

CHARACTERISATION AND QUANTIFICATION OF LOW-MOLECULAR-WEIGHT GLUTENINS IN DURUM WHEATS

Jean-Claude AUTRAN, Bernard LAIGNELET and Marie-Hélène MOREL

Laboratoire de Technologie des Céréales I.N.R.A.
9 Place Viala. 34000 Montpellier. France.

INTRODUCTION

Considerable amount of biochemical, genetic and technological investigations have been carried out on wheat protein fractions based on Osborne's solubility scheme as previously reviewed (Feillet, 1965; Bietz et al., 1973; Bushuk and Wrigley, 1974; Kasarda et al., 1976). However, gliadin preparations (even extracted without reducing agent and examined in different chromatographic media) contain aggregated fractions called either "aggregated gliadins" (Shewry et al., 1983), "high-molecular-weight gliadin" (Bietz and Wall, 1980; Field et al., 1983), or "low-molecular-weight glutenin" (Kanazawa and Yonezawa, 1973). By gel filtration of gliadin on Sephadex G-100, Jackson et al. (1983) identified in the void volume several subunits coded by genes different from those coding for other gliadin fractions. On the other hand, glutenin fractions have been shown to contain gliadin-like subunits (Bietz and Wall, 1973), giving other evidences of the limits of the solubility-based Osborne's classification. Other classifications have been proposed based upon sulfur content (Shewry et al., 1984; Shewry and Miflin, 1984), aggregative properties (Miflin et al., 1983) (Shewry et al., 1986), biological functions (Shewry et al., 1986), N-terminal amino acid sequences (Kasarda et al., 1984), chromosomal locations of genes coding for the proteins (Payne et al., 1984a).

It is now fully demonstrated that gluten proteins consist of 3 major storage protein families:

- one monomeric family that corresponds to classical gliadins (apparent molecular weights: 25000 to 70000) and that includes α + β types (genes mostly located on the short arm of chromosomal groups n° 6) and γ + ω types (genes mostly located on the short arm of chromosomal groups n° 1).

- one aggregative family, generally reported as "high molecular weight glutenin" or "HMWG", corresponding to native aggregates of apparent MW from 1 to several millions, which, upon the effect of reducing agents, yield subunits of apparent MW 65000 to 130000 (genes located on the long arm of chromosomal groups n° 1).

- one aggregative family that we shall refer to as "low molecular weight glutenin" or "LMWG", corresponding to large aggregates which, upon reduction, yield subunits with apparent MW of 12000 to 60000 only (the major types belonging to the 45000-50000 range), most of them having genes located on the short arm of chromosomal groups n° 1, in the same complex locus than γ + ω gliadin locus.

LMWG remain the least characterized group. They differ from HMWG by their subunit molecular weight, the chromosomal location of the genes coding for and the amino acid composition (lower glycine content (Shewry and Miflin,

1984)). Their amount in gluten is controversial; their different allelic types and their contribution to the gluten functional properties are poorly known; the physico-chemical basis of their aggregative behaviour is not explained.

Although our investigations have been carried out both on bread wheats and durum wheats, we choosed in this paper to illustrate the results in restricting ourselves to the durum wheat proteins as a model. Durum wheat LMWG have never been thoroughly examined (Payne et al. (1984b)). Moreover, durum wheat proteins afford an unique example of a clear cut relationship between a functional property which is essential in determining cooking quality of pasta (gluten viscoelasticity) and a genetic type i.e. the presence of a given allele at one locus coding for some γ -gliadins and LMWG (Damidaux et al., 1978; Autran and Berrier, 1984; Autran et al., 1986).

The aim of this paper is to characterize the durum wheat LMWG and to determine their quantitative importance in gluten proteins, more specifically, within ethanol-soluble fractions which have been considered for a long time as typical gliadins.

Based upon chromatography and densitometry from one and two-dimensional electrophoresis, an explanation for LMWG functional role in determining intrinsic quality differences among durum wheats is ultimately proposed.

MATERIAL AND METHODS

Plant material

The cultivars of durum wheat (*Triticum durum* Desf.) used were Agathe (good pasta quality and high gluten strength) and Calvino (medium pasta quality and poor gluten strength). They were grown in 1985 in the I.N.R.A. experimental field in Montpellier.

Wheats were milled into semolina in a pilot mill (yield 76 %) (Houliaropoulos et al., 1981).

Gliadin preparation

100 g. semolina were extracted at room temperature (20°C) with 1000 ml 70 % (v/v) ethanol/water (without reducing agent). After centrifugation at 38 000 g, proteins were precipitated from the supernatant by addition of 3000 ml 0.25 M sodium chloride. The mixture was allowed to stand overnight at 4°C and the precipitate was collected by centrifugation. Proteins were then dissolved in 200 ml 0.01 M acetic acid, dialyzed thoroughly against distilled water at 4°C, shell-frozen and freeze-dried.

Sequential extraction

Salt-soluble proteins, gliadin-I, gliadin-II, glutenin-I, glutenin-II were extracted at room temperature (approx. 20°C) from semolina (ratio of extractant to solid: 10:1) according to the sequential procedure developed by Landry (1979) with respectively: sodium chloride 0.5 M, ethanol 60 % (v/v),

ethanol 60 % + 2-mercapto-ethanol 0.6 %, acetic acid 1 % (v/v) + 2-mercaptoethanol 0.6 %, SDS 1.5 % (w/v) + 2-mercaptoethanol 0.6 %. Each step consisted in one extraction plus two washings of the residue. The ratio of each solubility group was obtained by protein determination on aliquots of the pooled supernatants. The remainder was dialyzed against distilled water and freeze-dried.

Size-exclusion chromatography of gliadin-I

Gliadin-I was separated by size-exclusion chromatography on Sephadex G-150 as described by Bletz and Wall (1980). The column (2.5 x 100 cm) was equilibrated with acetic acid 0.1 M. 100 mg of protein was dissolved in 1 ml of column solvent, clarified by centrifugation, and applied to the column. 5 ml fractions were eluted (flow rate: 15 ml/h.) at room temperature, monitored at 254 nm, dialyzed against distilled water and freeze-dried.

