# TAKING WHEAT APART AND PUTTING IT TOGETHER

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## Summary

Author reviews the recent results of research work done on the components of wheat, on the genetics and the plant breeding, and points to the significance and role of the individual components and of their interaction in breadmaking.

## Introduction

The problem of what makes a wheat flour "tick" in production of acceptable foods (bread, buns, biscuits, cakes, alimentary pastes, etc.) has kept more cereal chemists busy for a longer time than any other single issue. Much progress has been made in this field and some of it has been summarized in several, available publications (Pomeranz, 1968, 1971, 1973, 1980a, b, c, d; Pomeranz et al., 1970). Consequently, I would like to concentrate on some recent findings from our laboratories on studies that relate wheat (or wheat flour composition) to breadmaking. Before doing so, however, I will list the various approaches that can be used to demonstrate that relation, the methods that can be employed, the information sought, and the genetic dimension that undergirds some of that work.

## Approaches, methods, information

The four basic approaches are listed in Table 1. They include: use of statistics; studies of synthesis during maturation and breakdown during germination; separation and combination of dough components; and fractionation and reconstitution of isolated components.

Much has been computed by using a clean pad and sharp pencil; more by using a calculator; and even more by using a personal or institutional computer. Some paper pads are larger than others, some pencils sharper than others, and some calculators or computers more powerful than others. Much of the information is computed to determine statistically significant correlations; albeit, statistical significance does not always assure meaningfulness.

But most importantly, in calculating statistical significance much depends on what you feed into the computer, how you phrase the questions, what is the information that you seek, etc. Consequently, it comes as no surprise that some demonstrated by means of statistics that loaf volume potential is controlled by high molecular weight glutenins whereas the computations of others showed that medium-low molecular weight gliadins govern the potential (Konzak, 1977; Wall, 1979; Wrigley et al., 1982.)

**Table 1**

| Approaches |
|---|
| 1. Statistics — pad & pencil, computer |
| 2. Synthesis — maturation — breakdown — germination |
| 3. Dough — separation — combination |
| 4. Isolated components — fractionation, characterization, (synthesis), reconstitution |

A list of studies that correlated breadmaking quality with electrophoretic patterns of gliadins was presented by Mifflin et al. (1981). The authors stressed, however, that such analysis does not establish cause and effect relationships. It merely indicates linkages between "true quality genes" and structural genes for certain proteins. The relation is analogous to the linkage between mildew and rust resistance genes and barley prolamines, even though no relation between prolamines and pathogen resistance is postulated or visualized (Mifflin et al., 1981).

According to Mifflin et al. (1981), it is questionable whether true wheat glutelins are storage proteins. The prolamines represent 60% of the total wheat N and the true glutelin plus residue not more than 15%. Some glutelin preparations may be heavily contaminated by unextracted prolamines, have a predominantly prolamine-like amino acid composition and contain polypeptide and peptide fragments in common with gliadin.

Another approach involves identification of components synthesized during grain maturation or those degraded during germination. Much useful information was obtained from the studies of Mauritzen and Stewart (1965) on dough centrifugation. The most powerful approach has been the analytical one (Finney, 1978). It involves taking components apart, reconstituting them in original and various proportions and following the effects of such separations, reconstitutions, and combinations. While a great amount of excellent information has been obtained by the analytical approach, many questions remain unanswered and it is questionable how much additional, uniquely novel information can be obtained by the analytical approach. Specifically, the questions that arise concern the limit of fractionation by presently available

methods without damage to functional properties and the extent to which the approach can be used to follow interactions among components. While methods used thus far have not impaired functional properties as assessed by the optimized test, the possibilities of irreversible modifications cannot be excluded. Such modifications may not be discernible in the baking test which provides results of a whole series of compensatory and modifying actions and interactions.

The methods used to obtain information from the above studies are listed in Table 2. They are physical or biophysical, chemical or biochemical, various

**Table 2**

| Methods |
|---|
| 1. Physical — biophysical |
| 2. Chemical — biochemical |
| 3. microscopic — LM, TEM, SEM, attachments histochemistry |
| 4. Enzymic — immunochemical |

**Table 3**

| Information |
|---|
| 1. Indirect inferences |
| 2. Related to others |
| 3. Direct measurements |
| 4. Taking apart — putting together |

microscopic ones (light, transmission electron, scanning electron) alone or in combination with various attachments for identification and quantification of components. The methods can include the use of enzymic or immunochemical assays.

The information sought (and obtained) is listed in Table 3. Indirect inferences based on statistical calculations always leave the lingering questions—what is the basis for the correlations and what do they mean? I do not wish to repeat some of the many stereotype jokes in this field except to say that with enough samples you can make almost every two parameters statistically related and that there may not be enough recognition of the fact that while statistical computations provide a powerful quantitation of good data, statistics cannot replace good data and should not become an objective instead of a tool.

The information becomes much more meaningul, if related data are compared. Direct measurements (i.e. protein, wet gluten, etc.) are even more

meaningful; yet, they often fail (especially in destructive tests such as Kjeldahl protein determination) to evaluate qualitative effects, such as those resulting from inherent differences in gluten quality, effects of damage during maturation, storage, etc. Those differences can be accounted for by other methods, including the analytical approach of taking flour apart and putting it together as described before.

## Genetics

Before reviewing new findings from our laboratories, I would like to discuss briefly the contribution of genetics to unravelling the role of wheat flour components in breadmaking (Table 4.). Wheat composition and quality (as related to end-use properties and nutritional value) are controlled genetically. Detailed analysis of the controlling mechanism, however, is complicated by the polyploidy nature of wheat and the strong influence of the environment and cultural practices.

**Table 4**

| Genetics |
|---|
| 1. Classical plant breeding |
| 2. Isogenic lines |
| 3. A.A. sequence — DNA sequence |
| 4. Genetic engineering |

**Table 5**

| Recent studies |
|---|
| Synthesis — maturation |
| Breakdown — germination |
| Dough combination — freeze fracture |
| Fractionation — reconstitution |
| Statistics — direct measurements |
| Lipids |
| Plant breeding |

Several useful genes have been transferred from wild relatives to common wheat through induced homeologous pairing anc crossing over. Similarly, radiation-induced translocations have been useful in transferring disease-resistance genes to common wheat (Feldman and Sears, 1981).

The development by Sears (1954) of a series of wheat aneuploids in which a pair of chromosomes corresponding to a particular genome was missing

(nullisomic) and was compensated for by an extra pair of homeologous chromosomes (tetrasomic) provided researchers with a valuable tool to study the relationship between wheat chromosomes and specific plant of seed morphological or compositional characteristics. Those aneuploids have been studied, among others, to determine the chromosomes which govern protein content and composition. For a review on the relationship with wheat proteins see Kasarda et al. (1976) and Wrigley et al. (1982). Garcia–Olmedo et al. (1982) outlined the usefulness and limitations of different approaches to the genetic analysis of wheat endosperm proteins. Table 5 outlines recent studies from our laboratories in taking wheat apart and putting it together.

