and protein subunits shown in Figure 2.

[Protein composition-----Functional properties] ---------Breeding strategies

Link

(Genetic control of proteins)

With that as a background, I would like to discuss first, some of the ways that have been developed to characterize wheat proteins, next the relationships that have been elucidated between protein composition and dough properties and then to look very briefly at how this knowledge might be applied in breeding to target certain end-use specifications.

Protein composition analysis

One technique that has proved valuable in characterizing protein composition has been high performance liquid chromatography or HPLC. The application of HPLC to cereal proteins was pioneered by Bietz (10) and, since then, groups around the world have picked up the technique and run with it.

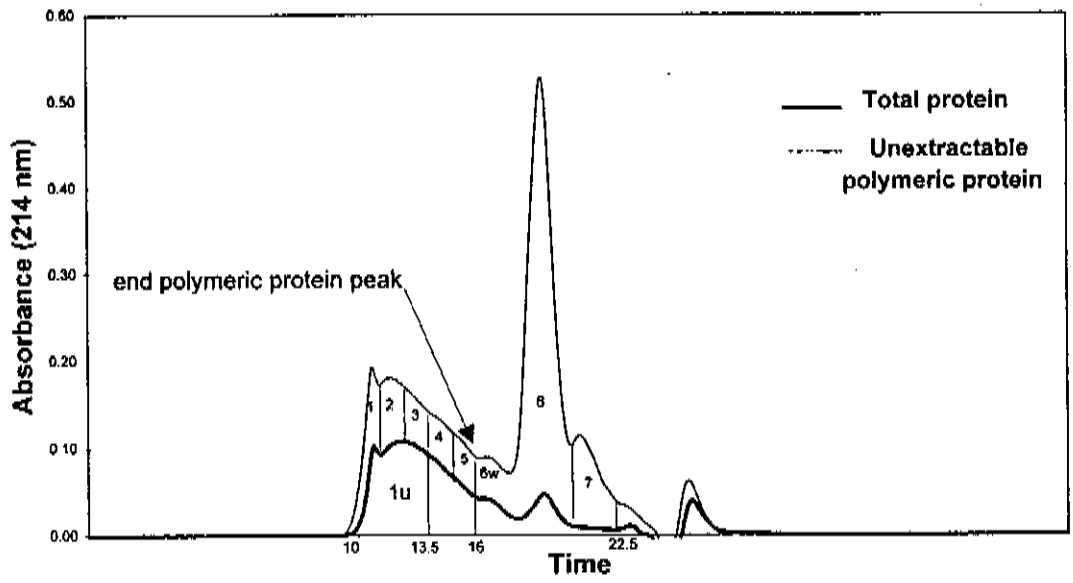


Fig. 3. SE-HPLC of total and unextractable (without sonication) flour protein (11).

In combination with ultrasound, size exclusion-HPLC is a powerful technique for characterizing wheat proteins. Two important quantities can be measured as shown in Fig. 3 (11). First, controlled sonication enables close to complete solubilization of wheat protein in a solvent such as sodium dodecyl sulfate (SDS)

solution. To achieve this, the largest glutenin molecules are broken down, but since, as polymer theory predicts (12, 13), the degradation products still fall within the size range of glutenins (14), this enables an accurate measure of the relative proportions of the polymeric proteins which are eluted in the first peak, gliadins in the second peak and albumin/globulins in the third peak. Second, although much of the glutenin is not resolved by present day columns, use can be made of the well-established relationship between solubility and molecular size. By extracting protein first without sonication, the protein in the residue can be quantitated and this serves as a simple yet reliable measure of the relative MWD. Recent work by Sapirstein and Johnson (15) and Lookhart and Bean (16) has streamlined this procedure.

It is now appropriate to discuss a few results based on SE-HPLC measurements that throw some light on the protein composition factors that govern flour functionality and, from this, the protein composition factors that control MWD. The focus will be on measurements using the Brabender extensograph. Admittedly this is only one procedure used in present day testing of functionality and extensograph measurements are chosen simply as an example to illustrate how these measurements can be related back to protein composition. Similar approaches may be used for other measures of functionality. The extensograph measures two main parameters, the resistance to extension and extensibility of a dough. Generally, it is found that, for a given variety, the maximum resistance to extension (R_{max}) is not strongly related to flour protein content whereas extensibility (Ext) is highly dependent on flour protein. This immediately illustrates a point. There are many parameters that are important in flour functionality. When we wish to change one of these by changing protein content or composition, we have to be aware of the effects on other parameters.

