Exploring and Improving the Industrial Use of Wheats

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Exploring and improving the industrial use of wheat (*Triticum aestivum*) produced in the European Union (EU) is of utmost importance and has been the topic of a major project supported by the Commission of the European Communities in the frame of the European Collaborative Linkage of Agriculture and Industry through Research (ECLAIR) program (1991–1995). This project was aimed at filling the growing gap between process development and an understanding of processing requirements and thus wheat quality requirements. A further objective was to develop new types of wheats capable of satisfying the future demands of the European industries and the export market (1).

The rationale behind the objectives was manifold. One of the main problems limiting the use of EU wheats is the lack of knowledge on processing requirements for specific uses, especially regarding recent developments of gluten/starch separation, wholemeal breadmaking, biscuit manufacture, flour blends, sour doughs, and sweet bakery products, etc. Also, the milling and baking industries require a higher quality of wheat because of modern developments in technology (e.g., frozen dough). Third, current methods of breeding are predominantly focused on white breadmaking. Finally, the quality of most wheat is not consistent because of too great a sensitivity to agronomic and climatic factors. In Southern Europe, the climate is often the factor limiting both yield and quality, whereas in the coastal regions of Northern Europe, where the crop can be cultivated intensively, sprouting puts a severe strain on both yield and quality.

Industrial use is likely to improve with a better knowledge of the various applications of wheat, processing parameters, and functional properties of wheat related to specific wheat protein constituents and their interactions. Quality determinants were evaluated to obtain a better understanding of the variability of composition, structure, and of their mechanism of action in the various industrial processes. Finally, the identification of improved breeding criteria (for specific applications or traits) and the development of rapid tests for use in breeding programs and trade could be obtained because of the availability of genetic stocks and wheat samples produced in highly controlled environments (Fig. 1).

Apart from purely scientific and technical aspects, a particularly innovative element of this project was the establishment of a multidisciplinary program bringing together physical chemists, biochemists, immunochemists, rheologists, and geneticists representing the broad range of the industries.

In addition, the main approaches were based on several recent advances that showed promise in terms of both more effective utilization and development of better European wheat varieties for the future. They included the following:

- The availability of isogenic, aneuploid and translocation stocks, which enable the pinpointing of gene products that are important in functional performance.
- The introduction of original approaches based on new concepts (e.g., intrinsic quality of wheat genotypes) or more recently characterized protein fractions (e.g., friabilin, LMW subunits of glutenin, HMW-albumin, S-protein).
- The acknowledgment that quality is not determined (and cannot be predicted) solely by protein composition but also by interaction of the proteins with various flour components such as starch, pentosans, and lipids.
- The development of modern physical and spectroscopic methods that can observe the behavior of individual components in a complex mixture.
- The demonstration of the potential of monoclonal antibodies to quantify specific components in a mixture and to probe their dynamics and distribution within various systems.
- The development of a range of physicochemical techniques that determine interfacial and aggregation behavior.

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Milling Quality

In contrast with the considerable effort devoted to improving wheats in terms of breadmaking quality, milling quality has received only minor attention. Although the physicochemical basis of milling quality is still poorly understood, milling quality is of great economical importance. Based on the amount of wheat produced annually in the EU, a 1% increase in milling yield represents 40 million ECU per year for the EU millers.

Accordingly, the milling quality project was aimed at developing new ways of understanding and predicting milling yield and identifying the nature and relative importance of factors determining milling quality (e.g., endosperm hardness, bran friability, endosperm ash content, etc.); investigating milling quality by morphological and chemical determinations; and producing a predictive (breeding) test for milling quality.

The first investigations by FMBRA on European sample sets used image analysis to examine morphometric parameters of the kernel. However, image analysis did not prove to have a good predictive value except for samples containing seriously shriveled grains (2–7). Thus, in general, endosperm content is not a factor that limits flour yield, and the belief that a positive correlation exists between grain size and endosperm content is certainly unjustified. When milling quality was described in terms of milling factors (bran friability, endosperm content, pericarp/endosperm separability), a comprehensive model was developed that better described the relative influence of both chemical and morphometric parameters on milling quality. For instance, ferulic acid appeared a far better marker for bran friability than did ash, so that bran friability could be calculated from the difference in ferulic acid content of pure endosperm and flour fractions (Fig. 2) (8).

Another important discovery that has drawn considerable interest from millers and milling scientists was the possibility of explaining 70–80% of the variation in milling quality by the potassium content of the kernel (that allows very good prediction of ash content of the flour and hence flour yield), bran friability, and kernel width.

On the other hand, an important breakthrough was made in the understanding of endosperm texture or hardness after the recent investigations of the proteins associated with the starch granules (friabilin, puroindoline) and their status as lipid-binding proteins (see below).

Starch/gluten Separation

To better control the process of starch/gluten separation, to investigate the causes for differences in quality of gluten extracted from wholemeal flour compared to gluten prepared from white flour, and to study the effect of processing aids (e.g., hemicellulases), a laboratory-scale decanter centrifuge (Fig. 3) was constructed by TUB (Berlin).

The integration of this decanter into the lab-scale separation system reduced residence time of gluten in the system, which affects gluten properties and allows gluten and starch to be separated from a range of raw materials including wholemeal flours (9). Glutens from wholemeal contain more low-molecular-weight (LMW) and less high-molecular-weight (HMW) subunits of glutenin than do glutens from white flours. It was clearly demonstrated that pentosans and hemicelluloses in flours have a strong effect on gluten yield and that flour processing properties are strongly determined by the way flour milling fractions are blended. This information is of great practical value for millers producing flour for the starch industry. Researchers at TNO showed that addition of 2% hemicellulose to flour decreased the gluten yield by 20%. This could be corrected by the addition of the enzyme hemicellulase. Also, hemicellulase addition to white flour increased gluten yield.

Differences in elastic behavior cannot be attributed to proteolytic activities, but the low pH of the process water is partly responsible.

Basis of Breadmaking Quality

This task was aimed at determining the underlying physicochemical basis for differences in gluten strength and breadmaking quality and thus providing feedback to plant breeding and grain trading programs.