## Plant breeding

Attempts to increase the variability, including improved composition and end-use properties, of new cultivated wheats by including mutations, by ionizing radiations, such as x-rays, or by chemical treatment has met with limited success. Another route involves enrichment of the gene pool of cultivated wheats by tapping the vast genetic resources that are to be found in the wild relatives of the wheats to produce amphiploids. Amphiploids are fertile interspecific hybrids with a complete set of paired chromosomes derived from each parent species. According to Feldman and Sears (1981), hexaploid wheat (genome AABBDD) originated as a hybrid between *Triticum turgidum* (probably the cultivated variety *T. turgidum dicoccum* or emmer wheat; genome AABB) and *T. tauschii* (genome DD). A spontaneous hybridization between *T. turgidum* and *T. tauschii,* the two progenitors of hexaploid wheat *T. aestivum,* probably took place in the field in Western Iran about 8,000 years ago (Feldman and Sears, 1981).

An example of putting hexaploid wheat together comes from investigations of J. V. Worstell of the U. Missouri, USA, under the guidance of E. R. Sears. Worstell developed a viable cross of wheat that averages about 26% protein, nearly double that of wheats grown commonly in the U. S. Midwest (J. V. Worstell, private communication, Dec. 8, 1981). The hybrid is a cross of the diploid *T. tauschii,* a grass indigenous to Southwest and Central Asia and *T. turgidum,* a high protein wild emmer wheat from Israel.

We compared the structure of the new high protein amphiploid wheat by light and electron microscopy to its diploid *(T. tauschii strangulata)* and tetraploid *(T. turgidum dicoccoides)* progenitors and to a commercially grown hexaploid hard red winter wheat (*T. aestivum* cv. Newton), all grown under the same conditions (Gaines et al., 1982.) Fig. 1, 2. Appearance and protein content in the central starchy endosperm among the cultivars was similar. The amphiploid subaleurone endosperm cells were densely packed with matrix

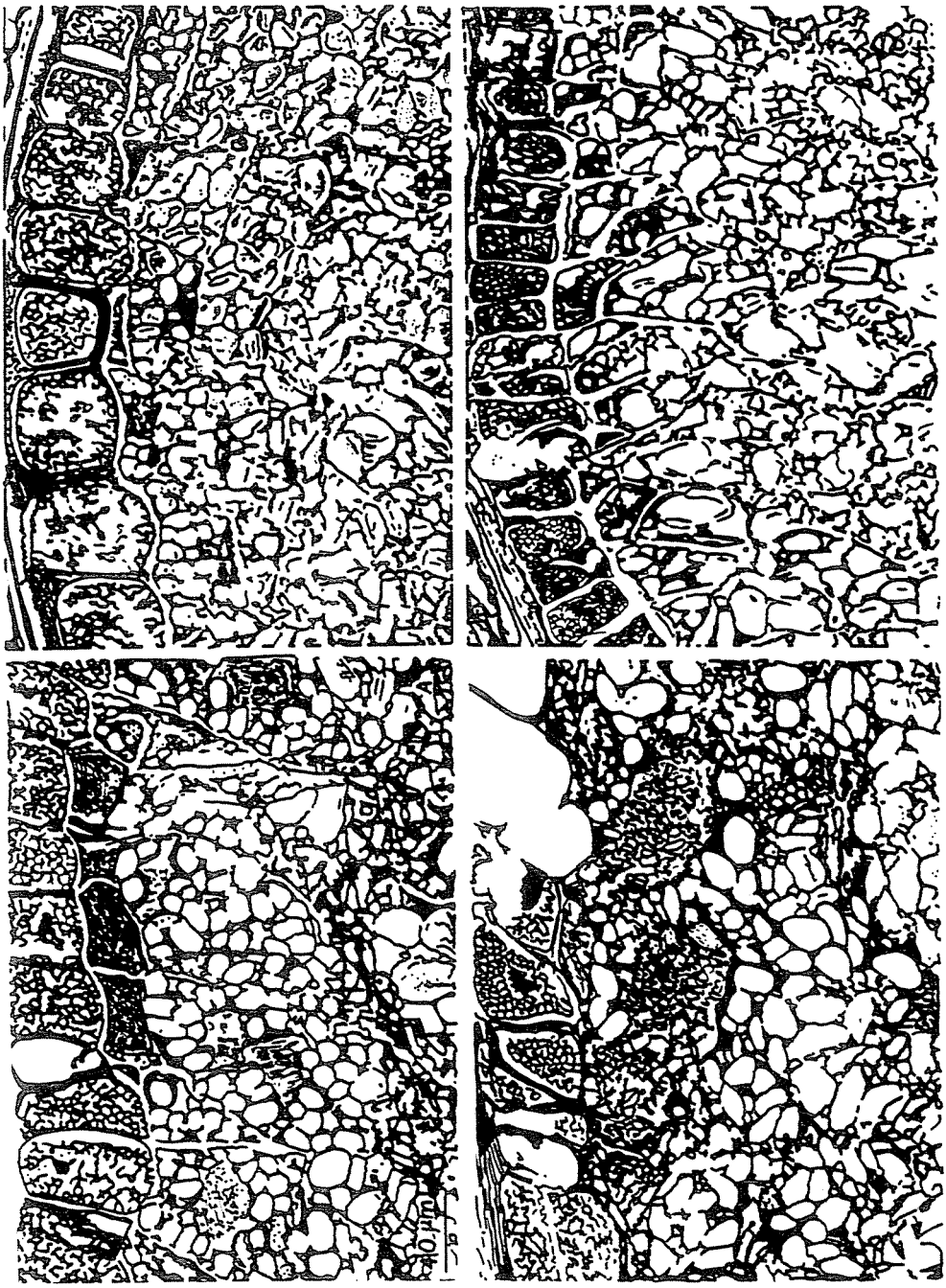

*Fig. 1.* Light micrographs of the aleurone, subaleurone, and central endosperm in the amphiploid (A), cv. Newton—*(Triticum aestivum)* (N); tetraploid *(T. turgidum dicoccoides)* (T); and diploid *(T. tauschii strangulata)* (D.) (Gaines et. al., 1982)