Ion-exchange chromatography of gliadin-I

Gliadin-I was separated by ion-exchange chromatography on Whatman CM-52 as described by Kasarda et al. (1983). The column (2.5 x 25 cm) was equilibrated with a buffer 5 mM sodium acetate / 1 M dimethylformamide (DMF) buffer (pH 3.5). 1 gram of protein was dissolved in 50 ml of column buffer, clarified by centrifugation, and applied to the column. 5 ml fractions were eluted (flow rate: 15 ml/h.) at room temperature with a gradient of 5 to 100 mM sodium acetate in the column buffer, monitored at 254 nm, dialyzed against distilled water and freeze-dried.

Electrophoresis

Fractions from column chromatography and sequential extraction were analysed by acid polyacrylamide gel electrophoresis (A-PAGE) in aluminium lactate buffer, pH 3.2, according to Bushuk and Zillman (1978) and identified according to the two-number Zillman and Bushuk's nomenclature (1979), but using the durum wheat γ -gliadin 51 as reference band (Damidaux et al., 1978) and by polyacrylamide gel electrophoresis in Tris-glycine buffer containing sodium dodecyl sulfate, pH 8.4 (SDS-PAGE) as described by Payne and Corfield (1979) slightly modified (Autran and Berrier, 1984) and named according to their mobility by reference to a specific "subunit 1000" (Berger et Le Brun, 1985).

Two-dimensional characterizations of the basic fractions were carried out using a NEPHGE x SDS-PAGE system as described by Holt et al. (1981) with a pH range of 7 to 10.5.

Densitometry

Black and white prints of the gels were scanned with a soft laser LKB Ultrascan densitometer. The densitometric curves were processed (baseline subtraction, peak identification, integration) with a LKB Gelscan software on Apple IIe microcomputer. Reproducibility of the densitometric analyses have been evaluated to $\pm 2\%$ when scanning the same electrophoretic pattern and to only $\pm 10\%$ when scanning different patterns of the same sample, which is

consistent with previous reports (Fullington et al., 1980, 1983). In all tables, estimated percentages correspond to the means of four determinations (for instance, two chromatographies, one electrophoresis and two scannings). Small traces of components were not taken into account and suppressed by baseline subtraction.

Other laboratory tests

Protein content (%N x 5.7) was determined by the Kjeldahl method. Glutens were extracted and submitted to Viscoelastograph measurements to determine firmness and elastic recovery as previously reported (Autran et al., 1986).

RESULTS

Protein composition and gluten properties

Protein content and gluten viscoelasticity of the samples are given in Table I. In accordance with previous works (Damidaux et al., 1978; Autran et al., 1986), cv. Agathe and Mondur, that belong to durum wheat genetic type " γ -gliadin 45" show much higher gluten firmness and elastic recovery than cv. Kidur and Calvinor that belong to type " γ -gliadin 42".

Table I : Protein composition and gluten characteristics.

Cultivar (genetic type)	Agathe ("45")	Calvinor ("42")
Protein content (% d.b)	15.4	16.0
Gluten firmness (mm)	2.37	1.50
Gluten elastic recovery (mm)	1.78	0.75
Protein composition : (in % total proteins)		
Albumins + Globulins	19.2	23.5
Gliadin-I	34.3	39.2
Gliadin-II	22.8	15.0
Glutenin-I	8.6	7.5
Glutenin-II	6.0	5.2
Residue	9.1	9.6

The samples differ with respect to the percentages of the six proteic classes: The gliadin-I ethanol-soluble fraction (without reducing agent), which prevails in both samples (34.3 and 39.2 %), corresponds to the classical "gliadin" of most of the previous works. The percentage of gliadin-II (ethanol-soluble in the presence of reducing agent), which is lower, clearly differentiates the two types of wheats (15.0% in the type "42" and 22.8% in the type "45").

All these fractions have been further characterized in SDS-PAGE, as illustrated on Fig. 1 in the case of cv. Agathe. In spite of their specific pattern, each fraction consists in subunits covering a wide range of apparent molecular weights with considerable overlaps with other fractions. Soluble proteins range from apparent MW 15 to 62.5 K daltons; gliadins-I from 22, 5 to 68 K and gliadin II from 37.5 to 110 K. Glutenin (I and II) patterns consist in four major regions:

- one low mobility region (apparent MW: 95-110 K) that corresponds to HMWG subunits, which, in the particular case of cv. Agathe, are likely to correspond to B genome bands referred to as the allelic block 6-8 (Branlard and Autran, unpublished results),

- a strong single band, with a mobility comparable to that of one salt-soluble component,

- one region with intermediate mobility subunits (apparent MW: 44.5-51.5 K) that is likely to correspond to a low-molecular-weight fraction of glutenin subunits (LMWG),

- one fast-moving region (apparent MW 15 to 40 K) with mobilities similar to some salt-soluble proteins and that we shall referred to as very-low-molecular-weight glutenins (VLMWG).

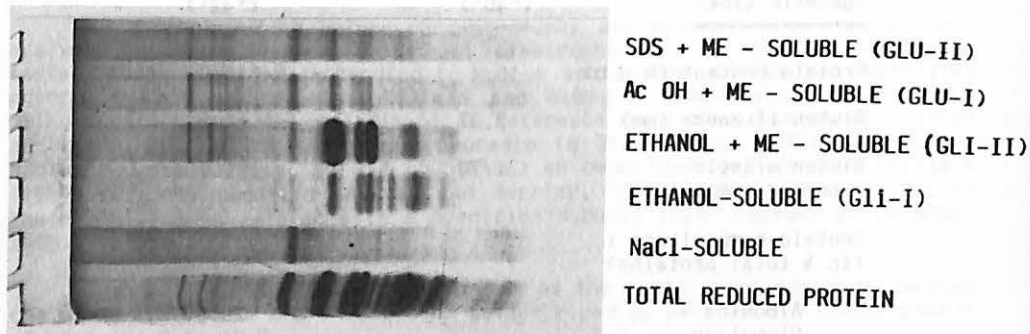


Fig. 1 : SDS-PAGE patterns of protein fractions from cv. Agathe.

Consequently, when considering a whole protein extract (slot n° 6, Fig. 1), it turns out that, with the exception of the HMWG subunits that can be easily located in the MW 95-105 K region, no subunit can be unambiguously assigned to a well-defined group or to a monomeric or an aggregative type.

Therefore, prior to one dimensional electrophoresis and to achieve a better assignment of the bands, a chromatographic step has been performed on the gliadin-I fraction.

Ethanol-soluble Proteins Chromatography

A relatively high resolution and a satisfactory recovery of the proteins (85-90 %) were obtained when using an ion-exchange chromatography on CM-52 with the acetic acid/sodium acetate/DMF buffer (pH 3.5).

