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Separation of durum wheat proteins by ultrathin-layer isoelectric focusing: A new tool for the characterization and quantification of low molecular weight glutenins

An isoelectric focusing method capable of resolving all groups of storage protein of the wheat seed, including the most basic low molecular weight glutenin (LMWG), was developed. Ultrathin polyacrylamide gels were used after drying and rehydration with 8 M urea, 50 mM DTE and 2.4 % carrier ampholytes (pH 4–9). Densitometric scanning of the isoelectric focusing gels permitted a more accurate and specific quantitation of LMWG components among various cultivars than patterns based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The two main genetic types (*i. e.* 'γ-42' and 'γ-45') of durum wheats were separated on the basis of the proportion in LMWG in storage proteins, but no significant difference was found within these groups. Advantages of the system as regards reliability, high resolution, ability to abolish protein oxidation and preventing reaggregation of LMWG were also discussed.

1 Introduction

Durum wheat (*Triticum durum* Desf.) storage proteins afford a 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 a locus coding for specific γ gliadin components [1, 2]. A breeding strategy based on γ-gliadin type determination by polyacrylamide gel electrophoresis (PAGE) has been developed and early generation durum wheat lines with a 'γ-45' allele could be selected as potentially strong gluten types, whereas lines with a 'γ-42' allele could be rejected as potentially weak gluten types [3, 4]. More recently, it was shown that the association involved not one, but a group of γ- and ω-gliadin proteins [5, 6] and that the genes coding for them (*Gli B1* chromosome locus) were tightly linked to genes coding for low molecular weight subunits of glutenin (LMWG) on *Glu-B3* chromosome locus [7]. The latter, because of their strong aggregating properties, are now considered more likely to be the direct causal agents of gluten viscoelasticity [8]. The monomeric γ-gliadins 42 or 45 have similar physicochemical properties among the various genotypes [9], and are perhaps best considered to be only genetic 'markers' of durum wheat quality [10].

The question arises as to whether the various contributions made to durum wheat quality by the different LMWG alleles might be accounted for by differences in functional properties, or simply due to differences in the amount of their components. Using sequential extraction, ion-exchange chromatography and densitometric scanning of sodium dodecyl sulfate (SDS)-PAGE gels, Autran *et al.* [11] demonstrated that the strong viscoelasticity of 'γ-45' types occurred simultaneously with a high proportion of LMWG ('LMWG-2' allele) in total proteins (27.7 %), while the low viscoelasticity of 'γ-42' types was associated with lower proportions (15.1 %) of LMWG ('LMWG-1' allele). They suggested that quantitative differences alone might explain the differences in gluten viscoelasticity between 'γ-45' and 'γ-42' types and recommended that a screen for the ratio of aggregated/monomeric components could predict gluten viscoelasticity in breeding programs aimed at improving cooking quality of durum wheats. However, difficulties have been encountered for a specific quantification of LMWG from a large series of samples for following reasons: (i) Unlike monomeric gliadins, LMWGs form highly aggregated complexes that cannot be resolved by native PAGE. (ii) Upon reduction of S-S bonds, unlike high molecular weight subunits of glutenins (HMWG) that can be clearly identified in SDS-PAGE, LMWGs overlap with gliadin components in the M_r 45 000–50 000 range and cannot be specifically quantified. (iii) LMWG subunits and gliadins have different p*I*s, but standard isoelectric focusing (IEF) procedures cannot be used for the separation of these proteins because LMWG subunits appear as the most basic protein components in wheat, and are extremely sensitive to oxidation.

In previous attempts at characterizing wheat storage proteins, Holt *et al.* [12] noticed that after extraction with SDS and β-mercaptoethanol as solvent, the most basic LMWG components failed to enter the gel and could not be resolved by IEF. A nonequilibrium pH gradient gel electrophoresis (NEPHGE) procedure based on the method of O'Farrell *et al.* [13] was indispensable to separate them from other less basic proteins, so that two different first dimensions were necessary to resolve all of the various wheat storage protein groups. According to Field *et al.* [14], however, if an extracting solvent containing 6 M urea and 55 mM cetyltrimethylammonium bromide (CTAB) in the presence of β-mercaptoethanol was used instead, a sample loading at the anode allowed a single focusing experiment to resolve all the extracted proteins. Nevertheless, Shewry *et al.* [15] underlined the need for reducing and pyridylethylating proteins before electrophoresis in order to obtain discrete bands, and noticed that the alkylation procedure may alter the initial protein p*I*s and even result in artefactural zones.

