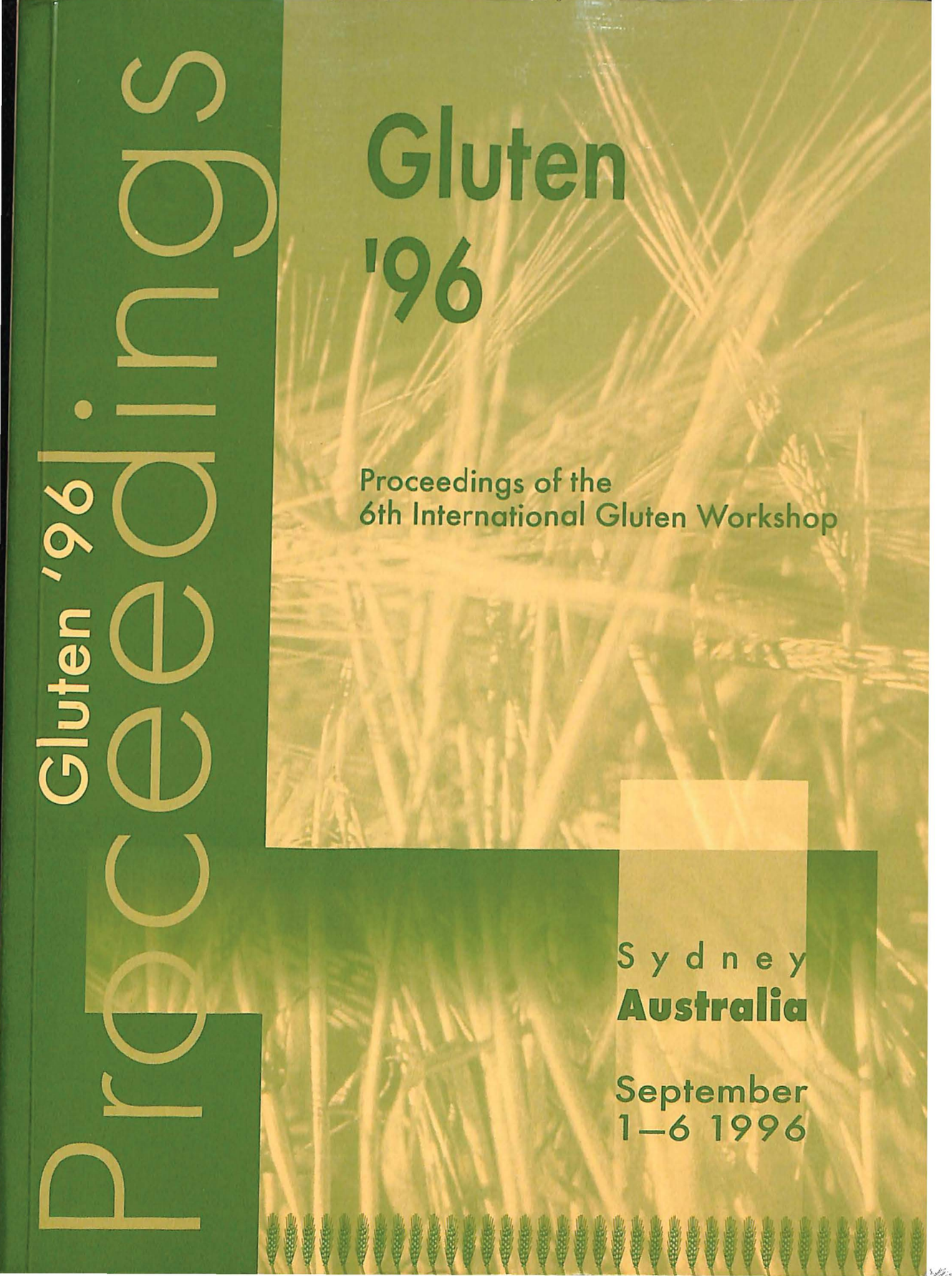

WHEAT KERNEL TEXTURE AND HARDNESS: WHAT DO GLUTEN PROTEINS DO ?