A typical separation of the ethanol-soluble proteins is shown on Fig. 2 in the case of cv. Agathe.

Fourteen fractions were collected and examined in A-PAGE without reduction (Fig. 3a) and in SDS-PAGE after reduction (Fig. 3b). The amounts of each fraction were evaluated: a) by Kjeldahl determinations on each recovered product and b) by measuring the areas under the chromatographic curve using a graphic tablet. Comparable results were obtained, excepted for ω -gliadins which gave higher values by densitometry. Mean values between the two techniques are reported on Fig. 1.

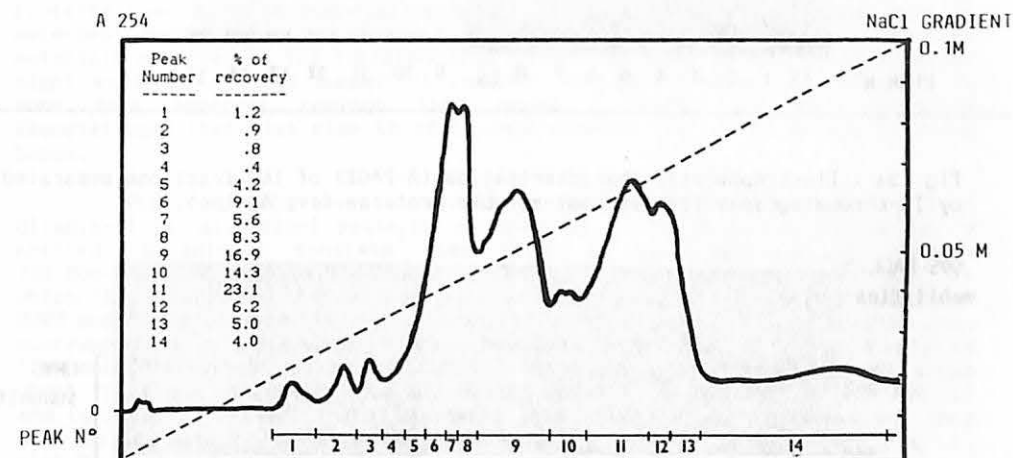


Fig. 2 : Ion-exchange chromatography in CM 52 (0.005 M sodium acetate buffer, DMF 1M, pH 3.5) of ethanol-soluble proteins (cv. Agathe)

In spite of overlaps between peaks and of the fact that most SDS-PAGE components correspond to several A-PAGE bands (for example, the reference subunit n° 1000 covers at least three major bands including one γ (51), one β and one α), several interesting trends in the fractions composition can be reported:

- The two first peaks contain ω -gliadin bands (A-PAGE mobilities 20 and 23 respectively) in a relatively pure state, which correspond to two close SDS-PAGE subunits with mobilities around 750.

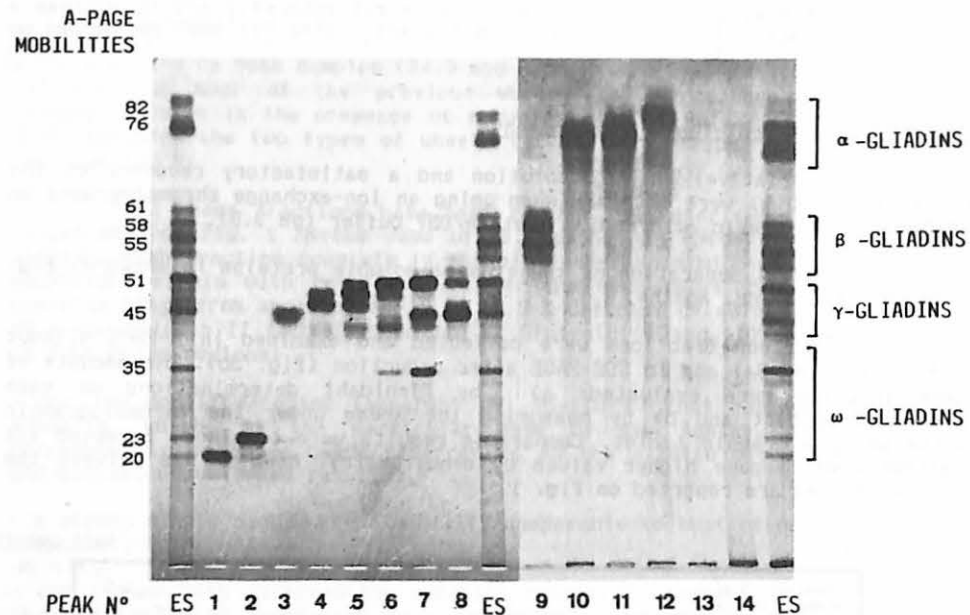


Fig. 3a : Electrophoretic characterization (A-PAGE) of the fractions separated by IE-chromatography from ethanol-soluble proteins (cv. Agathe).

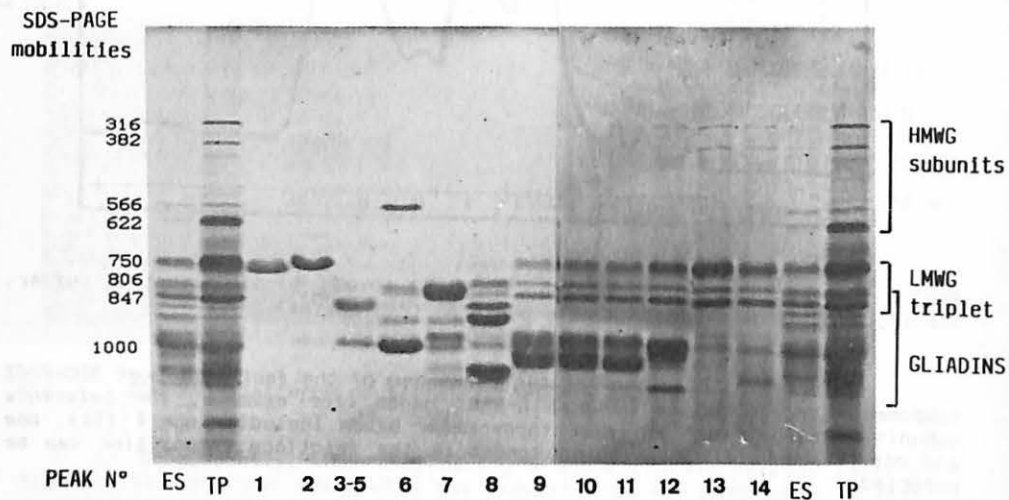


Fig. 3b : Electrophoretic characterization (SDS-PAGE) of the fractions separated by IE-chromatography from ethanol-soluble proteins (cv. Agathe).