In order to develop a fast and simple method for the specific separation of wheat LMWG from a large series of samples, and to permit accurate quantification by densitometric scanning of the gels afterwards, we have investigated a new IEF

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Abbreviations: **cv.**, cultivar; **DTE**, dithioerythritol; **GEVES**, Groupe d'Etude des Variétés et des Semences; **HMWG**, high molecular weight subunits of glutenin; **IEF**, isoelectric focusing; **LMWG**, low molecular weight subunits of glutenin; **NEPHGE**, nonequilibrium pH gradient electrophoresis; **PAGE**, polyacrylamide gel electrophoresis; **SDS**, sodium dodecyl sulfate; **TCA**, trichloroacetic acid;

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system. The best way appeared to be the use of rehydratable ultrathin polyacrylamide gels according to Radola [16], bound to a polyester sheet, according to Görg *et al.* [17]. It is well documented that ultrathin polyacrylamide gels offer many advantages, although they have little been used for separating plant proteins. On the other hand, dried and rehydratable gels afford a number of advantages over the conventional wet gels. As the gels are washed before drying, they are free of unpolymerized acrylamide monomer and of ammonium persulfate, which have been reported to interfere with the formation of uniform pH gradient in IEF and to oxidize proteins [16]. Such dried gels can then be rehydrated with various kinds of solutions. In particular, reducing agents that are necessary to keep proteins in the reduced state during the run [18], but that are potentially inhibitors of acrylamide polymerization, can be introduced at this rehydration step, saving the extra step of protein alkylation.

In the first part of our paper, we report and discuss the optimization of an IEF method especially aiming to resolve the most basic and aggregated proteins of the wheat seed, especially by checking the resolution of LMWG after two-dimensional analysis. In the second part, the identification and quantification of the LMWG components of various French durum wheat cultivars (cvs.) are presented and discussed.

2 Materials and methods

2.1 Wheat samples

The durum wheat samples used in this study comprised 10 licensed French cvs. and 10 genotypes submitted for registration, all grown and provided by Groupe d'Etude des Variétés et des Semences (GEVES, Guyancourt, France). Wheat seeds were milled into semolina, gluten was extracted from semolina and the viscoelastic index was measured on gluten as previously described [1].

2.2 Chemicals and reagents

All chemicals used were of reagent grade. Carrier ampholytes used for IEF were of 'Pharmalyte' type from Pharmacia.

2.3 Protein extraction

Wheat semolina (100 mg) was stirred for 30 min at 20 °C with 3 mL of distilled water and centrifuged for 5 min at 12 000 *g* in an Eppendorf microcentrifuge. The supernatant, containing essentially albumins and globulins, was discarded and the pellet was extracted for 3 h at 20 °C with 1 mL of a water/acetonitrile/mercaptoethanol (40/50/10 v/v/v) solvent. This solvent was expected to extract gliadins and to reduce the glutenin fraction (HMWG and LMWG subunits). After centrifugation, 4 µL of the supernatant were loaded onto IEF gels. 8 µL were loaded in the case of IEF gels used as the first dimension in two-dimensional analysis.