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This paper will briefly review the current knowledge of the bases of wheat grain hardness, with special emphasis on storage proteins. Based on the integrated information from various studies, I will try to indicate areas where I think research is needed to achieve a better understanding of the possible role of storage proteins in tissue mechanics and texture of wheat endosperm. Controversial results as well as open questions will be discussed with respect to future research.

HARDNESS OF CEREAL KERNELS

Hardness of cereals is defined as the force required to deform or crack the kernel (Anjum and Walker, 1991). The manner in which fracture occurs, fragment size and sifting behaviour depend upon kernel hardness (MacRitchie, 1980). Hardness and, more generally, mechanical properties, are among the most important characteristics of wheat kernel in various technological processes since they affect milling performance and the resultant flour quality (Pomeranz and Williams, 1990). Hard and soft wheats mill differently. Hard wheats require tempering to a higher moisture content than do soft wheats. Hard wheats produce coarse fragments that produce a semolina or a gritty flour and that flow more readily than finer ones. In contrast, soft wheats are reduced to a powder of fine particles whose irregular surface makes soft wheat flour agglomerate easily and thus sieve poorly. At the microscopic level, hard kernels tend to fracture at cell walls (or within the starch granules), whereas soft kernels fracture through the endosperm cells at the starch-protein interface, leaving the starch granules undamaged. Additionally, hard wheats are characterised by better bran cleanup and higher extraction rates for hard wheats, but hard wheat flours contain a significant amount of mechanically damaged starch granules.

Wheat endosperm also varies in appearance. It can be *opaque* if there is presence of air spaces which diffuse light and make the kernel appearance floury, or *vitreous* (translucent) if it is tightly packed. Although high-protein hard wheats tend to be vitreous, hardness must be distinguished from vitreousness since they are not the result of the same fundamental cause, and it is entirely possible to have hard wheats that are opaque and soft wheats that are vitreous. Hardness is caused by the genetically controlled strength of the bond between endosperm components (protein and starch). Vitreousness, on the other hand, results from the lack of air spaces in the kernel and is largely affected by environment, especially by protein content (Hoseney, 1992).

Typically, soft wheat flours are suitable for cakes, cookies or biscuits, as well as for starch-gluten separation and starch-derived products (*e.g.* paper), whereas hard wheat flours are preferred for bread-making, (especially, American or British white pan breads). Hardness also plays a major role in maize and sorghum since hard kernels have more desirable dry milling

properties (achievement of 100 % efficiency of separation of the components) as well as better adaptation to specific end-uses (corn-flakes).

THE BASIS OF KERNEL HARDNESS

Much effort was placed on the development of methods for accurate assessment of hardness (energy of milling, particle size index, milling time, pearling index, near-infrared reflectance,...). In parallel, at least four theories describing the fundamental (*i.e.* physicochemical or molecular) mechanism by which endosperm texture is expressed have been proposed in the last twenty-five years, which have stimulated considerable discussion.

In the early seventies, Simmonds (1971) and Barlow *et al.* (1973) showed that there was no difference in hardness (resistance to be pierced by a micropenetrometer) of either the starch or the protein between soft and hard types. They also observed that starch from hard types (prepared by flotation in a nonaqueous solvent) had a large quantity of proteins adhering to them, whereas soft wheat starch was relatively free of the adhering protein. They suggested, therefore, that hardness was related to the adhesion of starch and protein. Although soluble proteins (which could provide an explanation for greater adhesion in hard than in soft wheats) were purified or located from hard or durum wheats starches (Simmonds *et al.*, 1973; Lowy *et al.*, 1981; Rayas-Duarte *et al.*, 1995), the presence of a specific material responsible for adhesion was not really substantiated.

Stenvert and Kingswood (1977) suggested that the continuity of the starch-protein interface bestows endosperm hardness. Hard wheat might have a continuous protein matrix that physically traps starch so that resistance of the endosperm to deformation is greater simply because of more surface interaction, even if the strength of the protein-starch bond is the same. On the other hand, an unfilled or discontinuous matrix with many air spaces, results in a weak matrix, and thereby, a soft wheat. Although this theory looked reasonable, and was partially supported by further experiments of micro sectioning (Glenn and Saunders, 1990), its suffers a main deficiency in the sense that discontinuity appears mainly affected by environment and is more like a basis of vitreousness, whereas hardness is determined almost entirely by genetics (Hoseney, 1987, 1992).