Much of the effort was directed at determining and understanding the mixing requirements of UK-crown wheat varieties. Based on a test bake developed at CCFRA (Chorleywood), the work-input requirement ranged from 5 Whr/kg (18 kJ/kg) to 20 Whr/kg (73 kJ/kg) (Fig. 4). Samples with work-input requirements greater than 11 Whr/kg (40 kJ/kg) may not achieve their full potential in a breadmaking process based on a fixed energy input during mixing such as the Chorleywood Bread Process (CBP). These high work-input varieties were shown to be suitable for blending with weaker varieties, for exam-

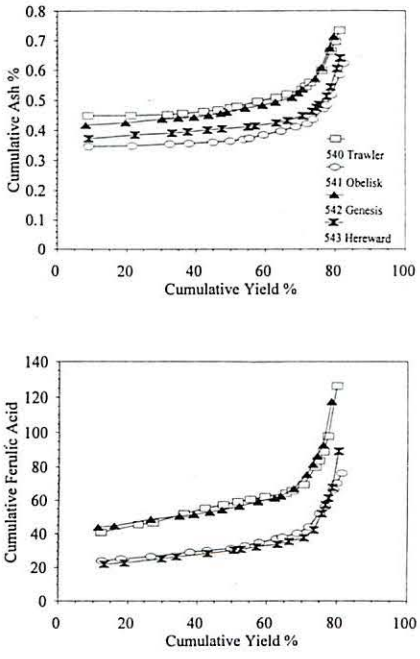


Fig. 2. Effect of ash and ferulic acid on bran friability (courtesy of TNO Biochemistry and Nutrition, The Netherlands).

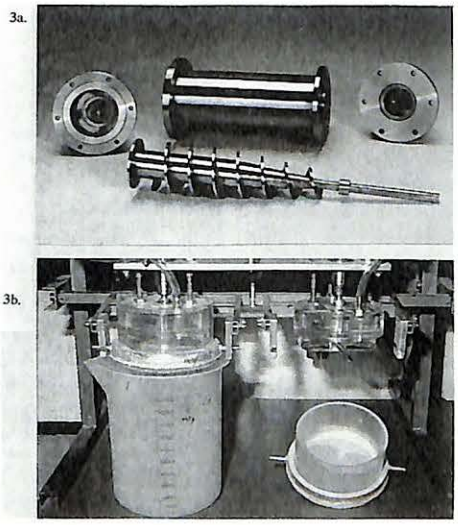


Fig. 3. Laboratory-scale system for the production of starch and gluten according to the new wheat starch process. **A,** Miniaturized bowl and screw of decanter centrifuge. **B,** Laboratory system for the extraction of B-starch from gluten (courtesy of Technical University of Berlin, Germany).

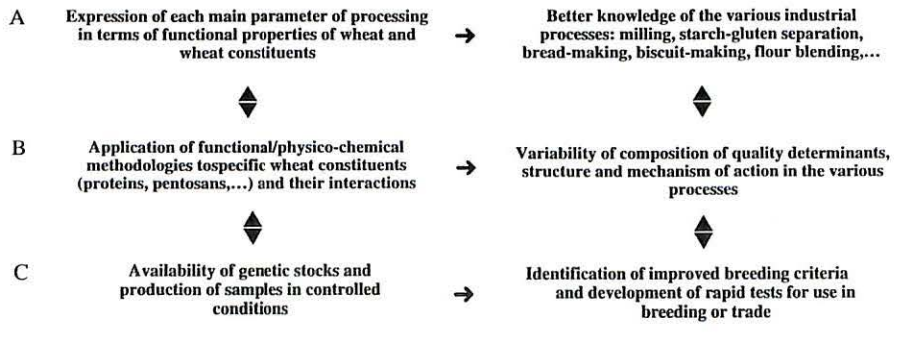


Fig. 1. Exploring and improving the industrial use of EU wheats.

ple a 50:50 blend of Fresco (extra strong) with Riband (weak) resulted in a quality similar to that of Avalon, a popular breadmaking variety during the 1980s.

The concept of glutenin macropolymer (GMP), defined by researchers at TNO, determines baking quality in CBP (UK) or RMT (German). (GMP changes from a linear polymer in flour to a three-dimensional structure in dough.) The breakdown rate of GMP during mixing depends on the baking strength and was shown to be related to the composition and incorporation rate of HMW subunits of glutenin (10,11).

In other experiments, a new impetus was given to the "gel protein" fraction as a tool in the prediction of baking quality. The elastic modulus or the breakdown rate of gel protein during mixing, rather than the amount of gel, proved to be useful for testing baking quality (12–14).

Loaf volumes of breads from wholemeal could not be predicted from those of white flour without making allowance for quality attributes such as hard or soft milling or extra-strong character. In general, protein content was more important than was gluten strength for wholemeal bread performance. However, the wholemeal loaf volume of a test sample could be predicted from measurements of the baking performance of the endosperm and bran/offal components relative to those of a control sample of Mercia ($r = 0.77$). This study suggested that both the endosperm and bran/offal control the baking performance of wholemeal flours.

In French or South-European baking procedures, dough extensibility was often found to be a more important and critical parameter. Dough extensibility was shown to be more associated with allelic variation of LMW and perhaps of gliadins than that of HMW subunits (see below).

Flour Blends

This project was aimed at predicting and improving the processing properties of flour blends. During mixing (15–19), the amount of GMP decreased, whereas during resting, the amount increased again. The decrease in GMP could be predicted by an exponential decrease using a flour variable (GMP content of flour) and a process variable. The relationship could explain 86% of the variation in the GMP content of dough. The increase in GMP could be described by a function of the amount of macropolymer in flour and the resting time. With a similar function, the quantity of the individual glutenin subunits in the polymer could be described (91% of the variation explained). These findings indicate that it is not so much the quality of the protein that determines the reassembly of the protein during resting, but rather the quantity of glutenin polymers. The large amount of variation that can be explained indicates that dough properties (GMP content of dough) can be predicted on the basis of a flour parameter (GMP content of flour) and a processing parameter (resting time). This represents a considerable advantage to the milling industry. Blending

