AACC INTERNATIONAL
2015 CENTENNIAL

2015 marks 100 years of AACC International members working together to share cereal grain science. The Centennial Committee, with the help of many expert members, is creating activities to celebrate achievements and look to the future of our science. The Scientific Milestones Sub-committee is coordinating a series of summary highlights—each focusing on an important area of cereal grain science. Experts in each area have identified key breakthroughs over the last 100 years, provided brief descriptions of the discoveries, and given examples of how these breakthroughs led to changes in our cereal foods. The experts also provide a few ideas on where future research may lead us. These brief highlights are meant to provide an overview and cannot be inclusive of the exhaustive research in these areas.

Centennial Scientific Milestones Sub-committee: Lauren Brewer, Rachel Prososki, and Steven Nelson.

Proteins

Peter Shewry and Rob Hamer

Introduction

Proteins are macromolecules consisting of chains of amino acid residues, with 20 different amino acids present in most proteins. Differences in the proportions and sequences of these amino acids result in massive diversity in protein structure and properties, enabling them to perform a wide range of structural, storage, and metabolic functions. Although different cultivars given types of cereals do vary in their detailed composition of proteins, all mature cereal grains contain between about 7 and 15% total protein, with storage proteins making up more than 50% of the total. Due to their high concentrations and functional properties in food systems, storage proteins play a large role in determining the quality and end use value of grains.

Two notable advances that stimulated the study of cereal proteins were Thomas Burr Osborne's pioneering work on plant proteins and efforts by the U.S. Department of Agriculture (USDA) to build and apply protein-based research.

In his monograph *The Vegetable Proteins* (1924), Osborne described the fractionation of plant proteins based on differences in solubility. Although refined through the years, the Osborne fractionation continues to be the starting point for many cereal scientists, and the terms he introduced for the fractions (albumin, globulin, prolamin, and glutelin) continue to be used.

Within the same decade, the need to understand cereal protein science grew with the need for quality assessment of crops. In particular, recognition of the importance of proteins in wheat led the USDA to establish programs on wheat grain quality that were subsequently adopted and developed by AACC International. The importance of wheat protein content, and subsequently also wheat gluten content, was established by Dill and Alsberg in the 1920s, including a paper published in the first volume of *Cereal Chemistry* (1924) on gluten isolation and properties. Although Dill and Alsberg also reported on N-to-protein conversion factors and on proteins from other cereals, their emphasis on wheat protein and gluten laid the basis for a systematic approach to identifying factors affecting wheat quality.

Protein science can be considered one of the pillars on which cereal science was built. In fact, one of the key reasons for founding the American Association of Cereal Chemists (now AACCI) was to create a forum to discuss grain and flour quality and analytical methods to assess these properties, which are largely determined by grain proteins.

Landmarks in Cereal Protein Research

Progress in cereal protein science has been founded on the combining of diverse scientific disciplines. Some major advances are summarized below in chronological order, with some key researchers mentioned in italics.

1926 Thomas Burr Osborne Medal established

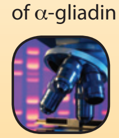
1928 First Osborne Medal was awarded to Thomas Burr Osborne