Doekes (1985) proposed another theory in which hardness is caused by the protein fractions that have an electric charge. If the charge is high, the proteins repel each other and the grain is soft. If the charge is low, there is no repulsion and the grain is hard. A doubt was expressed by Hoseney (1987), since there is no evidence that a mechanism based on the net charge of proteins could apply in a dry kernel.

Greenwell and Schofield (1986) reported that the soft wheat starch consistently contains a strong 15-kDa electrophoretic band on the surface of the granule that is either missing or found in only very low levels in hard wheat cultivars. To obtain this protein, flour-water doughs were formed, starch granules were washed out, and starch granule proteins were extracted with 1 % SDS at 50° C, precipitated with acetone and separated using a linear gradient PAGE. Essentially no exceptions have been found from several hundred wheats around the world. From this, Greenwell and Schofield suggested that the 15-kDa protein (*friabilin*) decreases adhesion at the starch-protein interface. Moreover, Schofield and Greenwell (1986) observed that the gene controlling friabilin segregated with the gene *Ha* controlling endosperm hardness on the 5Ds chromosome arm.

However, when an antibody was produced against the protein in view to develop a predictive test for hardness from wheat seeds, meal or flour, it was found that both types contained roughly comparable amounts of friabilin and that friabilin was a marker only as far as its attachment to the isolated starch (Rahman *et al.*, 1991). Although friabilin is very likely to be involved in some way with endosperm texture, to date, a direct causative role of friabilin has not been established. Rather, the occurrence of friabilin on water-washed soft-wheat starch appears to be a partitioning phenomenon involving bound polar lipids that occurs during dough development and subsequent starch isolation and recovery (Malouf *et al.* 1992; Jolly et al 1993). In fact, recent studies from Marion *et al.* (1994) demonstrated that friabilin

(i) presented high homology with the tryptophan-rich lipid-binding proteins named *puroindolines* and

(ii) were mainly present in the aleurone layer and at the periphery of starchy endosperm, a location that is not appropriate for its putative role in controlling albumen texture as functional marker of grain softness or "non-stick" agent. These recent results led Morris *et al.* (1994) and Greenblatt *et al.* (1995) to turn their investigations towards a possible lipid factor at the surface of starch granules.

A COMPLEX QUESTION: HOW THE STARCH-PROTEIN INTERFACE CAN BE THE SITE OF A CHEMICAL DIFFERENCE WHICH COULD MAKE THE BASIS OF ENDOSPERM HARDNESS ?

"The biochemistry of wheat hardness is one of the few subjects that remain, over the years, controversial and enigmatic" (Pomeranz and Williams, 1990), and there is still an agreement to consider that basis of hardness still remains to be fully elucidated. However, it is becoming clear that the molecular interface between the starch granule and the storage protein matrix surrounding it makes the heart of the question, but that this interface is much more complex than might at first be thought. In hard wheat the surrounding protein wets the starch granule surface better than in soft wheats, so that the most logical explanation is that in hard wheat, the matrix proteins adheres more strongly to starch than in soft wheats. But it is not known whether the nature of the bond involves an 'adhesion' or 'cementing' factor in hard wheats, or a 'non-stick' or 'release' factor that would impair starch-protein adhesion in soft wheats.