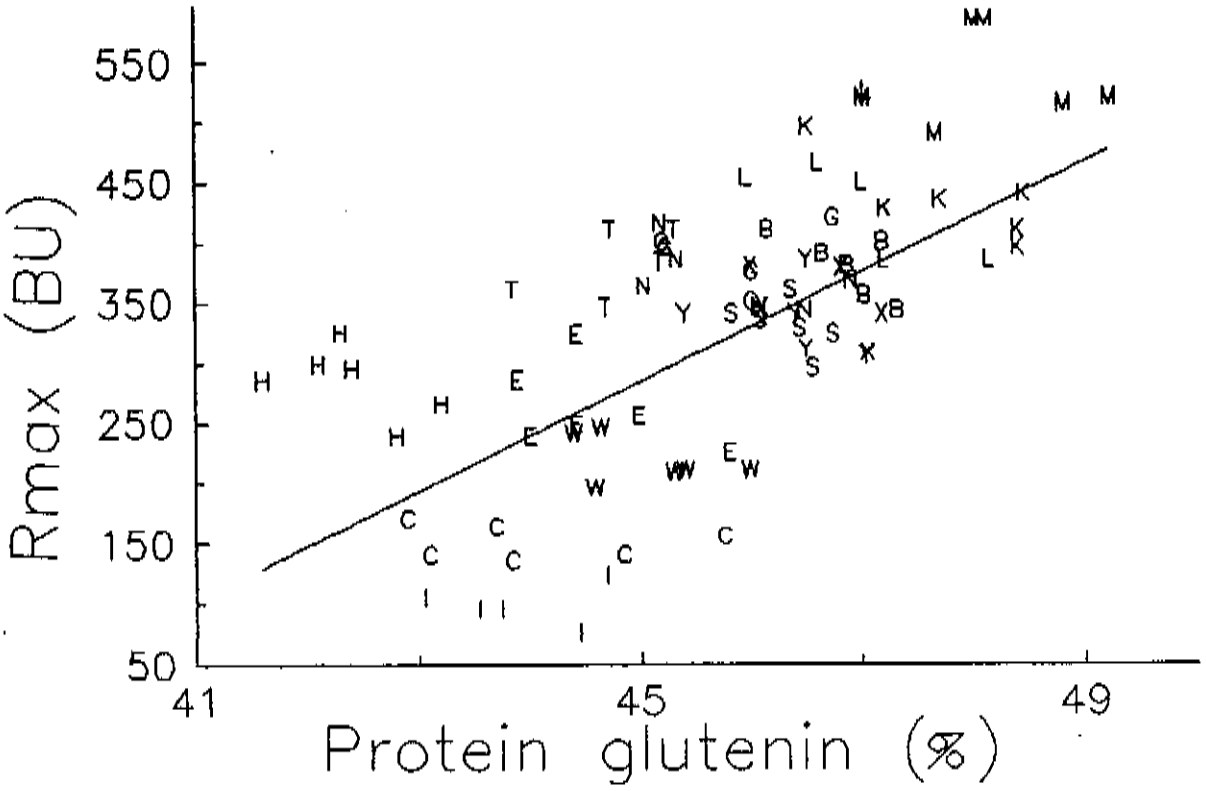


Fig. 4. Plot of Rmax vs the percentage of polymeric protein (glutenin) measured by SE-HPLC for 15 varieties grown at 6 nitrogen fertilizer levels (17).

Figure 4 shows a plot of R_{max} against the percentage of polymeric protein measured by SE-HPLC for 15 varieties grown at 6 different nitrogen fertilizer levels (17). This is an important set of materials that was grown by Lindsay O'Brien and this set, which encompasses a very wide range of properties, has proved valuable for elucidating protein composition-functionality relationships. In Figure 4, each variety is represented by a symbol. A number of features are to be noted. First, there tends to be a clustering of points for each variety. Next, the percentage of polymeric protein is a varietal characteristic, some varieties having generally low and others having high values. Finally, some varieties have clusters of points well above the line of best fit (e.g. the variety Halberd with the symbol H) while others have values well below the line (e.g., the variety Israel M68, having the symbol I). Analyses of the glutenin subunit composition of these lines showed

that the variety with exceptionally high R_{max} had an appreciably higher ratio of HMW/LMW – GS than the variety with lower R_{max} (17). What this result suggests is that the variety with the exceptionally high ratio of HMW/LMW-GS has a MWD shifted to higher values giving rise to dough strength additional to that predicted on the basis of the proportion of glutenin alone. This reiterates a point made earlier that the glutenin/gliadin ratio is not sufficient to explain dough functionality but requires additional information on the MWD of the glutenin