2.4 IEF in ultrathin gels

Two glass plates (230 × 120 × 2 mm) with 230 × 4 × 0.2 mm spacers were used to cast polyacrylamide gels by the flap technique of Radola [19]. An acrylate activated polyester sheet (Gel-Fix for PAGE, Serva, Heidelberg) was fastened to the upper plate by capillary attraction and the lower plate was pretreated with Repel Silane (Pharmacia). The gel solution (5 % T, 2.8 % C) in 45 mM Tris-HCl, pH 8.8, containing 10 % v/v glycerol was fully degassed before use. Polymerization of gel solutions (20 mL) was catalyzed by 60 µL of *N,N,N',N'*-tetramethylethylenediamine (TEMED) and 60 µL of a 15 % w/v ammonium persulfate. After polymerization for 20 min, the 0.2 mm thick gels were washed 3 × 10 min with water, 1 × 30 min with glycerol 10 % v/v, then dried overnight at room temperature in a vertical position. Before use, the gels were simply rehydrated by spreading 15 mL of an 8 M urea solution, containing 2.6 % w/v of Pharmalytes (Pharmacia) pH 4–6.5 and pH 6.5–9 (40/60) and variable concentrations of dithioerythritol (DTE), on the gel surface without stirring. After 30 min, the excess rehydration medium was wiped off using a piece of polyester sheet. IEF was performed at 13 °C using a 2117 Multiphor II apparatus (Pharmacia). 40 mM glutamic acid and 1 M sodium hydroxide were used as anolyte and catholyte, respectively. After a 400 V × h prerun at constant power (7 W), 5 µL samples were loaded at the anodic side of the gel, using an applicator strip, 7 × 1 mm (Serva). Focusing conditions were set to 2000 V × h at 7 W (maximum power) and then to 1500 V × h at 2800 V (constant voltage). The pH gradient was determined by cutting a series of gel segments (0.5 × 3.0 cm). After 30 min of soaking in 2 mL of 10 mM KCl, the pH of the solutions was measured with a conventional electrode.

2.5 Staining

IEF and SDS-PAGE gels were stained with Coomassie Brilliant Blue R-250 (0.05 % in 12 % w/v TCA solution) and destained with 10 % w/v TCA solution according to Chrambach *et al.* [20]. Alternatively, the method of Neuhoff [21] with Coomassie Brilliant Blue G-250 was used.

2.6 Densitometric scanning

Stained gels were scanned with a soft laser Ultroscan densitometer (Pharmacia). The densitometer curves were processed (baseline subtraction, peak identification, integration) with Nelson software (Stang Instruments, France) on an IBM PC-XT microcomputer.

2.7 Two-dimensional analysis

Two-dimensional analyses of wheat proteins were performed by combining our IEF procedure with conventional Laemmli [22] SDS-PAGE. Total analysis (IEF plus SDS-PAGE) could be achieved within one day. After the first-dimensional separation, proteins were located by soaking the IEF gel in water. Strips of 12 × 0.8 cm were cut, equilibrated for 75 s in 50 mM Tris-glycine, pH 6.8, containing 4 M urea, 2 % w/v SDS, 2 % v/v β-mercaptoethanol, 10 % w/v sucrose, and then slightly inserted into the stacking gel of SDS-PAGE. The IEF strip was sealed in position using a solution of 1 % w/v agarose in the tank buffer.

3 Results

3.1 Optimizing composition of rehydrated ultrathin IEF gels

After removing albumins and globulins by stirring durum wheat semolina in distilled water, the water/acetonitrile/mer-

captoethanol (40/50/10 v/v/v) extract was expected to contain a major part of the storage proteins, specifically including glutenins (LMWG and HMWG subunits). IEF of these proteins was investigated by comparing a standard laboratory-made horizontal IEF gel to a dried/rehydrated gel, using variable amounts of urea and DTE. In all cases, IEF was performed using 2.6 % w/v carrier ampholytes in the 4–9 pH range. 8 M urea was found as an optimal concentration to maintain all extracted proteins in solution. When lower concentrations (*e. g. 4* M) were used (not shown), some protein precipitate could be noticed near the anodic application site, irrespective of the DTE concentration. Standard IEF gels containing 8 M urea and no DTE permitted satisfactory resolution for the most acid proteins in spite of some streaks, whilst a more diffuse pattern was observed at the cathodic side, in the p*I* zone where LMWG were expected to migrate (Fig. 1A). Conversely, rehydrated gels gave discrete bands and much less background in both acidic and basic regions (Fig. 1B). A pH gradient analysis demonstrated that a slight drift occurred in the standard gel, compared with the rehydrated ones (Fig. 2). This effect, probably due to the presence of unpolymerized acrylamide monomer and ammonium persulfate, could contribute to the lack of resolution of the most basic proteins.