- The two major γ -gliadin bands 45 and 51 are mainly found in peaks 7 and 8 and are identified to subunits 806 and 1000 respectively.

- ω -gliadins bands (A-PAGE mobilities: 20 to 35) yield SDS-PAGE subunits 566 to 750; major γ -gliadins (43 to 51), subunits 806 to 1000; β -gliadins (55 to 68), subunits 806 to 1105; α -gliadins (76 to 86), subunits 928 to 1105.

The most interesting result is that only the chromatographic peaks 1 to 12 yield bands in A-PAGE, peaks 13 and 14 only streaks, while peaks 10 to 12 yield streaks and α -gliadin bands; this is not the case in SDS-PAGE in which intense subunits are visible in all fractions. It is clearly apparent that all peaks that yield streaks (along with some slot material) in A-PAGE show the strong triplet 750-806-847 (MW 44.5-51.5 K), and some faint subunits 316-382 of higher apparent molecular weight: mobilities 316-382 (MW 95-110 K) which agree to the above-mentioned HMWG subunits.

The strong triplet 750-806-847 occurs in all fractions eluted with a ionic strength higher than 60 mM NaCl and cannot be mistaken with γ -gliadin 750 or γ -gliadin 847 subunits, which are eluted far before and without overlap in A-PAGE patterns. Therefore, it must be concluded that these intense triplet subunits, strongly retained on the column and having probably a very basic characteristic, do not fall into any classical monomeric gliadin type. These proteins must have an aggregative behaviour since they yield bands only in detergent media and after use of a reducing agent. The streaks and slot materials observed in the corresponding A-PAGE patterns (and in whole gliadin) might evidence a large number of randomly combined subunits (excepted in some more apparent regions that could correspond to more probable associations) that give rise to continuous streaks instead of giving discrete bands.

When comparing the patterns of the chromatographic peaks 10 to 14 to gliadin-II or glutenin-I patterns of the Fig. 1, a striking similarity is noticed. Gliadin-II consists essentially of the same strong triplet 750-806-847. The same proteins are visible in the patterns of glutenin-I, in which they represent the central part of the patterns, intermediate between HMWG and VLMWG regions. It must therefore be concluded that this triplet does correspond to an equivalent of what has been identified in bread wheats as "low-molecular-weight glutenin" (LMWG). These proteins are present in durum wheat fractions extracted with a reducing solvent (gliadin-II or glutenin-I) and (gliadin-I) without reducing agent; from simple visual examination, they seem to account for a major part of the whole gliadin pattern (see Fig. 1, slot 3, or Fig. 3b, slot ES).

Very similar elution curves were obtained with other cultivars (patterns not shown). Unlike A-PAGE patterns which show different gliadin compositions between cultivars, apparent molecular weight distributions in SDS-PAGE were nearly identical. The only difference that has been noticed in the case of the "type 42" cultivar (Calvinor) concerned the composition of the LMWG group: the four fainter subunits 750-776-806-839 (apparent MW: 45-51.5 K) were present instead of the strong triplet that characterizes all "type 45" cultivars (Du Cros, 1987; Du Cros and Autran, unpublished results). These two types of LMWG groups are very likely to correspond to the allelic types referred to as LMW1 (linked to γ -gliadin 42 in "type 42" cultivars) and LMW2 (linked to γ -gliadin 45 in "type 45" cultivars) by Payne et al. (1984b) and Autran and Berrier (1984).

Complementary results have been obtained by a classical size-exclusion chromatography of the ethanol-soluble fractions on Sephadex G-150 (Fig. 4).

This result confirms the presence of aggregated material (excluded peak) in the ethanol-soluble fraction of durum wheat cv. Agathe. SDS-PAGE patterns of reduced proteins from this excluded peak indicates the presence of several types of subunits. The first eluted fractions (slots 1 and 2) contain HMWG, LMWG and traces of fast-moving bands while the end of the excluded peak (slots 3 and 4) essentially contain the LMWG triplet. As expected, the composition of the second chromatographic peak (slot 5) corresponds to major gliadin bands in the 25K-40K region. Accordingly, this result confirms that LMWG triplet has a tendency to form protein aggregates, of predominantly lower apparent size, however, than HMWG do.

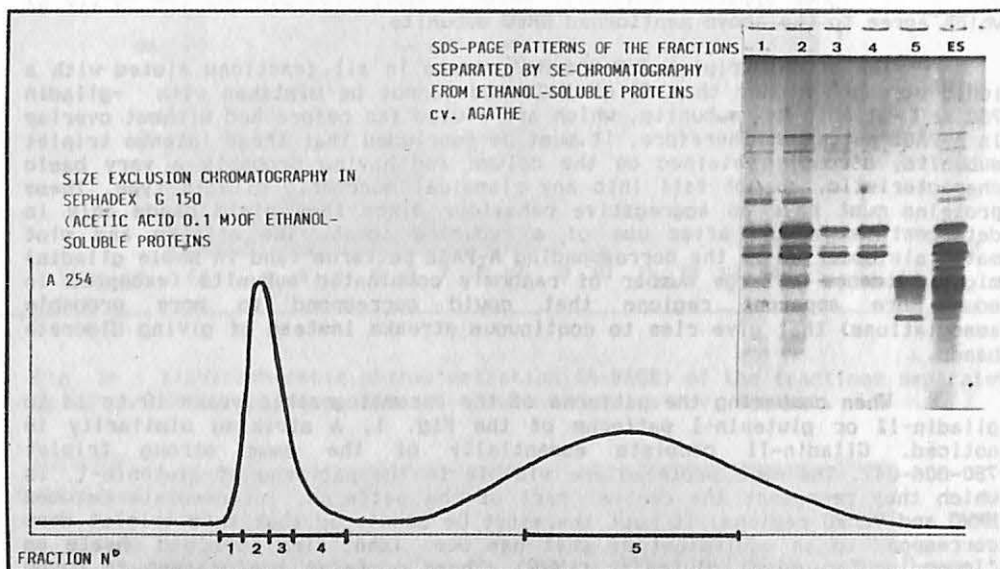


Fig. 4 : Size-exclusion chromatography of ethanol-soluble proteins (cv. Agathe) in Sephadex G 150 (acetic acid 0.1 M) and SDS-PAGE characterization of the fractions.