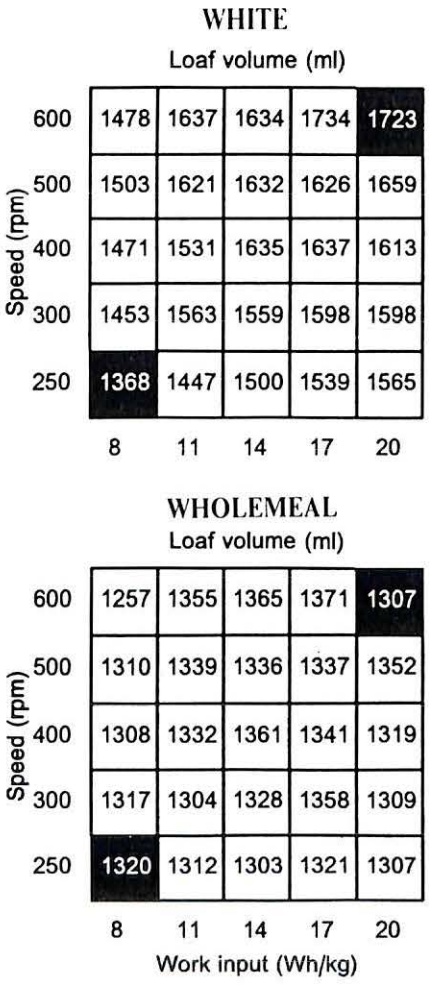


Fig. 4. Test bake matrix: work input/mixing speed/loaf volume for the extra strong variety Fresco (courtesy of Campden and Chorleywood Food Research Association, UK).

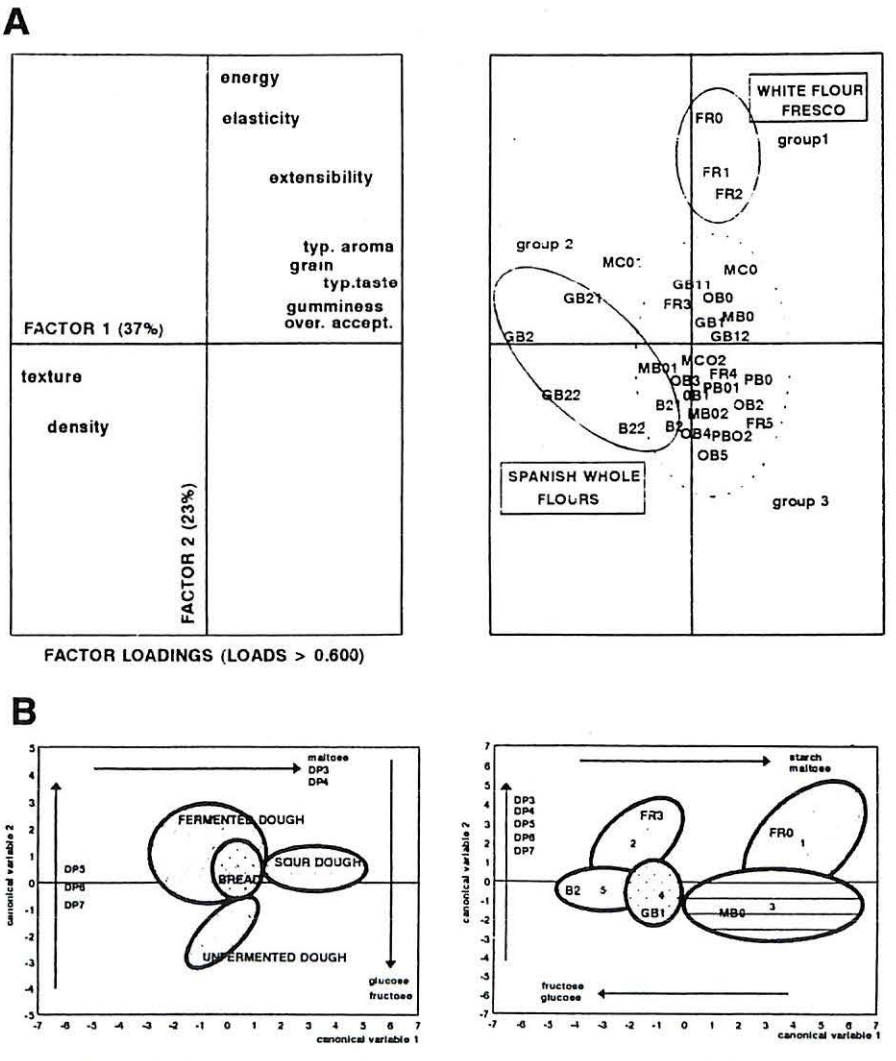


Fig. 5. A, Application of factor analysis to functional characteristics of flours inoculated with frozen lactobacilli using the sourdough process (left, variables and factor loading corresponding to the two first factors; right: distribution of samples in the plane defined by these two factors). **B,** Application of stepwise discriminant analysis to the study of effect of breadmaking step (sourdough process) and type of flour on total starch, sugars and low molecular weight dextrins (courtesy of IATA, Valencia, Spain).

of wheats into a grist still is an empirical process, relying heavily on the expertise of the miller. This makes it more difficult to achieve an optimal balance between costs and quality. These results indicate ways to increase cost effectiveness for the miller and provide clear directives for wheat breeders, growers, and traders.

In contrast, some LWM proteins that were isolated, characterized, and compared with other low M_r proteins (20–22) were shown to have effects on dough properties by specifically preventing reassembly of GMP.

Sweet Bakery Products

SME-Ricerche focused on the relationship between flour properties and the quality of sweet bakery products and rheological characterization of flour samples.

A small-scale baking test was first developed giving results in good agreement between the volume of the sample and the number of the classification method and between the volume of the sample and the protein content of the flour. A correlation was found between water absorption and volume after baking, particularly for high water absorption flours.

Dynamic rheological measurements carried out with a Bohlin rheometer characterized the region of linear viscoelasticity of flour slurries (40%). The rheological characterization was carried out in terms of relaxation spectrum H (l) according to a viscoelastic analysis. As the frequencies range was limited, G_e not only represented the elastic (permanent) network but also the viscoelastic (temporary) network. Used to discriminate flours, this new G_e modulus, G_e^*, indicated the quantity of structural units that are formed and the kinetics of the process.

The results obtained indicate that flours have different values of the level of structure formation and different values of the amplitude of deformation at which rupture of the system begins. For instance, a deformation at the strain of 2.5% is very high and all the flours show structural breakdown. The dependence of G_e^* slope from the angular velocity shows that flours have different behavior of structural breakdown.