The conflict between the theories proposed by the various authors having explored the basis of endosperm hardness results from the fact that hardness certainly covers several questions, each of which should have been the subject of a specific approach:

- *physico-chemical*: We poorly know the physico-chemical state of the surface of starch granules - a 'hairy billiard ball' according to Lineback and Rasper (1988), with portions of complexed and partially completed chains of amylose and amylopectin, extending through the boundary (Simmonds and O'Brien, 1981). Because tablets reconstituted from protein-depleted starch isolated from soft wheats gave similar tensile strength as those from hard wheats (Malouf *et al.*, 1992), it is more likely that the difference between soft and hard type lies in protein coating or lipid inclusions than in the starch polymers themselves. Although we know that, in wheat, the protein-starch bond is easily broken by an excess of water (*e.g.* in starch-gluten separation), hydrophobic interactions are likely to be involved, either *in vivo* (storage proteins could refold upon grain desiccation and bind to the surface of starch granules - in a similar way as lipids, which undergo transition from lamellar to tubular structures (Marion,

1992)), or *in vitro* (lipid-binding proteins could attach to the surface of granules during starch isolation). With respect to ionic bonds, whose importance was put forward during protein packing in protein bodies, perhaps their role should not be dismissed considering certain millers' views about lump formation and poor sieving in soft wheat flours.

- *biological*: The exact moment when starch-protein adhesion takes place in endosperm is not entirely clear. According to Pomeranz and Williams (1990), unripe grains of soft types are all vitreous, softness arising later, whereas, by freeze-fracture of unfixed endosperm, Bechtel and Barnett (1986) observed spaces between starch and protein matrix at least until 21 days after flowering, the association being visible when maturity was approaching. Furthermore, the starch-protein interface cannot be as simple as it was sometimes viewed. Endoplasmic reticulum and various organelles and vesicles are visible during proteins deposition and fusion of protein bodies into a protein matrix, while around starch granules, a number of soluble proteins, probably residues of the enzymic synthesizing systems are present as well as lipid and protein components derived from the amyloplast membrane (Simmonds and O'Brien, 1981). When the matrix is pressed against the growing starch granules, it is likely that remnants of these vesicles and membranes are present at the interface and play a role in the adhesion between storage proteins and starch observed in the mature kernel.

- *mechanical:* How to explain that the fracture will occur differently between (i) a hard or durum type endosperm that is opaque because of insufficient protein content (in this case air spaces that reflect light are present, but without impairing resistance to crushing, because the fracture will still take place at cell walls or within starch granules) and (ii) a soft type that is protein-rich and therefore vitreous (in that case, although no space is visible, the endosperm is crumbly and the fracture will still occur between protein matrix and starch) ?

- *genetic*: The locations of (i) the hardness gene *Ha*, (ii) the friabilin gene and (iii) a factor regulating free polar lipids (Morrison *et al.*, 1989), all on the short arm of chromosome 5D, cannot be coincidental, and makes compelling the evidence linking a mechanism involving some lipid-binding components with the control of endosperm texture. In contrast, the fact that some kernels texturally hard, found by Glenn and Saunders (1990) and Glenn and Johnston (1992) in soft genotypes, were containing the 15-kDa proteins, would preclude the role of lipid-binding proteins in hardness.

STORAGE PROTEINS AND ENDOSPERM HARDNESS

In comparison to the effort placed on functional (breadmaking) properties of wheat storage proteins, their contribution to kernel texture and hardness has received minor attention. However, given the quantitative importance, intracellular location and genetic determinism of storage proteins, it is likely that they play a major role (or at least some role) in endosperm texture.

On one hand, it must be recognised that no strong, clear-cut relationship has been so far demonstrated among either gliadins or glutenins of genotypes of different hardness levels. But, on the other hand, several facts could argue in favour of a direct role of storage proteins in endosperm hardness:

- Fused protein bodies and protein matrix, that are strongly pressed against starch granules during cell filling and endosperm dehydration, could interact with them provided they have adequate chemical composition and accessibility of reacting areas.

- Interactions would be made easier by a greater fluidity of protein bodies, a trait that could result from a specific protein composition (Simmonds and O'Brien, 1981).

- Studies on storage proteins of maize indicated much higher amounts or proportions of α-zein in flint genotypes and in the horny regions of endosperm (Paiva *et al.*, 1991; Dombrink-Kurtzmann and Bietz, 1993), with similar situation for γ-kafirins in sorghum (Mazhar and Chandrashekar, 1995), although the starch-protein bond in these cereals might be of different nature and stronger than in wheat (Hoseney, 1992).