Quantitation of the major proteic subunits in gliadin-I

All chromatographic fractions, as also whole gliadin-I, were analysed by laser densitometry. Considering that the dye binding capacity of proteic molecules to Coomassie blue in acid solution is roughly proportional to the basic amino acid contents of proteins, we assumed that most components of a given chromatographic peak would be similar in this respect so that optical density provided a valid measure of the relative amounts of protein. As in previous works using densitometric measurements of wheat proteins (Fullington et al., 1980, 1983), we thought this was a reasonable assumption, but recognized the possibility that bands within a given group could contain proteins with different amino acid compositions. When different kinds of proteins such as salt-soluble, gliadins, LMWG, HMWG, are compared,

densitometric results must be considered with care. However, since salt-soluble fractions (rich in basic groups) have little contribution to the total proteins that have been examined (gliadin-I), all the other groups being storage proteins with similar basic/acid amino acids ratios, we thought that reliable estimates could be made nevertheless.

Densitometric analyses have been carried out (Fig. 5) in view to estimate, in gliadin-I and its chromatographic fractions, the percentages of the major α -, β -, γ -, ω -gliadins or of LMWG and HMWG (traces being neglected). For example, in the case of cv. Agathe (see Fig. 2 and 3), peaks 1 and 2 were found to be 100% ω -gliadins; peak 7 consisted in ω -gliadin 35 (20%), γ -gliadins 43 (14%), 45 (21%) and 51 (45%); peak 9 consisted in 100% of β -gliadins; peak 11 consisted in LMWG triplet (83.7%), HMWG (5.9%), α -gliadins (8.5%) and traces (1.9%) of albumin-like fast moving material.

From the relative amount of each fraction in gliadin-I (see Fig. 2), an estimation was made of the amount of each proteic group in each major SDS-PAGE band or zone and the results were summed up for the whole gliadin-I. These estimations have been summarized on Table II in the case of cv. Calvino and Agathe respectively. A such estimation was not possible without

Table II : Global estimates of the different A-PAGE classes of gliadins and of HMWG and LMWG in ethanol-soluble fractions (gliadin-I) (in % of the total ethanol-soluble fraction)

	cv. Calvino (type "42")	cv. Agathe (type "45")
α -gliadins	19.9	14.0
β -gliadins	24.6	22.7
γ -gliadins	23.9 (*)	21.9 (**)
ω -gliadins	10.3	5.7
HMWG	4.3	5.9
LMWG	14.4	27.1
misc., fast-moving material	2.6	2.7

(*) including γ -42 : 10.1 %

(**) including γ -45 : 7.6 %

a preliminary chromatographic step because of the impossibility to assign a SDS-PAGE band to a well-defined group from a whole gliadin one-dimensional pattern.

The main trends that appear from these estimations are the following:

- In both types of cultivars (Calvino and Agathe), classical gliadin contains, as expected, high and similar percentages of α , β and γ fractions, much lower percentages (about 5% and 10%, respectively) of ω fractions, and some traces of fast moving components (residual salt-soluble fractions or fast-moving gliadins referred to as F42 or F45 in a previous work (Cottenet et al., 1984a)).

- The major γ -gliadins (42, 45 and 51), previously purified (Cottenet et al., 1983), represent respectively 10.1%, 7.6% and 8.7% of the total ethanol-soluble fraction. With respect to the total proteins, their respective ratios are 3.9%, 2.6% and 3.4%.

- Interestingly, both types contain small amounts of high-molecular-weight fractions (about 4% and 6%) and particularly large amounts of LMWG fractions for which they also strongly differ: about 14% in type 42 and 27% in type 45.

Densitometric analyses afford to demonstrate the occurrence of a high percentage of LMWG fractions (along with small amounts of HMWG), in ethanol-extracted proteins, besides what can be called "true gliadins" (i.e. those that behave as monomers and give rise to bands in A-PAGE gels).

These LMWG and HMWG contents are even likely to be underestimated since the determinations have been based on ethanol-soluble fractions that redissolved in the chromatographic buffer and on recovered chromatographic peaks only (insoluble or not eluted fractions usually consist more in aggregated fractions than in monomeric ones).

However, since this first estimation concerned the gliadins only, it was necessary to go further in estimating the percentages of LMWG and HMWG in the other fractions and in total proteins.

Quantitation of the major proteic subunits in whole durum wheat proteins

SDS-PAGE patterns (Fig. 1) of the five fractions (salt-soluble, gliadin-I, gliadin-II, glutenin-I, glutenin-II) were scanned and the percentage of each major band or zone was estimated as for gliadin-I hereinabove.

Salt-soluble fractions (as opposed to gliadins and glutenins) consist mainly in fast-moving components (mobilities >850) and in a strong slow-moving band (mobility: 622) and do not exhibit any particular band in the LMWG region. Since soluble proteins and true (monomeric) gliadins are likely to have been removed prior to gliadin-II extraction, the patterns of gliadin-II and subsequent fractions consist in aggregative components only, that fall respectively into HMWG (mobilities: 316 to 622), LMWG (750 to 847) and VLMW (> 900) subunits. In the case of cv. Agathe, the distribution of these three classes was: 16%, 58% and 26% respectively in gliadin-II; 36%, 37% and 27% in glutenin-I; 30%, 37% and 33% in glutenin-II.

Taking into account the percentage of each solubility fraction in total proteins (according to the data of Table I), the contribution of each major band can then be calculated, like the true percentages (with respect to the total proteins) that respectively fall to salt-soluble fractions, α -, β -, γ -, ω -gliadins, LMWG, HMWG and residue. From the synthesis of these data, presented on Tables III and IV, it appears that:

- Several fractions occur at similar ratios in both types of wheats: HMWG, VLMWG and residual proteins (about 10% each) and salt-soluble (about 20%, a little more in cv. Calvinor).

- The major differences between the two types of cultivars essentially concern gliadins and LMWG components. True gliadin bands represent 32% in cv.

Table III : Calculated contribution of each major SDS-PAGE subunit to the total wheat proteins (in % of the total proteins, not including residue).