The rheological characterization together with the standardized baking test allow a better definition of wheat flours suitable for the production of sweet bakery products. The added advantage of the rheological method developed is that it requires little sample and only a very simple sample preparation.

Microorganism and Flour Component Interactions

This task included three experimental approaches: changes in functional characteristics, biochemical changes in flour components and fermentation metabolites, and specific enzyme activities.

The results indicated that flour extraction rate was the main factor influencing breadmaking performance and biochemical patterns (23–25). However, there was not a single parameter defining bread quality, but physical, sensory, chemical, and biochemical properties interacted. This indicated that sophisticated techniques of data analysis were necessary (26,27) (Fig. 5). Microorganisms add further differences to those observed when using different flours, but their overall effects are smaller than the effect of flour variation. Overall, the strain of microorganism used is of lesser importance than is flour type or method of addition (sourdough or straight process) (28).

Dough fermentation results in differences in components and metabolites when compared with unfermented doughs, but the differences are less than those created during the sourdough step. The changes in flour components and fermentation metabolites are influenced by fermentation time. Baking does not add further differences to the fermentation step, with respect to starch degradation, nitrogen, and lipidic compounds (29). However, LMW dextrins seem to play a role in sourdough processes (30–32). Sourdough breads contain higher amounts of these dextrins than do control or straight processed breads, suggesting the possible role of sour dough addition in

N-terminal sequence of GSP and puroindoline-b

EVGGGGGSQEPPQERKLN (GSP)
EVGGGGGSQQCPQERKLS (puroindoline-b)

Partial sequences of major peptides isolated from lysyl-endoproteinase digests of GSP and their homology with puroindolines

VIQEAK (GSP)
VIQEAK (puroindoline-a)

GGEEHEV (GSP)
GGCEHEV (puroindoline-b)

DYVXE (GSP)
DYVME (puroindoline-b)

NFPV (GSP)
DFPV (puroindoline-a and -b)

QLQRAQS (GSP)
QLQRAQS (puroindoline-b)

EVGGGGGSQEP (GSP)
EVGGGGGSQQC (puroindoline-b)

unknown peptides A(L)AFP; ARTVQTA; SYVYEQ

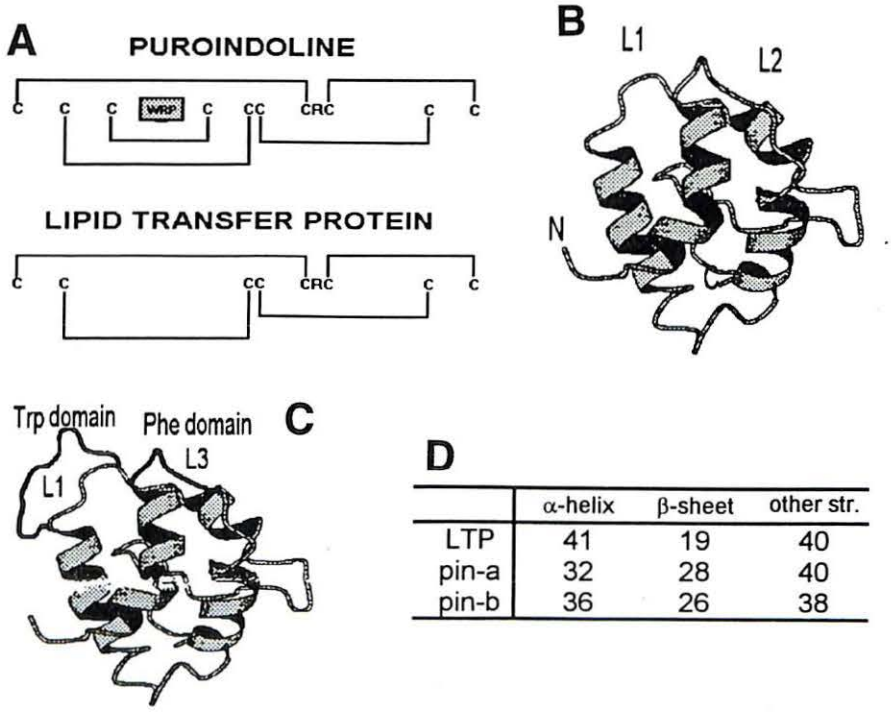
Partial sequences of chymotrypsin digests

RGQVFL (GSP)
RGEVFK (puroindoline-b)

LGIR (GSP)
LGIWR (puroindoline-b)

unknown peptide: SQIAPQ

Fig. 6. Sequence homologies between peptides provided by endoproteinase digests from GSP-friabilin and peptide sequences found in puroindoline-*a* and -*b* (courtesy of INRA-Nantes, France).



	α-helix	β-sheet	other str.
LTP	41	19	40
pin-a	32	28	40
pin-b	36	26	38

Fig. 7. Structure of wheat lipid transfer protein (LTP) and puroindolines. **A,** Disulfide bond pattern of puroindolines and lipid transfer proteins. **B,** Structure of wheat LTP from multidimensional ^{1}H NMR data. **C,** Three-dimensional model of puroindolines deduced from the 3D structure of wheat LTP. **D,** Secondary structure of LTP and puroindolines determined by Fourier transform infrared spectroscopy (courtesy of Marion, INRA-Nantes, France).

improving the keeping characteristics of breads (33).

Flour is the main quantitative contributor to proteolytic and amylolytic activities in flour lactobacilli mixtures. Increasing the ash content of flour leads to a higher enzymatic activity. Lactobacilli qualitatively influence the degradation of nitrogen and carbon compounds during fermentation. Effects are strain and species dependent. Metabolic and enzymatic reactions cause changes in those components. Significant regression equations were described for soluble nitrogen fractions, maltose, and dextrins. Monosaccharides undergo the most complex changes due to their active participation in metabolism.

This multiparameter study provided a sound basis for the development of an expert system for Spanish type sourdough bread production. Such a system would be of great value for flour millers and product developers.

Basis of Sweet Biscuit Quality

To increase productivity and quality and to create new products by optimizing ingredients and understanding the process was the aim of this task. The main results obtained by BSN (Athis-Mons) and INRA (Montpellier) include a better understanding of the rheological behavior of biscuit dough, which is viscoelastic at low strains and similar to a gel at higher strains.