- A significant effect of specific *Glu-B1* and *Gli-B1* alleles on hardness was showed by Félix (1996), confirming that hardness could be influenced by genes others than those located on the chromosome 5D.

- A relationship was found by Huebner and Gaines (1992) between hardness and at least one group of relatively hydrophobic gliadins.

- A differential adsorption of different protein fractions on wheat or other starches was showed by Eliasson and Tjerneld (1990).

RECOMMENDATIONS FOR FUTURE RESEARCH

Elucidating the basis of endosperm texture, particularly identifying a functional marker of hardness, has become more crucial than ever. It would allow to better control the current milling process and characterise the potential of raw materials in terms of adaptation to fragmentation and separation (of which breeders or genetic engineers could benefit). It would also strongly contribute to the development of new food or non-food end-uses of wheats, which require wheat fractions better defined histologically and more pure biochemically. To achieve this objective, I think it is worth considering the following points:

- It must be kept in mind that the degree of starch-protein adhesion is (unlike the environment-dependent vitreousness factor) a heritable character.

- The biochemistry of hardness cannot be dealt without a thorough knowledge and dynamics of storage structures in the developing endosperm, especially the origin, composition of protein bodies, other organelles and the enzymic systems involved, as well as the transitions they undergo in changing water environments. One should not attack this question as a chemistry exercise of simple reactions bringing together 2 or 3 elementary constituents.

- In particular, because of the extreme importance of biological structures and functions at the forming starch protein interface, any experimental procedure liable to alter this interface is a potential source of artefacts. When endosperm is hydrated, dough formed, starch isolated (*e.g.* in chloroform or benzene), treated by chlorine or protein-depleted by SDS or pronase, or mixed to purified proteins to explore adsorption phenomena, or when tablets are reconstituted from isolated components, valuable information may be obtained for stimulating discussion

or for developing new processes in technology, but artefactual conclusions may be obtained about the fundamental basis of hardness.

In parallel with studies that should proceed on lipid factors or lipid-binding proteins (as well as studies of other important features of wheat kernel, *i.e.* bran friability, adhesion between pericarp and aleurone layer, phenolic composition of cell walls,...), a multidisciplinary approach of the protein component of the starch-protein interface (re-examining the ancient concept of adhering/interstitial protein described by Hess (1954) through the use of modern methodologies) should be carried out with integration of the following aspects:

- Chemical composition of storage proteins from single kernels, using methodological advances (monoclonal antibodies, immunocytochemistry, availability of isogenic lines, etc.) with the aim to approach the nature of the starch-protein association.

- Micro spectrometry with spectral imaging, to obtain *in situ* spectra of small areas of endosperm, study the heterogeneity in functional groups and achieve a molecular chemical dimension to enhance the understanding of the systems (*e.g.* starch-protein interface) where highly localized compositional information may be of value (Wetzel and Reffner, 1993)

- Freeze-fracture electron microscopy of endosperm during development, to pick up new microstructural information, especially type, fluidity and fusion behaviour of protein bodies, that could be genetically determined and related to the expression of a hard or soft texture.

- Microfracture mechanics to establish a direct relationship between a cell product and actual hardness data based on both single caryopsis and bulk hardness tests (Glenn and Johnston, 1992; Dobraszczyk, 1994)

REFERENCES

Anjum, F.M., and Walker, C.E. (1991) J. Sci. Food Agric. 56, 1-13.

Barlow, K.K., Buttrose, M.S., Simmonds, D.H., and Vesk, M. (1973) Cereal Chem. 50, 443-454.

Bechtel, D.B., and Barnett, B.D. (1986) Cereal Chem. 63, 232-240.

Dobraszczyk, B.J. (1994) J. Cereal Sci. 19, 273-282.

Doekes, G.J. (1985) Getreide, Mehl und Brot 39, 259-263.

Dombrink-Kurtzman, M.A., and Bietz, J.A. (1993) Cereal Chem. 70, 105-108.

Eliasson, A.C., and Tjerneld, E. (1990) Cereal Chem. 67, 366-372.

Félix, I. (1996) Thesis, University of Clermont-Ferrand, France, 149 p.