Subunit mobilities	Main components	cv. Calvinor	cv. Agathe
281 to 316	HMWG	4.8	5.5
566	ω -gliadin	3.0	1.1
622	HMWG; alb-glob.	16.3	13.4
750	LMWG; ω -gliadin	4.8	16.3
776	LMWG	5.0	-
806	LMWG; γ -gliadin	7.2	6.6
839	LMWG; γ -gliadin	8.9	-
847	LMWG; γ -gliadin	-	9.8
872	β -gliadin; γ -gliadin alb-glob.	4.2	2.9
901	β -gliadin	3.9	4.1
928 to 1071	α -gliadin; β -gliadin VLMWG	15.1	16.1
1105	α -gliadin; β -gliadin; alb-glob.; VLMWG	8.2	6.3
>1250	alb-glob.; VLMWG	9.0	8.1

TABLE IV : Calculated contribution of salt-soluble proteins, gliadins, HMWG, LMWG, VLMWG and residue to the total wheat proteins (in % of the total proteins).

	cv. Calvinor	cv. Agathe
Salt-soluble	23.5	19.2
α -gliadins	8.0	5.0
β -gliadins	10.0	8.0
γ -gliadins	9.7 (*)	7.7 (**)
ω -gliadins	4.2	2.0
HMWG	10.2	11.5
LMWG	15.1	27.7
VLMWG	8.2	10.3
Residue	9.6	9.1
Ratio: aggregated/monomeric	1.35	2.58

(*) including γ -42: 3.9%
 (**) including γ -45: 2.6%

Calvinor, but only 23% in cv. Agathe. Conversely, LMWG occur at almost double ratio in cv. Agathe (28%) compared to cv. Calvinor (15% only).

- A more accurate illustration of these differences can be shown using two-dimensional electrophoresis (NEPHGE x SDS-PAGE) of most basic proteins of cv. Agathe and Calvinor (Fig. 5). Compared to the total protein extract, the subunits of LMWG groups are among the few that migrate toward the cathode in the 7-10.5 pH gradient. In the case of cv. Agathe, they give rise to three major spots (and several minor ones) with isoelectric pHS estimated between 7.5 and 8.0, while, in the case of cv. Calvinor, the four fainter bands of the LMW1 group give rise to five spots in a similarly basic pI region.

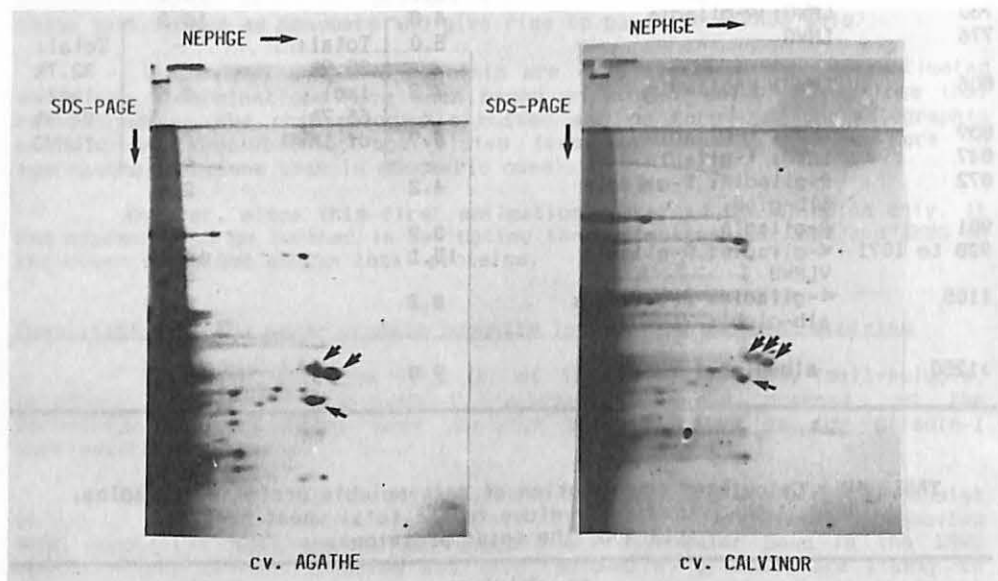


Fig. 5 : Two-dimensional characterization (NEPHGE x SDS-PAGE) of the most basic subunits of a total protein extract.

- LMWG make up a major fraction (and even one of the easiest fraction to identify from SDS-PAGE patterns) of both total reduced proteins and ethanol-soluble proteins. This result is illustrated on Fig. 6 by the densitometric tracings of these two samples, in comparison to the chromatographic peak 13 in which the percentage of LMWG triplet is 83 %.

- LMWG and HMWG are present in all gliadin and glutenin fractions. About 30-35% of LMWG are ethanol-soluble, 45-50% are ethanol+ME-soluble, 15-20% fall into the two glutenin groups. Concerning HMWG, 10-15% are ethanol soluble, 35-45% are ethanol+ME-soluble, 40-50% fall into the glutenin groups.

- In the two cultivars, the distribution of the aggregated groups (LMWG and HMWG) between the different solubility fractions is not the same: LMWG are less ethanol-soluble and behave more as "glutenin-types" in Agathe while the process is reversed for HMWG. In other words, the type "45" cultivar

contains more LMWG proteins than the type "42" and these proteins are less ethanol-soluble in the former than in the latter, what explains the large prevalence of gliadin-II fraction in cv. Agathe compared to cv. Calvinor.

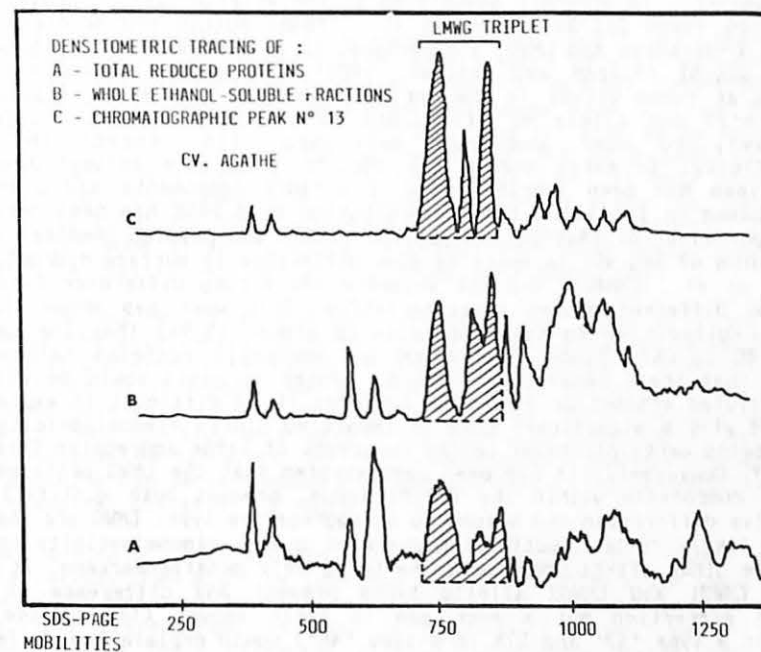


Fig. 6 : Densitometric tracing of : A - Total reduced proteins; B - Whole ethanol-soluble fractions; C - Chromatographic peak n° 13. (cv. Agathe).