An essential quality factor in biscuit-making is the uniformity of biscuit size. Weight, thickness, and density of biscuit are related to constituents absorbing water—proteins and damaged starch and pentosans. For instance, insoluble pentosans limit the hydration of other constituents and have a very negative effect on the thickness of biscuit.

On the other hand, insoluble or aggregative glutenins such as the glutenin macropolymer impart elasticity, which may contribute to biscuit shrinkage. Biscuit-type wheats should be selected on the basis of HMW composition such as 2-7-12 or LMW allelic types *o* or *m*, or simply on the basis of the gliadin-glutenin ratio (34).

Minor Protein Components Associated with Starch Granules

Although previous studies by Schofield and Greenwell (CCFRA, Chorleywood) showed an association of the 15K surface protein and friable endosperm, the role of starch granule protein in relation to functional properties of wheat and the relation of this protein to the hard and soft alleles of the hardness gene had still to be established.

In fact, CCFRA showed that anti-friabilin F7F antibody could not provide a predictive test on the endosperm texture in bread wheat but could be used in testing durum wheat purity (Durotest).

Using capillary electrophoresis, NEPHGE, N-terminal sequences, and im-

munoblotting, the basic friabilin components have been compared with the lipoproteins extracted by the detergent Triton X-114 by Marion at INRA-Nantes (35,36) (Fig. 6).

The results show the biochemical nature of friabilin and its location (37) and clarify the status of friabilin as lipid-binding proteins (38). For instance, a strong homology was demonstrated between some starch granule proteins (friabilin basic 2-3) and puroindoline *b*, the main lipid-binding protein in the tryptophan-rich domain (35,36).

Although friabilins are involved in endosperm texture, a rapid, sensitive diagnostic test has not been developed for this important quality parameter of bread wheat (39). Presence of friabilin on starch might occur during starch purification, but further work is needed to explain friabilin-starch interaction at the molecular level. A lipid-like factor may be involved in binding friabilin to starch on the surface of the granules (36).

Lipid-protein Interactions

In a study aimed at describing the mechanisms that play a role in the interaction between lipids and other components, lipid-binding proteins that recovered in the deterrent-rich phase after phase partitioning using Triton X114 have been extensively characterized by researchers at INRA-Nantes (35).

After its discovery, the following properties of puroindolin protein were found: mainly composed of helices at pH 4, strongly interacts with anionic phospholipids, and is stabilized by five disulfide bridges (40–42) (Fig. 7). Its structural flexibility controls the lipid binding specificity. Good foaming properties were also found for puroindoline, enhanced by the presence of lysoPC that forms a highly stable lipoprotein film at the air-water interfaces (43–45). Such a mechanism is probably important during the gas phase expansion of proof stage and baking of bread doughs (38). Phospholipid-puroindoline interactions observed in model systems is similar to the behavior of different membrane invading or membranotoxic proteins (46).

Using monoclonal antibodies techniques, the main puroindoline (puro-*a*) was found mainly in the aleurone layer, whereas puro-*b* was found mainly in the starchy endosperm (47).

Another work on interfacial behavior of dough during mixing was carried out at Gist-brocades. Using an overflowing cylinder, the breakdown of macropolymers during mixing could be clearly seen in the surface active behavior of dough samples. Added lipids have a strong influence on the surface behavior, but no difference is observed between soft and hard wheat types (48).

Characterization and Purification of Gluten Subfractions

Purification of gluten subfractions is an essential step to study their functional, rheological, and physicochemical properties, but it is difficult to obtain pure subunits and subfractions that retain their functional properties. LMW glutenins are closely linked fractions with molecular weight similar to those of gliadins and an aggregative behavior that make them difficult to handle. Results of this study at INRA (Nantes) include the isolation of gluten subfractions differing by their aggregation state with low polydispersity, based on adapting MacRitchie's procedure of differential solubility in increased acid concentrations.

New methods of purification of HMW and LMW subunits have been also developed in the course of the program by IACR-Long Ashton, INRA-Montpellier, and Universities of Viterbo and Padova. These methods include precipitation by acetone (that has the potential to yield large amounts of pre-purified protein groups) (49), preparative IEF, electroendosmotic electrophoresis, and adsorption chromatography on controlled pore glass beads (University of Padova) (50).

Novel methods of characterization were also developed at INRA-Montpellier, including acid-PAGE for glutenin subunits (51), IE-FPLC (52), and determination of the number of cysteines by mixed alkylation and electrophoresis (53,54). Figure 8 shows results obtained by mixed alkylation and electrophoresis.

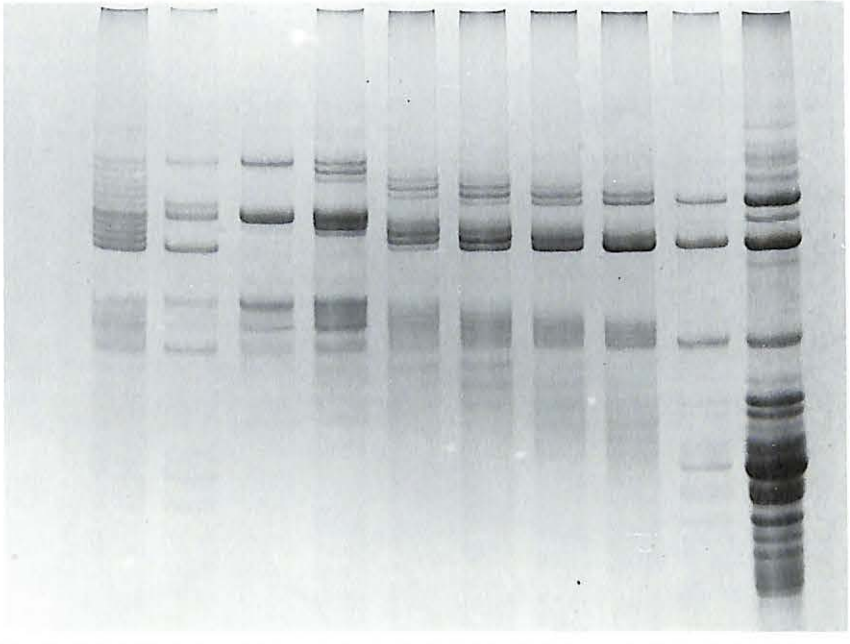


Fig. 8. Determination of the number of cysteine residues in high molecular weight subunits of wheat glutenin (variety Castan) by mixed alkylation and acid PAGE electrophoresis (from Morel and Bonicel, 1994).