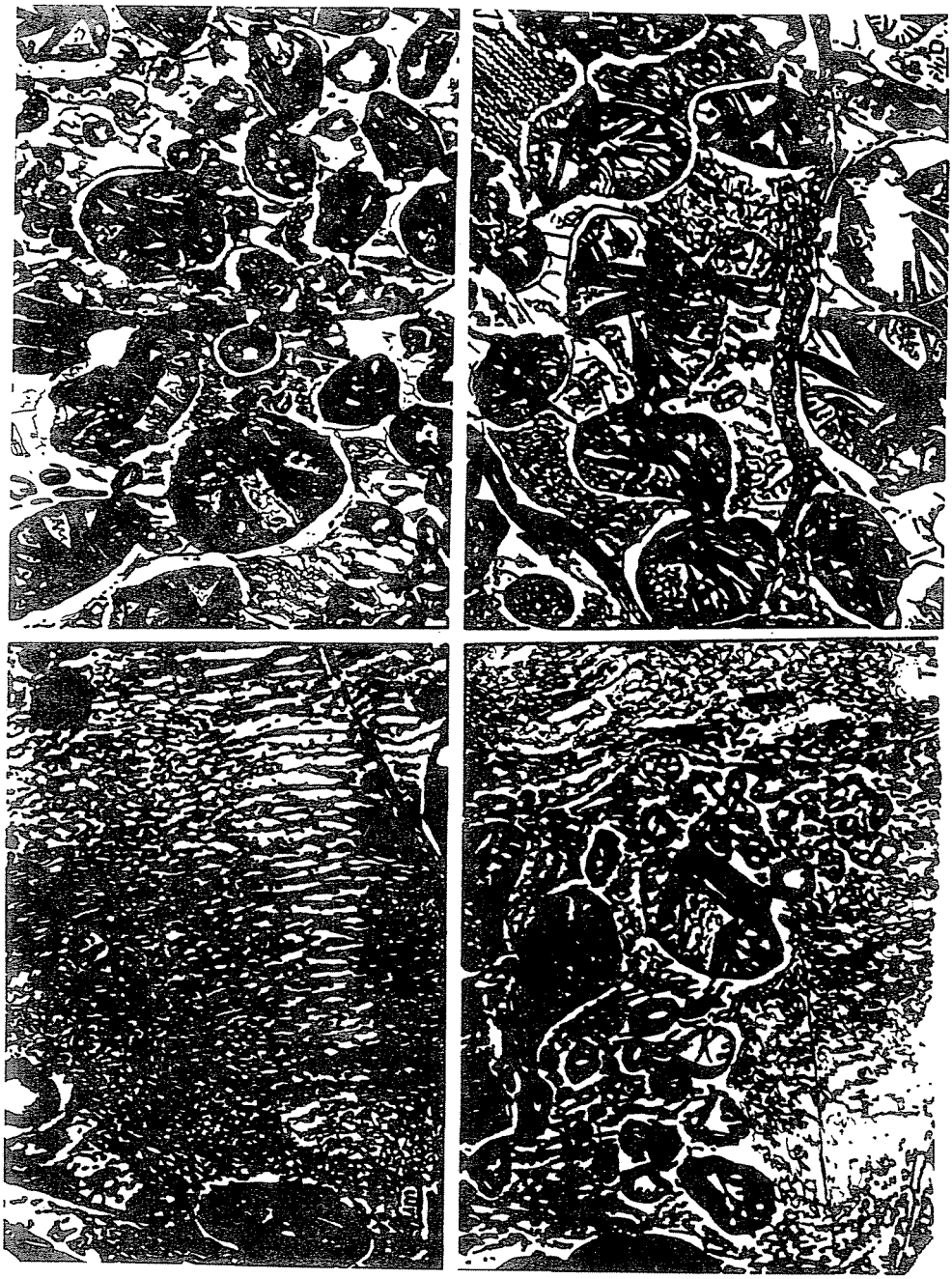

*Fig. 2.* Transmission electron micrograph of the subaleurone in the amphiploid (A), cv. Newton (N), tetraploid (T), and diploid (D). (Gaines et al., 1982)

protein and few small starch granules. Numerous small (< 1 μm) spherical osmiophilic protein inclusions and irregularly-shaped, less electron dense, inclusions were scattered throughout the matrix protein. The subaleurone of the tetraploid progenitor and cv. Newton possessed accumulations of matrix proteins with dense inclusions that were slightly larger and less numerous than in the amphiploid. Cytoplasmic remnants were more abundant in the tetraploid and hexaploid than in the amphiploid. The tetraploid matrix protein had a mottled appearance, unlike that in the other specimens. Starch granules, especially large A type, in the subaleurone region were more abundant in cv. Newton and the tetraploid than in the amphiploid. In the diploid subaleurone, the amount of matrix protein with relatively few, large (1–4 μm) irregularly shaped osmiophilic inclusions was diluted by great numbers of type A starch granules.

The screening of wild genetic resources for agronomically useful characteristics is only in its initial stages. Among the desirable characteristics that can be found in the wild relatives of cultivated wheats are increased resistance to pests, improved resistance to adverse climatic conditions and various types of stress, and increased yield. The usefulness of those traits and the possibility of incorporating improved quantity and quality of proteins and technological versatility are yet to be determined. The greatest challenge will be to "put together" a wheat that combines high productivity, resistance to adverse growth conditions, and acceptable end-use properties. To meet that challenge, a combination of classical plant breeding and genetic engineering may be required.

## Protein synthesis-maturation

Morphological and biochemical development of the wheat endosperm was reviewed by Simmonds and O'Brien (1981). Molecular aspects of seed protein biosynthesis were the subject of a review by Spencer and Higgins (1979).

Formation of storage proteins in the endosperm of maturing wheat has been intensively studied but has yielded contradictory results. Graham et al. (1962) showed that the earliest deposited endosperm storage protein was a single body enclosed by a membrane. Later during development, four or more bodies were present within the vacuole. The proteins were deposited into vacuoles from the rough endoplasmic reticulum (RER) via an unspecified mechanism called "internal secretion". These results were confirmed by Buttrose (1963), who suggested that the Golgi apparatus was linked to protein deposition by acting in a condensing function. Jennings et al. (1963) concluded that the protein body occurred singly and had a tightly appressed membrane, rather than occurring in vacuoles. It was subsequently postulated that protein

bodies formed within plastids called proteoplasts (Morton and Raison, 1963; Morton et al., 1964). The occurrence of single and multiple protein bodies within vacuoles of early developing wheat endosperm was confirmed by Barlow et al. (1974) and Harvey et al. (1974). They also described the presence of material within the vacuole which was interpreted as ribosomes, the site of the storage protein synthesis. During late stages of development, however, protein bodies formed via a different mechanism (Barlow et al., 1974). This other mechanism involved protein secretion into the RER lumen which resulted in the double membrane being pushed apart and the ends then joining to surround a protein body with a single membrane.