There is independent evidence that the HMW/LMW-GS ratio is related to the MWD. A plot of the percentage of unextractable polymeric protein as a function of the H/L MW-GS ratio showed a highly significant linear relationship (18). These results were obtained by using one wheat variety. The differences in the H/L MW-GS ratio resulted from different levels of sulfur fertilizer. The HMW-GS are sulfur-poor in comparison to the LMW-GS so that, as the level of sulfur is decreased, the highs increase relative to the lows. Another confirmation of the relationship between GS composition and MWD was obtained by analyzing fractions of glutenin separated by SE-HPLC at different elution times. In this case, 5 fractions were separated and the H/L –GS ratio was measured by densitometry of SDS-PAGE patterns under reducing conditions (11). The early eluted fractions (i.e., the fractions of highest MW) contain a higher ratio of H/L MW-GS and this ratio decreased with decreasing MW. This result is consistent with the earlier findings of Huebner and Bietz (19) and Kruger *et al* (20). Another interesting result was that the ratio of B/C LMW-GS also tended to decrease with increasing elution time and therefore decreasing MW (11).

One important breakthrough in understanding how flour functionality depends on protein composition has been the work on allelic variation of HMW-GS developed by Payne and coworkers (21). This has shown that certain alleles are associated with dough strength and breadmaking potential. On this basis, scores have been assigned to different alleles. For example, the subunit pair 5+10 at the *Glu-D1* locus is assigned a maximum strength score of 4. Other examples are subunits 17+18 and 7+8 at *Glu-B1* which are also associated with strength while subunits 2+12 at *Glu-D1* and 20x+20y at *Glu-B1* are correlated with weaker dough properties.

Some light on the origin of this allelic variation has been obtained by studying near-isogenic lines differing in the HMW-GS expressed at one locus (22). Three pairs of lines differing at *Glu-D1* were used. One line of each pair had subunits 5+10 and the other had subunits 2+12. As expected, the lines possessing subunits 5+10 each had higher dough strength as measured by Mixograph peak dough development time. There was no correlation between dough strength and either flour protein content or the percentage of polymeric protein. However, differences in the percentage of unextractable polymeric protein (UPP) matched closely the differences in dough strength. Since the UPP is a measure of relative molecular size, the result is interpreted to mean that the observed differences in strength due to allelic variation are related to differences in MWD. The allelic variants with 5+10 subunits have a MWD shifted to higher MWs than the counterparts with 2+12 subunits. How these differences in MWD are caused has not been clearly resolved and this is currently an area of close study. Although R_{max} was directly related to UPP, the orders of Rmax and extensibility were reversed, the 2+12 line having the higher extensibility. This suggests that a MWD shifted to too high values is detrimental to extensibility.

There is one other factor that has been proposed to influence the MWD of glutenin and that is the presence of chain terminators. Work, particularly by Kasarda, Lafiandra and Masci (23, 24) has drawn attention to certain modified gliadins that are found to be present in the polymeric protein structure. Some have been shown to have a single cysteine residue that is capable of forming one intermolecular disulfide bond and therefore can act as a chain terminator. These have been named D-subunits. We now know quite a lot about the structure of glutenin subunits as a result of the work of Shewry, Anderson and their co-workers (25, 26) in the rapidly developing application of molecular biology. For example, it has been shown by these workers that most glutenin subunits have at least two cysteine residues capable of forming interchain disulfide bonds and that these are located near the chain ends. These subunits can therefore act as chain propagators. The D-subunits are present in the polymeric protein fraction and have mobilities on SDS-PAGE in the region of gliadins. One example is a protein that has been reported to be present in wheat varieties from a number of countries (27). It has mobility in the omega-gliadin region for SDS-PAGE of reduced glutenin. Its N-terminal sequence is the same as omega-gliadins and it contains sulfur and has been postulated to act as a chain terminator. The attractiveness of chain terminators is that only small quantities might be needed to appreciably influence the MWD. They are therefore potential candidates for introduction or deletion by genetic engineering techniques in order to shift the MWD in a given direction.