1963 Starch gel electrophoresis of gliadins

1968 First chromosomal location of gliadin gene

1973 2D-electrophoresis of gliadins and SDS-PAGE of gluten proteins

1974 N-terminal sequence of α-gliadin



- Identification of cereal protein fractions (albumins, globulins, prolamins, and glutelins) and their separation (*T. B. Osborne*).
- Distinguishing between the effects of protein quantity and gluten quality, exploiting methods for separation of starch and protein (*D. B. Dill and C. L. Alsberg*) and of gliadin and glutenin fractions.
- Baking quality (measured by loaf volume) shown to be a linear function of flour protein, with the slope of loaf volume versus flour protein being a varietal characteristic (*K. F. Finney and M. A. Barmore*).
- Determining the quality of wheat flour dough using rheology, initially using manual approaches (*D. B. Dill and C. L. Alsberg*) and then instruments such as those developed by Brabender (e.g., mixing time [farinograph, mixograph] and dough extensibility [extensigraph]) and Chopin (alveograph). Parameters measured by these instruments are known to be related to protein (gluten) quality, and therefore, they are still used to standardize dough-making processes and measure wheat protein quality.
- Development of methods for separation of gliadin and glutenin fractions (*J. H. Woychik and J. A. Rothfus*) and their characterization using rheology and spectroscopy (*A. H. Bloksma*, J. S. *Wall, Y. V. Wu, D. K. Mecham, and W. Bushuk*), leading to an improved understanding of the structure of glutenin (*B. Sullivan and J. A. D. Ewart*).
- Development of analytical biochemistry for separating individual protein components, exploiting electrophoresis (notably SDS-PAGE) (*J.-C. Autran, J. A. Bietz, and R. Tkachuk*) and chromatography (notably HPLC) in combination with sonication for glutenin polymers (*J. A. Bietz, F. R. Huebner, G. L. Lookhart, and N. K. Singh*).
- Genetic studies of protein composition using high-resolution analyses and genetic stocks to establish genetic control of individual components and their polymorphism between cultivars (*K. W. Shepherd, C. W. Wrigley, P. I. Payne, E. V. Metakovsky, R. B. Gupta, and D. Lafiandra*).
- Determination of the amino acid sequences of gliadins and glutenins, including details of domain structures, based on direct analysis using Edman degradation (*D. D. Kasarda, J. A. Bietz, and S. Masci*) and mass spectroscopy (*A. Tatham, H. Wieser, and P. Koehler*) and on isolation of cDNAs and genes (*D. D. Kasarda, O. D. Anderson, N. G. Halford, J. A. Rafalski, J. Messing, and B. A. Larkins*).
- Determination of the conformations and secondary structures of individual gluten components and the development of 3D models (*A. S. Tatham, Y. Popineau, and D. D. Kasarda*).
- Improved methods for fractionation of gluten proteins, including the development of methods for small-scale testing and isolating functional fractions for use in reconstituted systems (*W. Bushuk, F. MacRitchie, F. Bekes, and R. Kieffer*).
- Determination of the structures of glutenin polymers, including patterns of interchain disulfide bonding (*H. Wieser, H.-D. Belitz, P. Koehler, A. Graveland, and D. D. Kasarda*), and higher aggregates (*P. L. Weegels, C. Don, and R. J. Hamer*).
- Physical chemical and spectroscopic studies of wheat gluten and gluten fractions enabling the development of models to explain structure–function relationships (*W. Bushuk, R. C. Hoseney, Y. Popineau, F. MacRitchie, P. S. Belton, and A. S. Tatham*).
- Use of transgenic systems to manipulate protein composition (*I. K. Vasil, P. R. Shewry, A. E. Blechl, D. Becker, and F. Barro*) (see below).
- Differences in dough strength related to rate of glutenin polymer accumulation during grain development and final polymer size (*J. L. Carceller, T. Aussenac, H. A. Naeem, and F. MacRitchie*).

Success Stories

1. Scientific breeding

Payne et al. (1979) and subsequently several other researchers established a relationship between differences in the allelic composition of one group of gluten proteins, the HMW subunits of glutenin, and dough strength, allowing breeders worldwide to readily select for dough strength in their breeding programs. These relationships have since been shown to be related to effects on glutenin polymer size distribution (*N. K. Singh and F. MacRitchie*). Similarly, Greenwell and Schofield (1987) reported an association of grain hardness with proteins present on the starch granule surface. These

1979 2D IEF/SDS-PAGE of wheat proteins

1984 First complete gliadin sequence (cDNA/protein)

1994 Formation of the AACCI Protein Division

2002 First complete cereal genome sequence (rice)

2012 Wheat genome sequenced

proteins were identified as puroindolines (*D. Marion*), and it has since been shown that allelic variation in their presence and/or properties accounts for about 70% of the variation in hardness in bread wheat (*C. F. Morris and M. J. Giroux*).

2. Redefining cereal protein relationships
The application of modern methods of protein analysis and the availability of complete amino acid sequences from gene sequences have allowed the Osborne fractions to be redefined (*B. J. Miflin and P. R. Shewry*). In particular, the gliadin (prolamin) and glutenin fractions of wheat gluten are known to comprise related proteins, which differ in that the gliadins are monomeric while the glutenins are polymeric. Likewise, the prolamin and glutelin fractions of barley and rye also comprise related components. Similarly, it has been shown that the major storage proteins of oats and rice, which are classically defined as globulins and glutelins, respectively, are related to the legumin-type storage proteins found in peas, beans, and other legume seeds. Related globulins, called triticins, are also minor components in wheat (*N. K. Singh and K. W. Shepherd*).