Barlow et al. (1974) found no evidence for protein transport from the RER and concentration into dictyosomes. Campbell et al. (1974) were unclear whether the storage protein was synthesized in the vacuoles or was synthesized elsewhere and transported to the vacuoles. It was hypothesized that the protein was synthesized in the cytoplasm and transported to the vacuoles either through the lumen of the RER or by a process similar to pinocytosis (Simmonds, 1978). Recently, Briarty and coworkers (1979) conducted an extensive stereological analysis on the developing wheat endosperm. They concluded that the route followed by storage proteins to the vacuoles was unclear, but the Golgi apparatus was not involved because it was absent 12 days after flowering (Briarty, 1978; Briarty et al., 1979). Campbell et. al. (1981) suggested that a direct connection exists between the RER and the protein bodies whereas Parker (1980) observed large amounts of membranous material associated with developing wheat protein bodies. Bechtel and Gaines (1982) from our laboratories demonstrated recently the presence of dictyosomes secreting densely stained vesicles throughout endosperm protein body formation for hard red winter wheat, *Triticum aestivum* L.; winter feed barley and spring malting barley, *Hordeum vulgare* L.; and oats, *Avena sativa* L. The contents of the Golgi vesicles and protein bodies were digested with proteases. The results implied that the Golgi apparatus plays an important role in the concentration and transport of storage proteins into vacuoles. Subsequently, Bechtel et al. (1982a) completed an indepth microscopical analysis of protein body initiation and development in wheat starchy endosperm. That study addressed three questions concerning the early stages of protein body formation in wheat:

1. When and where are protein bodies initiated?
2. What organelles are involved with protein body formation and development? and
3. What kinds of intracellular transport system, if any, is involved in the transfer of storage protein from its site of synthesis to its deposition into vacuoles?

Two days after flowering (DAF) the endosperm was a thin layer of coenocytic cytoplasm lining the embryo sac. By 4 DAF the endosperm had cellularized and completely filled the embryo sac. Enough differentiation had occurred by 6 DAF to distinguish cells destined to become the aleurone layer, subaleurone region and central endosperm. Protein bodies were initiated at about 6–7 DAF and were found first near the Golgi apparatus. Wheat was ready for combine harvest at 34 DAF. Enlargement of the small protein bodies near the Golgi apparatus occurred by several mechanisms: 1. fusion with one or more of the dense Golgi vesicles and/or with other protein bodies, 2. fusion with small electron-lucent Golgi-derived vesicles, 3. pinocytosis of a portion of the adjacent cytoplasm into the developing protein body, and 4. fusion of protein bodies with one another, mainly at later stages of grain development. Of the four mechanisms described, the pinocytotic vesicles and fusion of protein bodies were the most frequent and consistent processes observed. Direct connections between RER and protein bodies were not observed. The results confirmed a role for the Golgi apparatus in the initiation of protein bodies. Also the lack of RER derived vesicles suggested a soluble mode of secretion of storage proteins involved in the enlargement of protein bodies (Fig. 3).

Accumulation of protein during the middle and late stages of development was the object of a second study (Bechtel et al. 1982b), in which the deposition of protein into vacuoles in the starchy endosperm of hard red winter wheat was studied using transmission electron microscopy and enzymatic digestion of thin sections. Protein bodies that formed in the cytoplasm were transported to the central vacuole(s) were the protein body membrane and tonoplast fused and deposited the granule of protein into the vacuole. The protein granules in the vacuole enlarged by three mechanisms: 1. addition of membranous vesicular material of various types, 2. addition of flocculent material, and 3. fusion of the granules with other newly deposited protein granules. The fusion process occurred rapidly after 17 days after flowering and resulted in the conversion of the spherical protein granules into irregularly-shaped protein masses that eventually became the matrix protein. Enzymatic digestion of thin sections revealed that the contents of dense-cored Golgi vesicles and protein bodies were susceptible to protease VI and pepsin but not susceptible to α-amylase. The vacuolar protein granules were almost completely digested with protease VI and pepsin. The only undigested regions were peripheral densely-stained inclusions which were thought to be the last added protein.

The results indicated that protein matrix formation in mature wheat (and probably in other cereal endosperm proteins i.e. rye, triticale, and barley) may be indicative of potential breadmaking qualities. Fusion of protein granules with other newly deposited granules increased noticeably during the 14 to 17

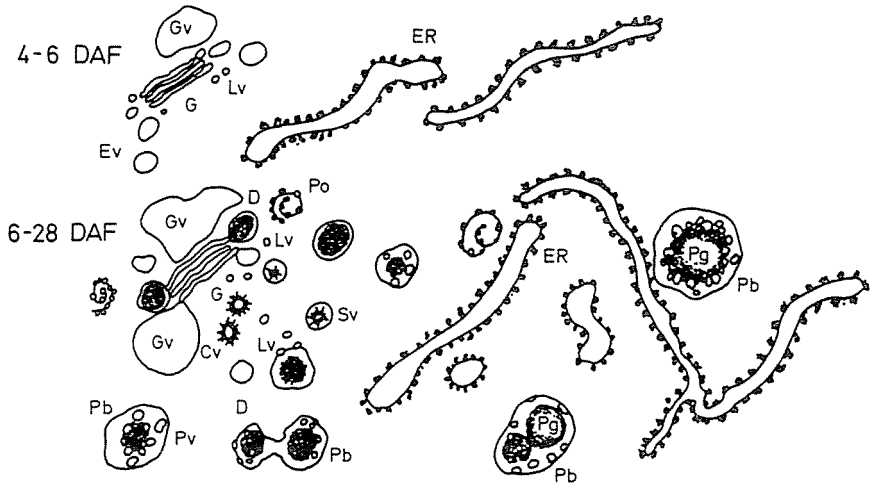


*Fig. 3.* Diagrammatic scheme of protein body initiation in wheat starchy endosperm. Before protein bodies were initiated (4–6 DAF), the Golgi apparatus (G) secreted electron-lucent vesicles (Ev), small lucent vesicles (Lv), and Golgi vacuoles (Gv). After protein bodies were initiated (6 DAF) and throughout protein body formation (6–28 DAF), the Golgi apparatus secreted vesicles with star-shaped inclusions (Gv) and dense cored vesicles (D) in addition to the vesicles secreted prior to protein body initiation. Rough ER was present throughout endosperm development. Polysomes (Po) associated with the Golgi apparatus and ER. Pinocytotic vesicles (Pv) were associated with protein bodies (Pb) frequently while lucent vesicles (Lv) and dense cored vesicles (D) were not. Protein bodies (Pb) with protein granules (Pg) often fused with one another to form large protein bodies (Bechtel et al., 1982b)

DAF period and peaked at the 21 to 28 DAF period. Fig. 4, 5. Finney (1954) found that wheats harvested 10 to 14 days preripe (about 20 to 28 DAF) had maximum loaf volume potentialities and superior physical flour properties than the same wheats harvested at other times, including harvest maturity. Fig. 6 shows the relationships among kernel dry weight, water content, timing of protein body initiation, and protein per kernel. Optimal loaf volume potential takes place during rapid fusion of protein granules. There seems to be indirect evidence that increased protein body fusion, up to a certain level, is concomitant with increase in loaf volume potential.

## Breakdown—germination

Some insight into the composition of wheat flour components can be gained from study of their breakdown during malting—germination—sprouting. While most of our data are from recent studies of barley, a brief mention of the results seems appropriate.