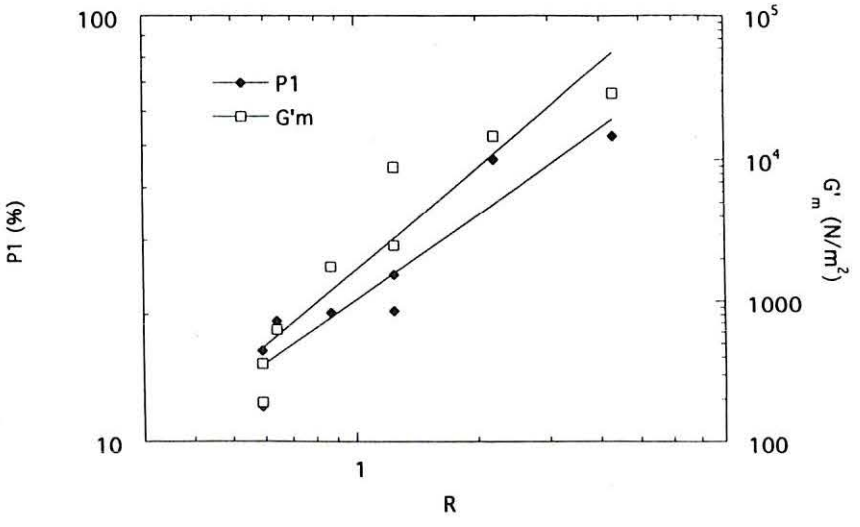


Fig. 9. Relationship between the content of gluten fractions in large-size glutenin polymers (PI), their plateau modulus (*G'm*), and their proportion of less mobile chains (R), obtained by spin-labeling of cysteine residues (courtesy of INRA-Nantes, France).

Physicochemistry and Functionality of Gluten Subfractions

Physical studies were carried out to gain more detailed information of secondary structures (55–59). A study conducted at IACR-Long Ashton showed that incorporating purified HMW subunits (e.g., 1Bx20) into a dough using a 2-g mixograph resulted in an increase of dough strength, whereas simple addition resulted in a decrease (60).

The rheology of various gluten subfractions was investigated at INRA-Nantes by dynamic assay in shear and revealed a behavior typical of transient network structure and large differences in storage and loss moduli between the fractions, including a strong correlation between the plateau modulus and the proportion of the largest glutenin aggregates (excluded SE-HPLC peak) (61,62) (Fig. 9). The determination of the effect of different HMW alleles was facilitated by the availability of near-isogenic lines that differ only in HMW composition (63,64). Viscoelasticity of gluten seems therefore controlled by the amount of the larger glutenin aggregates, the size of which can be precisely determined through dynamic light scattering (65). A two-level gluten structure was tentatively proposed: an aggregate level (directly involved in the observed viscoelastic behavior) and a network level resulting from the connection of the large aggregates through non-covalent interactions. The effects of factors affecting gluten viscoelasticity (e.g., temperature) are complex because they may play a role at both levels, whereas the SS/SH process seems to act only through the aggregation state (66).

FTIR and ^{1}H NMR relaxation studies at IFR-Norwich indicated that HMW subunits were not elastin-like in their interaction with water (67–69). For example, hydration increases the mobility of the HMW subunit 20, which in turn favors the more extended and β-sheet type structure (70,71). It was suggested that the origin of elasticity of HMW subunits was in intermolecular hydrogen bonding, which arises because of the high density of glutamine residues along the central repetitive domain (72).

Electron spray mass spectrometry was used to determine molecular weights of HMW subunits with known gene sequences. At INRA-Nantes and ENSBANA-Dijon, electron spin resonance (ESR) also provided information on molecular flexibility and confirmed that polymerization of subunits resulted in less mobile polypeptide chains and more rigid proteins (73–77). Also, with either TEMPO probing or labeling of cysteine and lysine residues, ESR results suggested the presence of two liquid phases in gluten (the organized lipids and the aqueous-protein phase) that differ in polarity (73,74). X-ray scattering and scanning tunneling microscopy studies were carried out at IACR-Long Ashton (59) to determine dimensions and flexibility of HMW molecules, indicating that subunits behave in solution as semi-rigid rods (56,57).

Dynamics of Dough Development

The aim was to study the behavior and interactions of wheat components in doughs and baked goods using antibodies for polysaccharides and gluten proteins and microscopic techniques (78,79).

At IFR-Norwich, polyclonal antibodies have been produced in rabbits against bran pentosan extracts, using lectins absorbed on microtitration plates to capture pentosans that would not be readily immobilized in the conventional way. Second, mice were injected to produce antibodies against arabinoxylans (AX) and boosted with AX alone and AX-protein conjugates. These antibodies are currently used with a new silver enhancement SEM technique (Fig. 10) to probe the wheat and bread samples and structural changes that occur during baking.

Multilocal Experiments and Genotype × Environment Interaction

The evaluation of yield potential and expression of quality attributes of wheats in different environments was another important facet of the program. A network of variety trials were carried out with a rigorous and uniform methodology in wheat growing areas (80,81).

Laboratories and breeding companies Involved in the North-Western (INRA-Clermont, ITCF-Paris, Club des 5-Paris) and Southern-Europe (Produttori Sementi-Bologna, ISC-S. Angelo Lodigiano, INRA-Clermont, ITCF-Paris, Club des 5-Paris, EERM-Jerez de la Frontera, ENMP-Elvas) networks have carried out technological analyses highlighting potential yield and quality characteristics of the recently released European cultivars as well as correlation between quality traits and agronomic factors, effect of nitrogen fertilization, and characterization of growth environments.