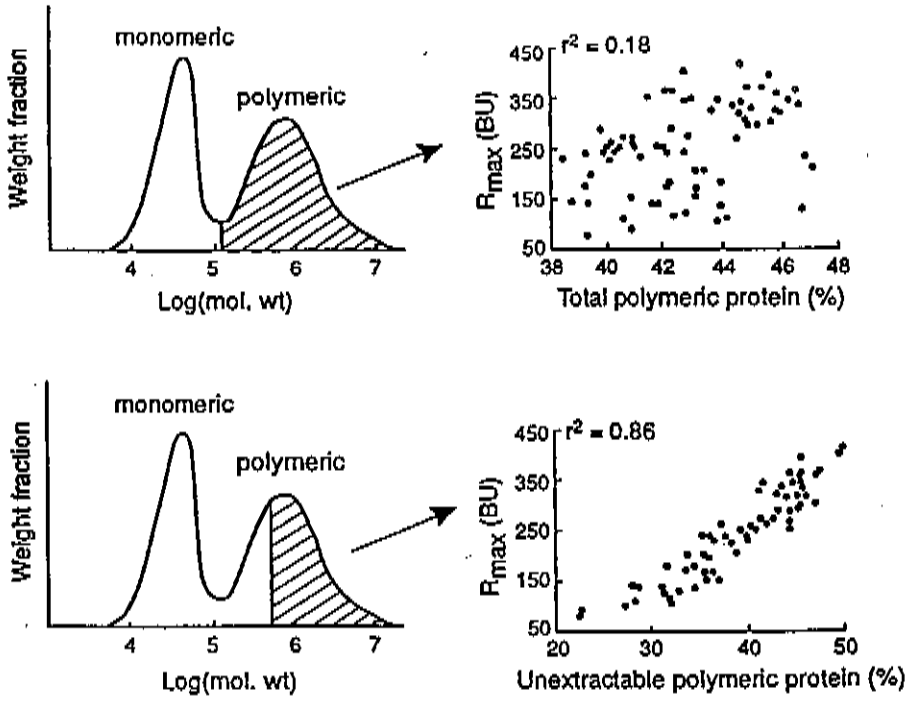


Fig. 5. Schematic representation of the total and unextractable polymeric protein and plots of these two variables for a set of 74 recombinant inbred lines (29, 5).

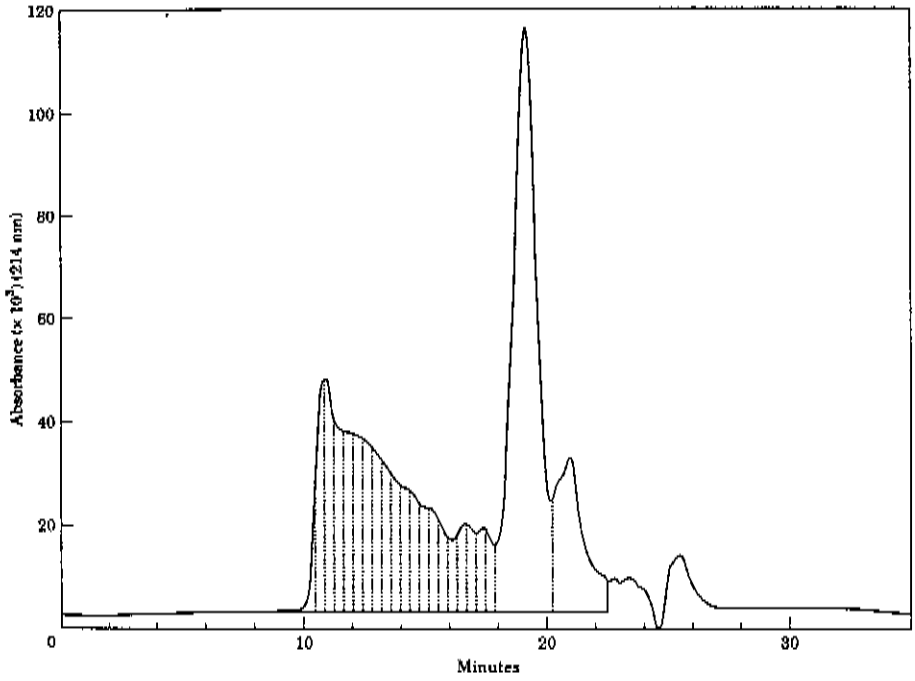


Fig. 6. SE-HPLC profile for protein from one of the 150 lines divided into 0.4 min intervals (30).