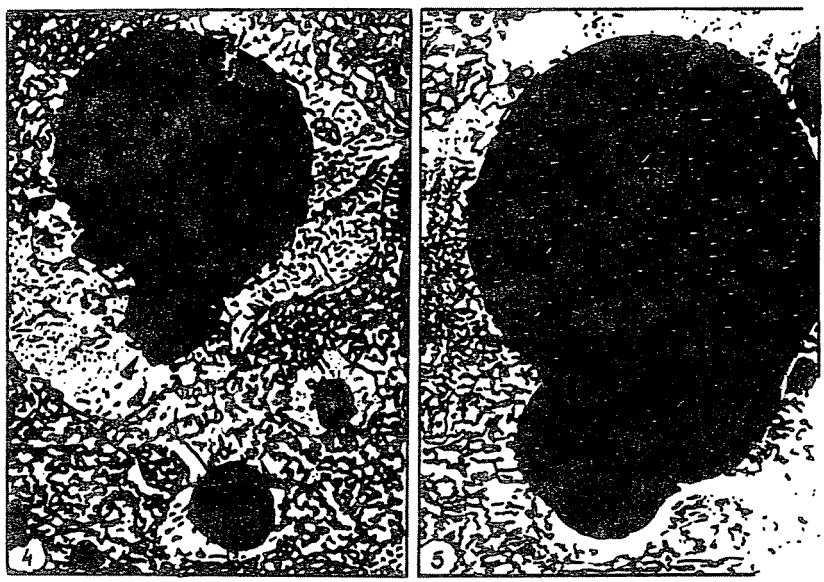


*Fig. 4.* Fusion of three 10 DAF protein granules (arrows) into a larger granule (X 22, 700) (Bechtel et al., 1982a)

*Fig. 5.* Large protein granules in vacuole of 10 DAF endosperm. Note dense line between fused granules (X 11,500) (Bechtel et al., 1982a)

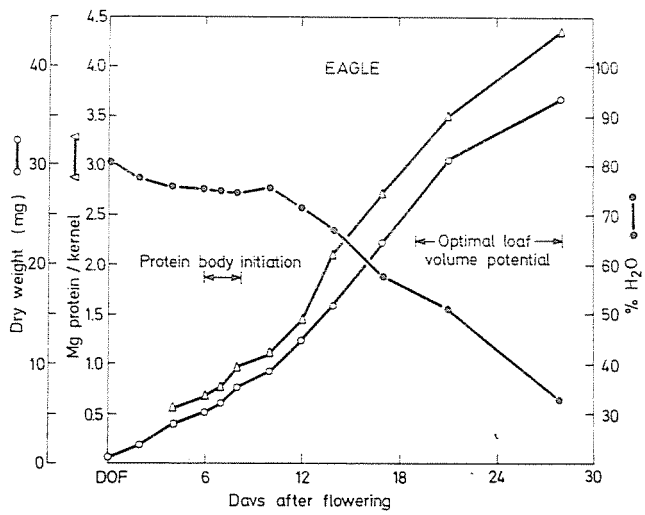


*Fig. 6.* Graph of dry weight, moisture content, and protein content of hard red winter wheat Eagle during caryopsis development (Bechtel et al., 1982b)

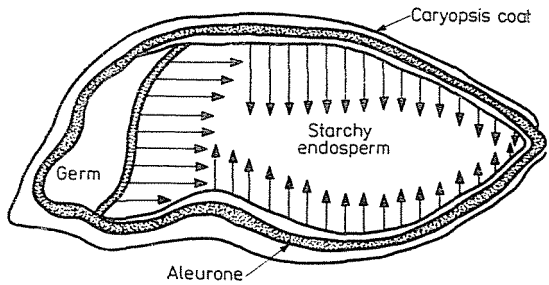


*Fig. 7.* Diagrammatic summary of hydrolytic modification of cell walls, protein, and starch in kilned barley (Fretzdorff et al. 1982a)

A study on classifying malt by sieving and air centrifuging—elutriating techniques indicated that particles from various parts of the barley kernel differed in protein content, diastatic power, and α-amylase activity (Pomeranz et al., 1976). More recently, modification in a kilned malt was studied by a combination of histochemistry, light microscopy, and transmission and scanning electron microscopy (Fretzdorff et al., 1982a). Hydrolysis of cell walls, proteins, and starch was most extensive in the starchy endosperm area adjacent to the scutellar epithelium. Some hydrolysis occurred in areas adjacent to the aleurone layer; hydrolysis decreased as the distance increased from the embryo end to the distal end and from the aleurone layer to the center of the starchy endosperm. While no rigid sequence of hydrolysis was observed, generally, cell-wall hydrolysis was more extensive than protein hydrolysis and starch hydrolysis seemed to take place gradually in the late stages of malting and kilning (Fig. 7). Finally, it is of interest to mention a study on distribution of alpha-amylase in field-sprouted wheats and its relation to end-use properties of wheat flours (Finney et al., 1981). In that study, wheat was taken apart by milling. Alpha-amylase, as percent of wheat amylase, increased in patent flour and decreased in bran, as alpha-amylase increased from 0 to 2 D.U. per gram of wheat.

## Statistics—direct measurements

I stressed previously the importance of statistical relationships in elucidating the role of wheat flour components in breadmaking. Some of the background information is based on direct demonstration of the role of certain wheat flour lipids. It has been suggested that lipids may affect baking in many ways (Pomeranz, 1971). During progressive stages in the baking process, the lipids may: 1) modify gluten structure at the mixing stage; 2) catalyze

oxidation of sulfhydryl groups; 3) catalyze polymerization of proteins through a process that involves lipid peroxidation; 4) act as lubricant; 5) improve gas retention by sealing gas cells; 6) prevent interaction between starch granules during gelatinization; 7) give some structural support to the gluten; 8) retard water transport from proteins to starch; 9) retard starch gelatinization; and 10) act as antistaling agent. It is possible that some of these effects (e.g. retardation of starch gelatinization and the antistaling effects) are due to the same mechanism. Breadmaking quality is a composite character (dough development and stability, loaf volume potential, etc.) resulting from the action and interaction of several factors. One cannot, therefore, expect a simple linear correlation between a single component and breadmaking quality. Either multiple correlations (with all the complexities) can be computed or partial correlations such as keeping one or more factors constant (i.e. effects of protein content in determining effects of protein quality) can be applied.

It is well established that, in petroleum-ether defatted flours, nonpolar lipids are detrimental and polar lipids, especially glycolipids, are effective improvers (for a recent review see Chung and Pomeranz, 1981.)

Recently, we completed studies designed to correlate lipid content and composition with genetic differences in breadmaking quality of wheats (Chung et al., 1982). Such correlations are well established for wheat proteins.

First, a preliminary study (Chung et al., 1980) established the conditions of lipid extraction that differentiate hard red winter (HRW) wheat flours that vary in breadmaking potential. The ratio of nonpolar lipids to polar lipids extracted with petroleum ether or Skellysolve B gave the best differentiation. This preliminary study was then extended to HRW wheats grown in the Great Plains of the United States and to their straight-grade flours (Chung et al., 1982). The study was confined to HRW wheats, because several studies implied that sound wheats of a certain class and unexposed to extremes in environment might best differentiate wheats according to breadmaking quality.