The concept of stability of quality was also refined. Several genotypes grown in the same set of environments exhibited different responses to the various growing conditions in terms of yield and quality. Study of quality is possible only if varieties are assessed in a multilocation trial. SEN and NWEN networks were thus well adapted to this task. Considering that all genotypes grown in such a trial were submitted to the same environment effects, stability of quality was defined according to the agronomic or dynamic concept: the variation of environments induced a variation of quality, common to all genotypes

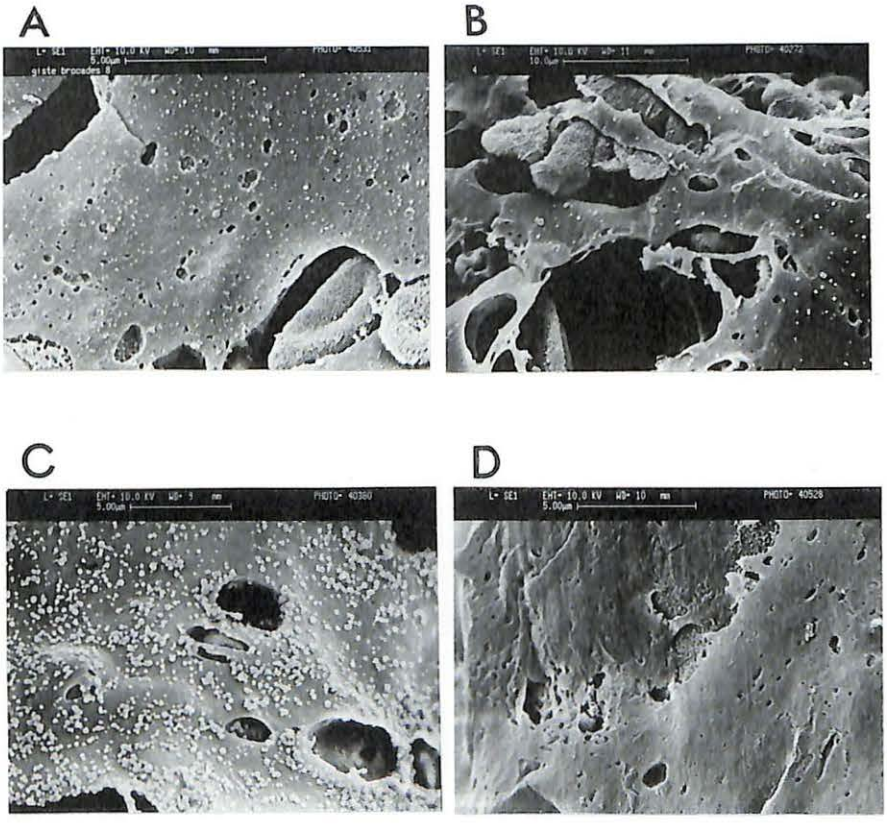


Fig. 10. Labeling of white bread samples without bread improver **(A)** or with pure xylanase **(B–D).** Samples were labeled either with anti-arabinoxylan Mab 0418 (1, **B**), anti-gliadin Mab 0033 **(C),** or only colloidal gold (1 nm) labeled antimouse IgM **(D)** (courtesy of Institute of Food Research, Norwich, UK).

tested. Thus, environmental variation of quality observed for one genotype was jointly due to location and genotype × environment (G×E) interaction effects, but stability was based only on G×E interactions.

The work on G×E interactions also allowed identification of the main determinants of protein content and composition. For instance, INRA-Clermont and ITCF gained information on kinetics of dry matter and protein accumulation in the grain in four wheat genotypes of contrasting quality (82). Results demonstrated differences in speed and duration of accumulation of both dry matter and protein fractions.

Genetics of Wheat Storage Proteins

The LMW subunits of glutenins are the least characterized group of wheat proteins. Owing to the development by the French and Italian geneticists of near-isogenic lines or chromosomic substitution lines such as those from cv. Courtot, advances in this topic have been made possible (83).

Genetic variability for B, C, and D groups was better described as well as genetic linkage between loci coding for gliadins and glutenins on group 1 chromosome (84–86). Because no identification system allowed satisfactory description of LMW subunits, a multiple system (SDS-PAGE/IEF/A-PAGE) was developed by INRA-Montpellier to characterize LMW subunits (34). A two-step A-PAGE/SDS-PAGE technique was developed at INRA-Clermont-Ferrand (87) to reveal the polymorphism of ω-gliadins. A two-dimensional A-PAGE × SDS-PAGE method was developed at ISC-S. Angelo Lodigiano/Rome and INRA-Montpellier to allow detailed description of LMW subunits in various cultivars following a genetic approach based on the relationship between the alleles at the *Gli-1* and *Glu-3* loci (88,89). Also, evidence was provided that a few B-group LMW subunits are encoded at loci other than *Glu-3* (90,91), while some *Gli-A2*-encoded α-gliadins were found to occur in the two-dimensional patterns of C-group LMW subunits (90), suggesting that a few α-gliadins are able to interact covalently with polymeric glutenin.

The effects of both HMW, LMW, and gliadins on gluten properties were much better understood (84,92,93). For instance, new relationships between γ- or ω-gliadin alleles and technological quality were established by ISC-S Angelo Lodigiano (84,94) and INRA-Clermont-Ferrand (87, 92,95,96).

A specific effect on breadmaking quality of D-type LMW subunits of glutenin (97–99) and of fractions controlled by genes on *Gli-D1/Glu-D3* loci was shown at University of Viterbo (100). Concerning specifically the impact of null alleles on dough characteristics, it was found at INRA-Clermont (101) and University of Viterbo (102) that the absence of storage proteins encoded by the *Gli-D1/Glu-D3* loci resulted in increased dough strength and larger size glutenin polymers (64). However, studies at ISC-S Angelo Lodigiano suggested that the impact of the null allele at *Gli-D1/Glu-D3* loci on gluten strength was highly positive in genotypes possessing HMW glutenin subunits 2+12, and negligible or negative in those containing HMW subunits 5+10. More interestingly, storage proteins encoded by these loci (and LMW glutenin in particular) were found to play a major role in conferring extensibility to dough and could account for the superior breadmaking characteristics of bread wheat as compared with durum wheat. Furthermore, researchers at INRA Montpellier suggest, based on interactions of allelic variation at the *Glu-3* and *Glu-1* loci, to screen lines containing the *Glu-B3* "*o*" or "*m*" allele when aiming at breeding wheats giving extensible doughs (34).