Glenn, G.M., and Johnston, R.K. (1992) Food Struct. 11, 187-199.

Glenn, G.M., and Saunders, R.M. (1990) Cereal Chem. 67, 176-182.

Greenblatt, G.A., Bettge, A.D., and Morris, C.F. (1995) Cereal Chem. 72, 172-176.

Greenwell, P., and Schofield, J.D. (1986) Cereal Chem. 63, 379-380.

Hess, K. (1954) Kolloid Z. 136, 84.

Jolly, C.J., Rahman, S., Kortt, A.A., and Higgins, T.J.V. (1993) Theor. Appl. Genet. 86, 589-597.

Hoseney, R.C. (1987) Cereal Foods World 32, 320-322.

Hoseney, R.C. (1992) 'Principles of Cereal Science and Technology', AACC, St Paul, Minn., USA, 327 p.

Huebner, F.R., and Gaines, C.S. (1992) Cereal Chem. 69, 148-151.

Lineback, D.R., and Rasper, V.F. (1988) 'Wheat Chemistry and Technology' (Y. Pomeranz, Ed.), AACC, St Paul, Minn., USA, pp. 277-372.

Lowy, G.A.D., Sargeant, J.G., and Schofield, J.D. (1981) J. Sci. Food Agric. 32, 371-377.

MacRitchie, F. (1980) 'Advances in Cereal Science and Technology' (Y. Pomeranz, Ed.), AACC, St Paul, Minn., USA, Vol. 3, pp. 271-326.

Malouf, R.B., Lin, W.D.A., and Hoseney, R.C. (1992) Cereal Chem. 69, 169-173.

Marion, D. (1992) 'Cereal Chemistry and Technology: a Long Past and a Bright Future' (P. Feillet, ed.), IRTAC, Paris, pp. 57-62.

Marion, D., Gautier, M.F., Joudrier, P., Ptak, M., Pézolet, M., Forest, E., Clark, D.C., and Broekaert, W. (1994) 'Wheat Kernel Proteins: Molecular and Functional Aspects', September 28-30, Viterbo (Italy), pp. 175-180.

Mazhar, H., and Chandrashekar, A. (1995) J. Cereal Sci. 21, 155-162.

Morris, C.F., Greenblatt, G.A., Bettge, A.D., and Malkawi, H.I. (1994) J. Cereal Sci. 20, 167-174.

Morrison, W.R., Law, C.N., Wylie, L.J., Coventry, A., and Seekings, J. (1989) J. Cereal Sci. 9, 41-51.

Paiva, E., Kriz, A.L., Peixoto, M.J.V.V.D., Wallace, J.C., and Larkins B.A. (1991) Cereal Chem. 68, 276-279.

Pomeranz, Y. and William,s P.C. (1990) 'Advances in Cereal Science and Technology' (Y. Pomeranz, Ed.), AACC, St Paul, Minn., USA, Vol. 10, pp. 471-548.

Rahman, S., Jolly C.J., Skerritt, J.H., and Higgins, T.J. (1991) Chem. Aust. 58, 397.

Rayas-Duarte, P., Robinson, S.F., and Freeman, T.P. (1995) Cereal Chem. 72, 269-274.

Schofield, J.D., and Greenwell, P. (1986) 'Cereals in a European context', Bournemouth, UK, 1-3 July, (I.D. Morton, Ed.), Ellis Horwood Ltd, 8, 2, pp. 407-420.

Simmonds, D.H. (1971) Wallerstein Lab. Commun. 34, 17-34.

Simmonds, D.H., Barlow, K.K., and Wrigley, C.W. (1973) Cereal Chem. 50, 553-562.

Simmonds, D.H. and O'Brien, T.P. (1981) 'Advances in Cereal Science and Technology' (Y. Pomeranz, Ed.), AACC, St Paul, Minn., USA, Vol. 4, pp. 5-70.

Stenvert, N.L., and Kingswood, K. (1977) J. Sci. Food Agric. 28, 11-19.

Wetzel, D.L., and Reffner, J.A. (1993) Cereal Foods World 38, 9-20.