Lipids were extracted (with petroleum ether) from 21 samples of HRW wheats and 23 samples of experimentally milled straight-grade flours that varied in breadmaking potential. Wheat protein content varied from 11.5 to 15.7%, flour mixing time from 7/8 to 9 min, and LV per 100 g of flour from 523 to 1,053 cc. The total lipids from 10 g of flour (db) were fractionated into polar lipids (PL) and nonpolar lipids (NL); total lipids were analyzed colorimetrically for carbohydrates, mainly galactose (GAL). Significant linear correlations were found between LV and the following variables: PL content ($r = 0.877$ for wheat and 0.888 for flour), NL/PL ratio ($r = -0.902$ for wheat and $-0.907$ for flour), and lipid GAL ($r = 0.745$ for wheat and 0.905 for flour). PL, NL/PL ratio, and lipid GAL were curvilinearly related to mixing time requirement. The correlation coefficients of LV with PL, NL/PL ratio, and

lipid GAL generally were somewhat improved when LV and lipid contents were corrected to a constant protein basis.

In summary, we have found that several petroleum ether-extractable lipid fractions or their ratios are related to genetic differences in mixing requirement and LV potential. The NL/PL ratio and the amount of PL and lipid galactose of either wheat or flour were more highly correlated with LV than with mixing time. Significant correlations of LV and the lipid content, when both were corrected to a constant protein basis, indicated that PL are related largely to protein quality and to a limited extent to protein quantity. The petroleum ether-extractable PL, especially glycolipids, are a function of or are somehow involved in governing protein quality. Therefore, the determination of PL or lipid-galactose content in addition to a protein assay could be used to estimate LV and mixing time of mixing time of sound HRW wheat grown under similar conditions.

Some conclusions, based on the LV-lipid content relation seem of interest. About 180 mg of native PE-extractable PL per 100 g wheat or flour appeared to be required for satisfactory LV of about 875 cc at 12% protein. An increase of 10 mg of PL per 100 g flour was accompanied by an increase of 35 cc LV, which would require about 500 mg of good quality protein. Functionality of PL is, therefore, about 50 times more effective as a LV improver than good quality proteins. We have to recognize that flour proteins are structurally the backbone of dough whereas flour lipids primarily strengthen that structural backbone and bring out the best in its performance.

Recent findings from our laboratories provide another interesting information: in terms of LV and crumb grain, 9 to 12% shortening may be required to replace 0.2% polar free flour lipids (Chung et al., 1981). Thus, polar wheat flour lipids are about 50 times as effective as shortening lipids.

## Dough combinations

With all respect to the useful information that can be obtained from statistical computations, the most useful and reliable information has been obtained, thus far, from fractionation and reconstitution studies. Before describing some of our recent findings in this field, I would like to mention some studies on combinations of dough ingredients. They are based on microscopic investigations reported by Fretzdorff et al. (1982b).

Breadmaking involves complex, multiple interaction(s) of wheat flour components. Such interactions can be followed by physical, chemical, and microscopic methods. Microscopy is a particularly useful and powerful instrument for studying the ultrastructure and functional relationships of the interactions „in situ." Several workers, i.e. Evans et al. (1978), and Chabot et

al. (1979) have recently used transmission electron microscopy (TEM) and scanning electron microscopy (SEM) in flour, dough, and bread studies. Bechtel et al. (1978) concluded, on the basis of TEM studies, that protein strands provided a matrix network in a mixed dough and that in baked bread most of the starch was gelatinized into fibrous strands interwoven with thin protein struds. Fig. 8, Fig. 9.

Dehydration of freeze-drying while specimens are prepared for microscopic studies may produce artifacts or mask surface details. Moreover, exposure to buffers, fixatives, and dehydrating agents before drying may alter the protein matrix and liberate starch granules from the maxtrix. To examine the relationship between starch granule structure and baked goods structure, the components of the system should be, practically, undisturbed and the best


*Fig. 8.* Top center of loaf immediately after baking. Note gelatinized starch (f) between thin protein strands. Note lack of vacuoles in protein (p). Gas vacuoles (g) (Bechtel et al., 1978)

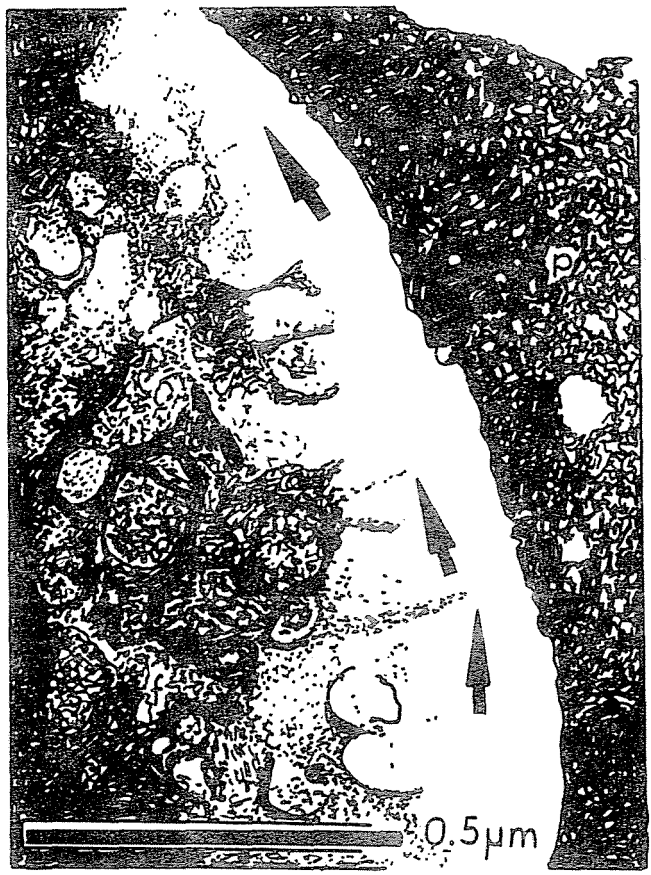


*Fig. 9.* Highly magnified protein (p)—gelatinized starch (f) interface. Note fine connections between starch and protein (arrows) (Bechtel et al., 1978)

treatment is no treatment (Chabot, 1979). For those reasons and because water, next to starch, is the main quantitative ingredient of dough and bread, it is desirable to study dough and bread with minimal, or preferably without, chemical fixation and dehydration. The freeze-fracture technique, therefore, is a promising method to investigate water distribution in dough and bread.