HMW subunits 7+9 and LMW subunits encoded by the *Glu-B3* "*k*" allele (which is associated with the *Gli-B1* "*k*" gliadin allele coding for γ-gliadin 40) were also

found to exert a positive effect on dough extensibility, suggesting that dough quality can be improved by using these alleles in combination with HMW subunits 5+10 and 1 or 2*, which were shown to increase dough elasticity.

In addition, a new *Glu-D1* subunit 5*, different from subunit 5 normally associated with subunit 10, was also discovered by researchers at the University of Viterbo in cv. Fiorello, which lacks the additional cysteine residue, typical of subunit 5, at the beginning of the repetitive domain (103). This raised doubts on previous results excluding subunit 5 as being responsible for differences in breadmaking quality observed in the pairs 5+10/2+12.

Sprouting Resistance

Prevention of sprout damage is important to the EU. The average costs of sprout damage, which generally occurs once every five years (leading to 10% loss in yield and reduction of the amount of breadmaking quality by 50%), is 50–60 million ECU per year. The approach envisaged by researchers at TNO was entirely new in both concept and methodology. Instead of detecting levels of amylase, work has focused on developing a bioassay to monitor inhibitors of germination and purifying a fraction containing a germination inhibitor (which proved to be distinct from abscisic acid) to allow rapid detection at an early stage and perhaps prevention (104,105). In addition, the determination of the broadness of the genetic basis for dormancy should allow to select for sprouting resistance in breeding programs.

Conclusions

As a whole, this ECLAIR project was a truly integrated precompetitive program (106). It has clearly contributed 1) to fill the gap between process development and its understanding in terms of processing requirements and wheat quality requirements and 2) to stimulate breeding and development of wheats capable of satisfying the present and future demands of European industry. The main results of this study are: 1) A better understanding on physico-chemical bases of the industrial processing of wheat and flour, 2) the development of improved methods for the rapid and efficient analysis and characterization of lines in early stages of breeding and of wheat samples in trade, 3) a genetic base of strong-type lines that breeders can now utilize to eventually introduce new varieties of wheat with all the desired agronomic and technological characteristics, particularly the stability of the expression of quality in various environmental conditions of development of the plant, and 4) a better identification of quality determinants, the genes of which should be identified, cloned, sequenced, and possibly transferred.

Moreover, due to the tremendous exchanges of knowledge among participants and to the great success of the program in a social sense, a European network with huge scientific power and excellent degree of communication has been developed during the last four years, enabling optimism about future research and development programs on wheat science and technology.

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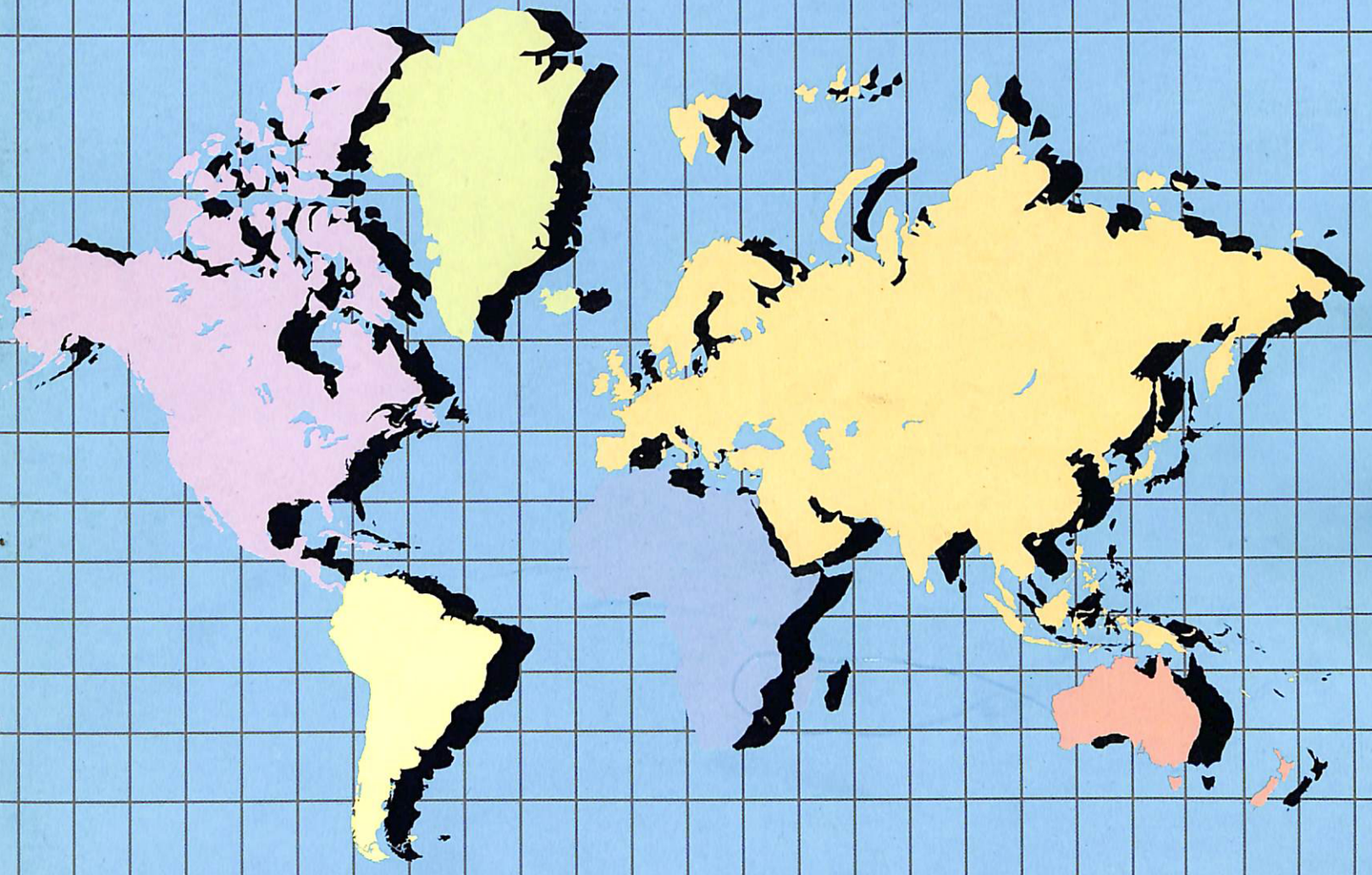

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