3. Modifying protein composition by transgenesis
The development of transgenesis has allowed structure–function relationships to be defined in planta, by increasing or reducing the levels of individual gluten protein components (notably HMW subunits of glutenin) (*I. K. Vasil, P. R. Shewry, and A. E. Blechl*). Current work is focusing, for example, on developing wheat that is safer for celiac disease patients, using RNAi and other technologies to knock out either individual gliadin groups or all gliadins or to reduce the occurrence of celiac-active motifs in their sequences (*D. Becker and F. Barro*).

4. Elucidating gluten structure and viscoelastic properties
Detailed analyses of individual gluten proteins, gluten protein fractions, and whole gluten protein using circular dichroism, infrared, and nuclear magnetic resonance spectroscopies provided information on the secondary structures of gluten proteins and their interactions, leading to the development of hypotheses to explain their elasticity (*A. S. Tatham, D. D. Kasarda, F. MacRitchie, and P. S. Belton*). R. J. Hamer and T. Van Vliet proposed the presence of even higher level structures (the hyperaggregation model), leading to the discovery of the glutenin particle (*C. Don and R. J. Hamer*) and the demonstration that starch systems with noncereal protein particles give rise to elasticity and strain hardening (*L. E. Van Riemsdijk and R. J. Hamer*). These studies provide explanations for effects of processing (*C. Don and R. J. Hamer*), heat stress (*C. Don and G. Lookhart*), and genetic variation (*C. Don and D. Lafiandra*) on gluten structure and function.

Application of Cereal Protein Research in Industry

The progress in cereal protein science has positively impacted the cereal food industry in various ways:

- The development of extrusion cooking led to the replacement of conventional baking for a variety of cereal-based products (e.g., snacks, breads, crisps/chips, *cracotte*/crackers).
- The industrial separation of gluten and starch has been used since the 1980s to produce "vital" gluten for bread baking, enabling the production of improved and more consistent quality white and whole meal breads.
- Bread improvers have been developed based on gluten mixed with other additives (i.e., enzymes), enabling the replacement of potassium bromate and allowing the development of bake-off products. Insights into the structural changes in gluten during dough mixing have opened up new ways of optimizing the mixing process and, thus, better ways of coping with quality variations in wheat flour and improving final product quality.
- New insights into gluten protein structure and properties have led to more highly energy- and water-efficient processes for gluten and starch separation (*S. H. Peighambardoust, A. J. van der Goot, R. J. Hamer, and R. M. Boom*).
- Industrial gluten is widely used in formulations for meat replacers (mix of soy and gluten).

Future Outlook

Toward the safest wheat possible
The food industry is looking for sustainable proteins and protein production. More vegetable proteins will likely be used (e.g., textured proteins), and the demand for cereal protein products with acceptable texture and taste is expected to grow.

There also are concerns about increases in the incidence of adverse reactions to wheat consumption, including celiac disease, wheat allergy, and nonceliac gluten sensitivity. Recent developments in identifying specific epitopes linked to celiac disease may allow us to reduce their incidence through breeding, thus reducing exposure in consumers. Despite some negative reporting, bread remains the staple food in most temperate regions, with demand increasing in regions in which wheat is not traditionally grown and consumed (such as sub-Saharan Africa and Asia). Prolyl-specific peptidases from microorganisms will be exploited therapeutically to enable occasional gluten consumption by celiac patients or to produce gluten-free materials such as wheat starch.

Toward securing the supply of high-quality cereals

It is essential to increase cereal production to meet the growing global population. At the same time, there is increasing variation in climate and a need to reduce chemical inputs, costs, and environmental impacts. We, therefore, need to focus on developing new types of cereals that have stable yields and qualities over diverse environments and are less reliant on agrochemical inputs (notably nitrogen fertilizer). Insights into the relationship between abiotic stresses, protein composition, and gluten function will enable us to breed more stress-resistant varieties.

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