The structures of isolated flour components of mixed doughs (containing several combinations of ingredients), of fermented doughs, and of bread crumb were examined by Fretzdorff et al. (1982b) by the freeze-fracture technique. While the shapes of the small and large starch granules were unaltered in doughs, the gluten and water-soluble structures appeared completely different in the complex-dough system. In general, water was distributed in three forms: 1) coating around starch granules and yeast cells, 2)

droplets, and 3) large areas; all of which changed with protein development. Protein development was followed from a protein network in a flour-water dough to a sheet-like protein in a complete dough (containing flour, water, yeast, sugar, salt, shortening, malt, and oxidant). Both compositional and physical (dough development) effects were indicated. A transition stage between the two structures appeared after sugar was added. Fermenting a flour-water-yeast-salt dough did not affect the protein network structure, but fermenting a complete dough altered the sheet-like protein to a fine network. In bread, regular dense-structured sheets were observed. Fig. 10. In most


*Fig. 10.* Fermented flour—water—yeast—salt—dough showing extensive starch (S)—protein (P) interaction (arrows) (Fretzdorff et al., 1982b)

doughs protein-starch interaction was clearly visible; thin "pearl chains" or thin protein strands connected starch and protein. Those interactions intensified after fermentation. In bread crumb, protein and starch were tightly connected. It would be of interest to determine whether the observed structures are related to the existence (or co-existence) of fibrilar and sheet-like protein networks in bread doughs (for a review, see Miflin et al., 1981).

## Fractionation and reconstitution

This brings me to the last, and as I repeatedly said most powerful, method of taking wheat or wheat flour apart and putting it together as well as demonstrating what is the function of each of the separated fractions in breadmaking. This part is based on some recent studies of Finney and coworkers in our laboratories.

Finney et al. (1982) and Jones et al. (1982) fractionated and characterized gluten proteins and studied their functional properties in breadmaking. The fractionation scheme is described in Fig 11. Gluten proteins from hard winter wheat flour of good (RBS–76) and poor (76–412) breadmaking quality were solubilized in dilute lactic acid and separated by ultracentrifugation into four protein fractions (Jones et al., 1982). The high-molecular-weight glutenin proteins sedimented at 100,000 G as a dense, relatively insoluble pellet (35 min for 76–412 flour; 2 hr for RBS–76 flour). The low-molecular-weight glutenin proteins sedimented at 435,000 G as a gel (6 hr for 76–412 flour; about 10 hr for RBS–76 flour). The high-molecular-weight gliadin proteins also sedimented at 435,000 G as a clear viscous solution (6 hr for 76–412 flour; about 10 hr for RBS–76 flour). The low-molecular-weight gliadins of both flours remained in the corresponding supernatants. Removing total free lipids from flour before washing out and solubilizing the gluten materially increased the sedimentation

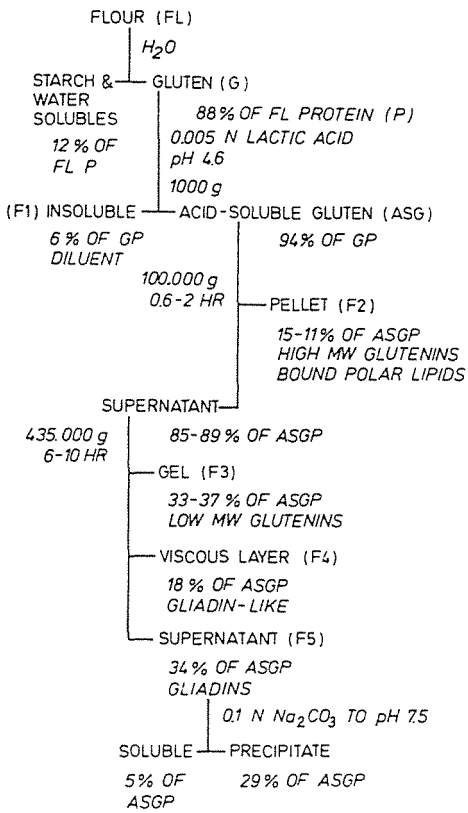


*Fig. 11.* Scheme to fractionate wheat flour into crude gluten protein and starch plus water solubles and to fractionate the acid soluble gluten into two glutenin and two gliadin fractions (Finney et al., 1982)

rate of the high-molecular-weight glutenin (pellet) proteins of the poor quality bread flour 76–412.

The corresponding gel and viscous layer plus supernatant fractions of the good and poor quality flours were interchanged singly in reconstitued flours containing the starch plus water-soluble fraction and baked into bread (Finney et al., 1982). The gel glutenin proteins of the acid-soluble gluten proteins controlled mixing requirement and baking absorption, and the viscous layer and supernatant gliadin proteins controlled loaf volume and crumb grain. It was suggested that the relative ease with which the high-molecular weight pellet glutenins sedimented already after 35 to 120 min at only 100,000 G, indicates that they are relatively free compared to the low-molecular-weight gel glutenins that required an additional 6–10 hr at the high centrifugal force of 435,000 G to sediment. The gel glutenins appeared to interact tenaciously with the gliadin proteins. The extent to which the bound polar lipids interacted with the pellet proteins, both intra- and inter-molecularly to produce very high molecular weight aggregates, may render them as relatively non-interactive with the gel glutenin and gliadin proteins, so that the pellet glutenin proteins are relatively free to sediment at relatively low centrifugal forces (Finney et al., 1982).

The ease of sedimenting the pellet glutenins indicates that they are not involved in formation of additional lipoprotein complexes and that the free lipids become bound probably by interacting with reactive gel glutenin and free gliadin proteins. Similarly when the dough is formed, reactive gel glutenins probably interact with reactive gliadins. Thereby, the gel glutenin proteins become bound. Fig. 12. When the centrifugation forces are greater than the protein interaction forces but less than the molecular forces that keep the relatively small gliadin proteins in solution, then the gel glutenins sediment.

It was postulated (Finney et al., 1982) that the tenacity with which gliadin proteins interact with free lipids and glutenin proteins may be the physical-chemical criterion of why poor quality bread wheats are poor and good ones are good.

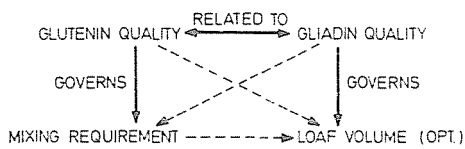


*Fig. 12.* Diagram of likely direct and indirect relationships between glutenin quality, gliadin quality, and the functional properties mixing requirement and loaf volume (optimum). Indirect relationships are indicated by broken lines. Wide lines indicate strong relationships. Also, not shown, glutenin quality governs mixing tolerance and dough absorption (attributable to flour proteins). Oxidation requirement and dough stability are strongly related indirectly to mixing requirement (Finney et al., 1982)

## The end of the line?

Is this then the end of the line of our understanding the role of wheat flour components in breadmaking? I certainly do not believe so. Many questions are unanswered. And while we have no answers to those questions, we can suggest new methods of attacking them so that we can come up with the answers. Every single answer (finding) that I have discussed has raised several questions. This is of course what research should do. Some questions about putting storage proteins together during wheat development and maturation were posed by Spencer and Higgins (1979). They concern 1) the changes in site of protein synthesis as the wheat develops or as different proteins are laid down, 2) relationships between proteins deposited in vacuoles and in protein bodies, 3) transportation of proteins to storage organelles, 4) site of final protein body assembly, 5) identity of transcriptional and translational control factors as they relate to synthesis of specific storage proteins, and 6) identity of various types of protein bodies, their relation to end-use properties, factors that govern-control their synthesis, and potential modification of synthetic patterns or of synthesized proteins.