A more detailed examination of the quantitated results shows that:

- The triplet zone of a total proteins pattern of cv. Agathe (subunits 750 to 849) is essentially constituted by LMWG subunits: 84% LMWG, 3% β -gliadins, 10% γ -gliadins, 3% ω -gliadins. The equivalent quadruplet in cv. Calvinor is made up of 67% LMWG, 5% β -gliadins, 24% γ -gliadins, 4% ω -gliadins.

- When considering a total ethanol-extract (34.3% in Calvinor), the percentage of true gliadins (those that migrate in a regular A-PAGE gel) with respect to the extracted proteins, is largely overestimated: only 79% of these extracted proteins in cv. Calvinor and 65% in cv. Agathe are true monomeric gliadins, the remainder being assignable to aggregated proteic material.

- The ratio LMWG / gliadins affords an excellent discrimination of the two types of cultivars: respectively 0.47 and 1.22 for Calvinor and Agathe. The same is true for the ratio: aggregated fractions (HMWG+LMWG+VLMWG+residue) / monomeric fractions (gliadins): respectively 1.35 and 2.58. Such ratios, that keep closer to physico-chemical basis of technological quality than did the glutenin / gliadin ratios previously proposed, should be successfully used in view to predict gluten characteristics at the breeding stage

DISCUSSION

In durum wheats, gluten viscoelasticity - an important factor of pasta cooking quality - is strongly associated to the allelic type of proteins that are coded by locus *Gli-B1* (Payne et al., 1984b; Autran and Berrier, 1984): ω -gliadins, γ -gliadins and LMWG, and perhaps, to a lower extent, to those coded by locus *Glu-B1* (Autran and Feillet, 1985). Two major alleles have been discovered at locus *Gli-B1* in the world collection: allele 42 (γ -gliadin 42 and LMWG n°1) and allele 45 (γ -gliadin 45 and LMWG n°2) that correspond respectively to poor and good cultivars with respect to gluten viscoelasticity. In early works, the quality difference between these two genetic types has been attributed to γ gliadin components and a breeding strategy based on γ -gliadin type determination by A-PAGE has been developed. However, purification (Autran and Feillet, 1985) and physico-chemical studies of γ -gliadins 42 and 45, in spite of some difference in surface hydrophobicity (Cottenet et al., 1984b), did not evidence any strong difference capable to explain so different gluten characteristics. This work has shown that the ratio of γ -gliadin 42 to total proteins is higher (3.9%) than the ratio of γ -gliadin 45 (2.6%). Since γ -gliadins are monomeric proteins (although we recognize that their behaviour in dough, gluten or pasta could be different than in diluted ethanol or in A-PAGE buffers) it is difficult to explain how they could play a significant role in imparting gluten viscoelasticity, this property being certainly based on the occurrence of large aggregates (Mifflin et al., 1983). Conversely, it has been demonstrated that the LMWG proteins, that are major components within the *Gli-B1* locus, present both qualitative and quantitative differences and belong to an aggregative type. LMWG are therefore much more likely to be functional markers of gluten viscoelasticity in durum wheats, the other *Gli-B1*-coded proteins being only genetic markers. It is not known if LMWG1 and LMWG2 allelic types present any difference in their functional properties but a such gap in their amount (14% of the total proteins in a type "42" and 27% in a type "45") could explain the differences in durum wheat gluten characteristics.

In bread wheats (*Triticum aestivum*), it has been shown (Payne and Lawrence, 1983; Branlard et Le Blanc, 1985) that baking quality is more associated to *Glu-1*-coded proteins (HMWG subunits) than to *Gli-1* ones. In durum wheats, an opposite situation seems to prevail. In spite of some relationship between HMWG subunits and gluten or pasta quality (Huebner and Bietz, 1986), LMWG would be primarily involved in gluten viscoelasticity in durum wheat gluteins. We can therefore hypothesize that all aggregative protein fractions could contribute to quality, that HMWG would act in bulk with little varietal specificity, and that LMWG would be the major functional markers of genetic differences in durum wheat gluteins. The fact that the world durum wheat collection essentially consists in two different allelic types at *Gli-B1* locus may explain the occurrence of two clear cut groups of cultivars with respect to gluten viscoelasticity. In bread wheats, by contrast, the occurrence of many allelic types at *Glu-A1*, *Glu-B1* and *Glu-D1* loci may explain the continuous evolution in gluten quality without two distinct classes of cultivars as in durum wheats.

CONCLUSIONS

1) Taking durum wheat proteins as model, this work confirms that Osborne-type fractionation, which is still used in many studies, is unsatisfactory, at least when investigating the physico-chemical basis of quality. The ethanol-soluble fraction, considered by many authors as well-defined gliadins, consists in different types of proteins: some that migrate in A-PAGE gels and that correspond to monomeric molecules and those that can be studied in SDS-PAGE gels upon reduction only. The latter are far from being negligible in ethanolic extracts since minimum estimates of the only LMWG triplet are between 18% and 33% according to the durum wheat type. It must be emphasized that analysing A-PAGE bands gives information only on a part of the extracted proteins, the remainder giving rise to streaks and slot material. It is even possible that some ethanol-soluble fractions that have been recently found correlated to quality (40) actually correspond to LMWG types.

We subscribe to a proteins classification which will be based on aggregation characteristics in A-PAGE buffers: true gliadins (from 23% to 32% of the total proteins in this work) correspond to monomeric proteins separated in discrete bands in A-PAGE, while glutenins (from 33% to 50%) are separated in SDS-PAGE gels only after a preliminary treatment by a reducing agent, even if some of them (particularly LMWG fractions) have been partly extracted by aqueous ethanol. To calculate the ratio aggregated / monomeric components in gluten proteins can be recommended to predict gluten viscoelasticity, instead of the classical glutenin / gliadin ratio.