To summarize the protein compositional factors that govern the MWD of glutenin, there appear to be three main ones:

1. The ratio of HMW/LMW-GS (and to a lesser extent, the ratio of B/C LMW-GS)
2. Allelic variation at *Glu-1* loci (e.g. the presence of HMW-GS 5+10 vs 2+12)
3. The presence of chain terminators

In terms of the MWD of the total protein, the factors include those that influence the relative amounts of polymeric and monomeric proteins as well as those that affect just the glutenin that we have already considered. Of course, it needs to be emphasized that these are the genotypic factors only. The MWD is also known to be strongly influenced by environment. The effects of differences in sulfur fertilizer level have been mentioned. Nitrogen availability, heat stress and water stress are among other environmental factors that influence MWD. There is one point that should be mentioned at this stage. When proteins or subunits are deleted in a wheat line and, providing the protein concentration is maintained, it appears that the extra protein is distributed among all the other components. This conclusion comes from a number of studies. To mention one, in lines produced by Lawrence (28) in which HMW-GS were progressively deleted from a maximum of 5 to 0, the quantity of HMW-GS decreased linearly with decreasing number of subunits. This kind of result suggests that the proportion of a given class of proteins or subunits depends to a large extent on their number and therefore the number of coding genes. According to this, a wheat variety with a high glutenin/gliadin ratio will have a large number of glutenin subunits and/or a relatively small number of gliadins. Conversely, a low glutenin/gliadin ratio might be associated with fewer glutenin components and a large number of gliadin components. Of course, this generalization needs to be qualified to take into account differences in expression of the proteins or subunits.

Protein composition-functionality relationships

Returning to the relationships between protein composition and flour functionality, specifically continuing the focus on Extensograph properties, Fig. 5 shows plots of R_{max} against the percentage of polymeric protein (PPP) and the percentage of unextractable polymeric protein (UPP) for a set of 74 recombinant inbred lines (29, 5).

This typifies a general result that R_{max} is not always highly correlated with PPP but is invariably correlated with UPP. The diagrams on the left are schematic representations of these plots. The interpretation is that not all the glutenin contributes to dough strength but only a fraction above a certain molecular weight. This is consistent with what is found for synthetic polymers. For example, only a fraction of polymer above a critical MW is found to contribute to tensile strength. Extensibility, on the other hand, is found to depend on the flour protein content and correlates highly with the percentage of flour glutenin.

In order to pinpoint more precisely the protein fractions that contribute to the Extensograph parameters of R_{max} and Ext, the SE-HPLC profiles for some 150 samples comprising 30 wheat lines, each grown at 5 locations, were divided into elution time intervals of 0.4 min as shown in Fig. 6 (30). The percentage areas of the chromatograms measured up to each elution time were then correlated with R_{max} and extensibility for each of the 150 samples. As the elution time increased, the correlation coefficients increased and reached maximum values for both R_{max} and Ext, thereafter decreasing. For R_{max}, the maximum value occurred at an elution time or MW cut-off when approximately 60% of the protein had eluted. The maximum correlation coefficient for extensibility corresponded to the cut-off point for polymeric protein and, in this case, the correlation was with the percentage of polymeric protein in the flour.

Although the maximum in the correlation coefficient for Rmax was clearly identified, the correlation coefficient was relatively low, explaining only some 24% of the variation. In polymer science, two quantities are found to contribute to strength – the fraction of polymer above a critical MW and the MWD of this fraction. The low values of the correlation coefficients for R_{max} possibly means that the other variable, the MWD of this protein, is not being accounted for. Since most of this fraction elutes in the void volume, it is not possible to determine its MWD. There is thus a need to find methods for measurement of the MWD of glutenin. There are presently some promising approaches being developed in this area, including the work of Preston and Stevenson using Field Flow Fractionation (31).

Extensibility, unlike Rmax, is found to depend on the flour protein content and correlated highly with the percentage of flour polymeric protein (FPP), this parameter accounting for some 74% of the variation (30). It needs to be remembered that these considerations on Extensograph parameters only apply within what may be called normal behavior; i.e., within a certain range of Extensograph properties that are normally encountered. Outside this range, they may not necessarily apply. Tables I and II show some results that may throw light on the property of extensibility, a property that breeders have found difficult to manipulate.