If we are to study molecular aspects of storage proteins, their synthesis, deposition, utilization, etc., it is essential that the individual storage proteins be well defined and characterized. Even definition of the proteins has proved difficult to do and has been subject of much controversy. Much of the early work on storage proteins was directed toward their classification into a few distinct groups. The picture that has emerged, as a result of applying new sophisticated analytical techniques, is increasing complexity and micro-heterogeneity. Unfortunately, new knowledge has been gained at the expense of comprehension of what that knowledge implies. While the new knowledge forces us to discontinue our previous simplistic views, we hope that it will eventually provide us with a consistent and comprehensible (if not simple) law and order. It is hard to visualize that haphazard deposition of heterogenous components is consistent with the way protein synthesis is controlled and with the way proteins in mature grain are made available to fulfill their biological function as reserve substances for the germinating and developing seed.

Why are we "blessed" with such an abundance of gliadin and glutenin proteins? Why does the plant need them even though they, apparently, have no specific physiological function, as enzymes? If they are not uniquely needed, how did they originate and why have they survived after thousands of years? Is it only because of the polyploidy nature of chromosomes in hexaploid wheats? In barley and rye, all the prolamines are coded by genes on one chromosome; in hexaploid wheat, however, the structural genes for prolamines are on at least two chromosomes, of each of the three genomes (Mifflin et al., 1981).

According to Wall (1979), numerous studies that involved amino acid composition, molecular weight peptide maps, and N-terminal sequences have shown that present gliadin nomenclature is inadequate to describe true relationships and that a more suitable system must be developed. Mifflin et al. (1981) suggested that classification should be based on structural loci in the genome and their chemical structure, as determined by the base sequence of the genes. Based on those considerations, Mifflin et al. (1981) suggested to classify prolamines into high-molecular-weight (glycine-rich), S-poor, and S-rich fractions. These three fractions differ in physical and chemical properties and in their ability to form aggregates. They postulated that differences in relative amounts of these prolamines account for different processing properties of wheat, rye, and barley.

In light of the postulates of Mifflin et al. (1981), how much significance should be attached to the role of the glutenin: gliadin ratio in breadmaking (Lee, 1975)? The effects of gliadin and glutenin are not additive. Their effects are the result of interaction. One of the interesting pieces of evidence on their interaction stems from the fact that one cannot mix a dough to optimum consistency if all gliadin is replaced by glutenin. Similarly, how are those findings related to proposed structures of gluten proteins: aggregating linear molecules vs. linear chains crosslinked by disulfide bonds that govern resistance of dough to elastic deformation? How do these structures relate, in turn, to various degrees of disulfide bond reactivity? And, finally, how is the picture modified—complicated by the possibility that some, or even most, storage proteins are actually glycoproteins?

We have gained considerable information on wheat flour lipids. Our knowledge involvement in the shortening response is meagre, to say the least. With the recent increase in use of liquid oils in combination with surfactants, to replace shortening, the question still remains why can shortening do it alone; yet, oils require surfactants.

Some surfactants can be effective improvers; they do so, however, under certain conditions only. Only flour polar lipids can contribute to the production of an acceptable loaf of bread—with or without shortening, in untreated and defatted flour, in the production of regular white bread and whole wheat or dark bread, and in bread that is based on products of wheat milling only or in bread that is nutritionally enriched by the addition of soy flour or other protein-rich supplements. What is so unique about wheat flour polar lipids?

For years, we had the simplistic view that gluten proteins are responsible for dough mixing properties and oven spring and starch for changes in the later stages in the oven and in subsequent shelf life. There is much new information to indicate that this is an oversimplification. Starch interacts in the dough and in oven spring and protein contributes to freshness retention. What then is

there in the starch that is responsible for the contribution? If there are varietal differences in starch contributions, what are they? Neither differences in starch granule size nor in gelatinization temperature explain completely meaningful differences among breadmaking wheats.

Lipids are an important factor in breadmaking. Yet, there is a practical limit to the extent to which they can contribute and we are woefully limited in our capacity to convert poor breadmaking wheats into good breadmaking wheats. Lipids must have a partner for best results. That partner are the gluten proteins and the secret of effectiveness in governing functional properties of polar lipids in breadmaking is interaction with proteins. What is the precise mechanism of interaction and precisely which proteins are incolved?

How important are the other components: minerals, soluble and insoluble pentosans, etc.? We generally restrict ourselves to interactions of binary systems: lipids with proteins, lipids with starch, proteins with starch. We do so for the sake of simplicity and ease of conceptualizing the interactions. Are there important multisystem interactions? Similarly, we limit ourselves to stationary interactions. Bread production involves a series of dynamic changes and interactions of constantly modified partners. How do we get a handle on following those changes?

I have emphasized, thus far, some specific and often limited areas. It goes without saying that we need a basic knowledge of wheat components—of their characteristics, of their synthesis and deposition, and of the control mechanisms that operate at the genetic, biochemical, and physiological levels. This knowledge will help define the limits within which the components can be modified (quantitatively and qualitatively), and will help establish selection criteria for plant breeding programs that relate to grain improvement (Spencer and Higgins, 1979). This knowledge will also determine the direction and scope of future developments and the possibility of modifying wheat flour components to assure their optimized utilization by new processes and technologies.

Which brings me to the final questions. No single wheat flour component can make it alone in breadmaking. Admittedly, some are more powerful and more important than others. Gluten proteins certainly belong in this category. Similarly, some approaches and techniques are more powerful than other in elucidating the role of wheat flour components. Fractionation and reconstitution certainly belong in this category. Yet, gluten alone cannot make an acceptable bread and concentrating our research efforts on studying gluten proteins will not answer all the questions. We must study all the components and all reasonable interactions. This is a tall task. Judicious selections of important components and interactions and luck in making those selections will be the prerequisite for progress. Similarly, fractionation and reconstitution alone, as an approach, will not answer all the questions. We must

conduct a series of interrelated studies that include dough fractionation and use some of the newer tools that are becoming available for biological investigations. Those tools include poverful separation methods (various centrifugal analyzer methods, gradient density ultracentrifugation, use of novel membranes, high pressure liquid chromatography, affinity chromatography); spectroscopy (photoacoustic, NMR fine resolution and imaging, NIR reflectance and transmission), fluorescence probes and polarization; use of new chromogenic and fluorescence probes; chemical and enzymic modifications; immunochemistry, and combinations of microscopic techniques.

Much of the new knowledge is predicated on interdisciplinary research and on designing the research in such a manner that it covers the whole range; from nonconventional, bold new ideas to practical demonstrations with full interaction between theoretical and applied researchers. I am confindent that this combination will teach us much how to take wheat apart and how to put it best together so that maximum yields of acceptable raw materials for the best loaf of bread are assured.

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