2) The glutenin subfractions do not exhibit the same solubility characteristics. LMWG might be less aggregated than HMWG since about one third of total LMWG has been found in ethanol extracts (less than 15% for HMWG). However, the differences, if any, in functional properties of these two fractions and of their different allelic types among durum wheats are not known but should be clarified shortly, more especially from sequences determinations.

3) LMWG appear as a major fraction among gluten proteins although they have been certainly underestimated in this work since non eluted and residual fractions (that are likely to mainly consist in aggregated proteins) have not been considered. However, LMWG do not contribute to the same ratio of total proteins in the different durum wheat genetic types. They can be estimated to 27% in type "45" but to 14% only in type "42". Because of their aggregative behaviour and without considering any difference in the functional properties between their allelic types, the only difference in their contribution to the protein pool could explain the opposite characteristics of "42" and "45" durum wheat types with respect to gluten strength and elasticity.

LITERATURE CITED

- Autran J.C. and Berrier R. (1984) In: "Gluten Proteins", Proc. 2nd Int. Symp. Workshop Gluten Proteins (Graveland A. and Moonen J.H.E., ed), T.N.O., pp. 175-183
- Autran J.C. and Fellet P. (1985) In: "Proteins Evaluation in Cereals and Legumes", E.E.C. Workshop, Oct. 23-24, Thessaloniki, Greece
- Autran J.C., Abecassis J. and Fellet P. (1986) *Cereal Chem.* 63, 5, 390-394
- Berger M. et Le Brun J. (1985) *Industries des Céréales* 37, 17-25
- Bietz J.A., Huebner F.R. and Wall J.S. (1973) *Bakers Digests*, 2, 26-35, 67
- Bietz J.A. and Wall J.S. (1973) *Cereal Chem.* 50, 537-547
- Bietz J.A. and Wall J.S. (1980) *Cereal Chem.* 57, 6, 415-421
- Branlard G. and Le Blanc A. (1985) *Agronomie*, 5, 6, 467-477
- Bushuk W. and Wrigley C.W. (1974) In: *Wheat: Production and Utilization* (Inglett G.E., ed.), Avl, Westport, Conn, pp. 119-158
- Bushuk W. and Zillman R.R. (1978) *Can. J. Plant Sci.* 58, 505-515
- Cottenet M., Autran J.C. et Joudrier P. (1983) *C. R. Sci. Paris* 297, Série III, 149-152
- Cottenet M., Fellet P. et Autran J.C. (1984a) *C. R. Acad. Sci. Paris*, 299, Série III, 11-16
- Cottenet M., Kobrehel K. et Autran J.C. (1984b) *Sciences des Aliments*, 4, 551-556
- Damidaux R., Autran J.C., Grignac P. and Fellet P. (1978) *C. R. Acad. Sci. Paris* 287, Série D, 701-704
- Du Cros D.L. (1987) *Journal of Cereal Science* (In press)
- Fellet P. (1965) *Ann. Technol. agric.*, 14, H.S. n° 1, 1-94
- Field J.M., Shewry P.R. and Mifflin B.J. (1983) *J. Sci. Fd Agric.* 34, 370-377
- Fullington J.G., Cole E.W. and Kasarda D.D. (1980) *J. Sci. Fd Agric.* 31, 1, 43-53
- Fullington J.G., Cole E.W. and Kasarda D.D. (1983) *Cereal Chem.* 60, 1, 65-71
- Holt L.M., Astin R. and Payne P.I. (1981) *Theor. Appl. Genet.* 60, 237-243
- Houliaropoulos E., Abecassis J. et Autran J.C. (1981) *Industries des Céréales* 12, 3-13
- Huebner F.R. and Bietz J.A. (1986) *Journal of Cereal Science* 4, 379-388

- Jackson E.A., Holt L.M. and Payne P.I. (1983) *Theor. Appl. Genet.* 66, 29-37
- Kanazawa H. and Yonezawa D. (1973) *J. Agric. Chem.* 47, 17-22
- Kasarda D.D., Bernardin J.E. and Nimmo C.C. (1976) In: *Advances in Cereal Science and Technology* (Pomeranz Y., ed.), Vol. 1, Association of Cereal Chemists, St Paul, Minn, pp. 158-236
- Kasarda D.D., Autran J.C., Nimmo C.C., Lew E. J.-L. and Shewry P.R. (1983) *Biochim. Biophys. Acta*, 747, 138-150
- Kasarda D.D., Laflandra D, Morris R. and Shewry P.R. (1984) *Kulturpflanze* 32, S 41-60
- Landry J. (1979) *C. R. Acad. Sci. Paris*, 288, 907-909
- Mifflin B.J., Field J.M. and Shewry P.R. (1983) In: "Seed Proteins" (Daussant J. and Mosse J., ed.), Chap. 12, Acad. Press, London, pp. 255-319
- Payne P.I. and Corfield K.G. (1979) *Planta* 145, 83-88
- Payne P.I. and Lawrence G.J. (1983) *Cereal Res. Com.*, 11, 1, 29-33
- Payne P.I., Holt L.M., Jackson E.A. and Law C.N. (1984a) *Phil. Trans. R. Soc. Lond. B* 304, 359-371
- Payne P.I., Jackson E.A. and Holt L.M. (1984b) *Journal of Cereal Science* 2, 73-81
- Shewry P.R., Mifflin B.J., Lew E.J.-L. and Kasarda D.D. (1983) *Journal of Experimental Botany* 34, 148, 1403-1410
- Shewry P.R. and Mifflin B.J. (1984) In: *Advances in Cereal Science and Technology* (Pomeranz Y., ed.), Vol. 1, American Association of Cereal Chemists, St Paul, Minn. pp. 1-83
- Shewry P.R., Mifflin B.J. and Kasarda D.D. (1984) *Phil. Trans. R. Soc. Lond. B* 304, 333-339
- Shewry P.R., Tatham A.S., Forde J., Krels M. and Mifflin B.J. (1986) *Journal of Cereal Science* 4, 97-106
- Zillman R.R. and Bushuk W. (1979) *Can. J. Plant Sci.*, 59, 281-286

Proceedings of the 3rd International Workshop on

GLUTEN PROTEINS

Budapest, Hungary

May 9-12, 1987

Editors:

R. LÁSZTITY

F. BÉKÉS

World Scientific