Table I. Extensograph-Protein Composition Data for Two Wheat Lines (Averages for 5 Sites)

Line	Rmax (BU)	Ext (cm)	FPP (%)	UPP (%)
RAC704	452	18.1	5.3	50.2
DD118	454	23.5	5.9	51.3
L.s.d.	104	1.65	0.35	1.9

Correlations Ext – FPP: r = 0.84*** Ext – UPP: r = 0.24

The results in Table I were obtained for two wheat lines with contrasting extensibility that were grown at 5 locations (32). The results are shown as the average over the 5 locations. Although the extensibility of the two lines are very different, R_{max} is similar for the two and as might be expected, there is no difference in the UPP which gives a measure of the relative molecular-weight distribution. The difference in extensibility between the two lines appears to be simply explained by differences in the amounts of flour polymeric protein, this being significantly higher in the line with higher extensibility.

Table. II. Extensograph-Protein Composition Data for Two Wheat Lines (Averages for 5 Sites)

Line	Rmax (BU)	Ext (cm)	FPP (%)	UPP (%)
RAC746	398	17.4	4.5	51.2
VF304	274	22.4	4.6	45.4
L.s.d.	99	3.4	0.2	2.2

Correlations: Ext - FPP: r = 0.78*** Ext - UPP: r = -0.82***

Table II shows results for another pair of lines with contrasting extensibility (again averages over 5 locations). It is seen that there is an appreciable difference in R_{max} between the two lines, the line with lower extensibility having the higher R_{max}. For this pair of lines, although extensibility is highly correlated with the FPP for each, there is no difference in this parameter between the two lines. On the other hand, there is a significant difference in the UPP. This result suggests that the lower extensibility of the line RAC746 results from the MWs of the protein being shifted to too high values.

Application of protein composition-functionality relationships in breeding

Wheat breeders make their crosses and selections based on yield, disease resistance and other agronomic considerations. The task of the cereal chemist is to provide the compositional information for optimum processing quality in relation to the end-use requirement. The ultimate aim is to design a wheat with predictable properties by completely specifying the required alleles that control protein composition. In order to use the findings about protein composition – functionality relationships for input into breeding, we return to the link; i.e., the scheme for the location of genes that code for the main functional proteins (Fig. 2).

Continuing our focus on Extensograph properties, if we wish to increase R_{max}, this is relatively simple in theory. We need to increase the proportion of protein above a critical MW. This can be done by:

1. Increasing the proportion of total glutenin in the protein
2. Shifting the MWD to higher values and this is achieved by
   - Increasing the ratio of HMW/LMW-GS
   - Substitution of "strong" alleles.
   - Elimination of chain terminators.

The first requirement may be achieved by choosing alleles that are associated with relatively small numbers of gliadins at *Gli-1* and *Gli-2*. The second requirement is met by maximizing the number and expression of

HMW-GS, introducing alleles associated with strength (e.g., 5+10 at *Glu-D1*, 17+18 at *Glu-B1*) and eliminating loci that are associated with modified gliadins that act as chain terminators. If we want to reduce dough strength, we need to reverse these selections although, generally, it is not a simple case of selecting alleles for one property but arriving at a balance of properties that conform to the particular end-use requirements.

If we wish to increase extensibility, the requirements appear to be not quite so simple. Like Rmax, an increase in the percentage of flour glutenin is required but with the MWs not shifted to too high values. These conditions can be achieved by:

1. An increase in the flour protein content
2. An increase in the proportion of glutenin in the protein
3. Prevention of MWD shifting to too high values. This can be achieved by:
   - Decreasing the ratio of HMW/LMW-GS
   - Substitution of HMW-GS; e.g., replace 5+10 by 2+12
   - Introduction of chain terminators

We see that, for both R_{max} and Ext, each may be increased by increasing the proportion of glutenin in the protein. However, each appears to have different requirements for the MWD. This illustrates a point previously alluded to. Many different properties are involved in wheat quality – dough strength and extensibility, dough mixing requirements and mixing stability and product quality such as bread loaf volume to name a few. Changing the protein composition to alter one parameter in a certain direction will usually produce changes in others so that a compromise needs to be reached. This has to be taken into account when designing the optimum allelic composition for a given end-use requirement. In addition, environmental effects need to be taken into account. As more information becomes available in regard to reducing the effects of environmental conditions, this can also be factored into the allelic composition. Cereal science is at an exciting stage where, as a result of the concerted efforts of many, challenging areas for future researchers continue to be opened up.

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