However, even in the presence of 8 M urea, which is supposed to break down hydrogen bonds, reaggregation of protein through disulfide bonding cannot be ruled out as a consequence of air oxidation during runs in horizontal gels, especially for LMWG proteins that are extremely sensitive to such phenomena. In order to avoid any possible reoxidation of the sulfhydryl groups, either the surface of the gel could be protected from oxygen, or a reducing environment could be maintained in the gel. Because the addition of DTE in the rehydration medium seemed the easiest option and was found to give reproducible gels, the second possibility was investigated further using various concentrations. As illustrated on

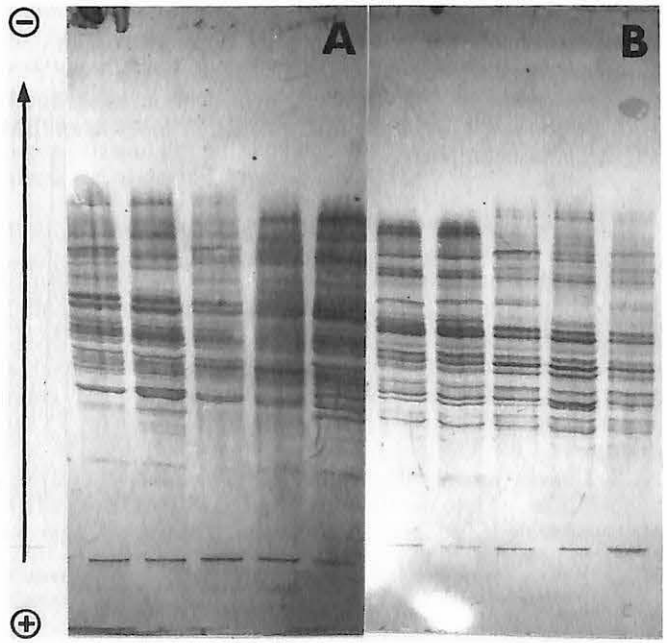

Figure 1. IEF patterns of wheat storage proteins extracted by water/acetonitrile/mercaptoethanol (40/50/10) solvent from five durum genotypes. (A) Standard ultrathin gel (5 % T, 2.8 % C, 8 M urea, carrier ampholytes 2.6 %, pH 4–9); (B) Dried ultrathin gel, rehydrated with 8 M urea and 2.6 % carrier ampholytes (pH 4–9).

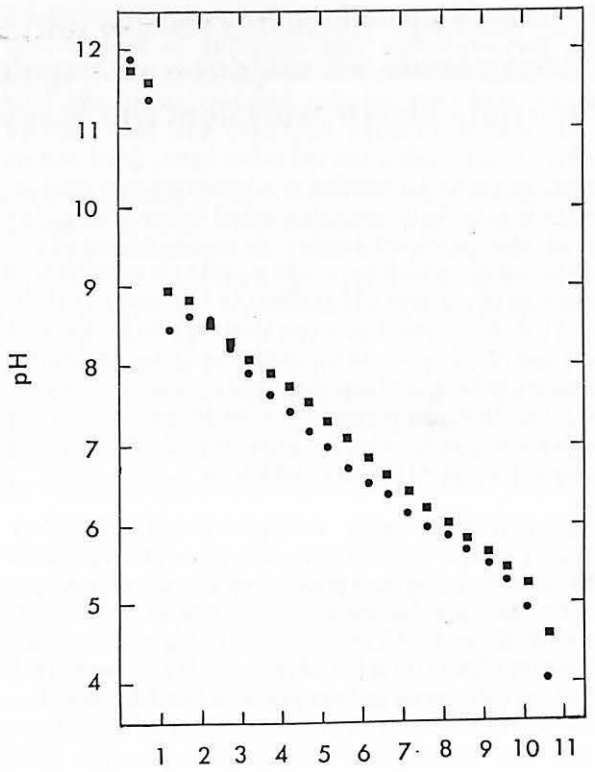

Figure 2. pH Gradient profile of gels A) –●– and B) –◆– from Fig. 1.

Fig. 3, the addition of DTE produced a great improvement of the IEF protein patterns. Positive effects on band sharpness were noticeable, even for DTE concentrations as low as 5 mM (Fig. 3A), indicating that such a concentration seems likely to keep LMWG proteins in a soluble form by preventing disulfide bond reconstitution. Increasing DTE concentrations to 50 mM and 100 mM caused a spreading of the protein patterns towards the cathodic side (Fig. 3B and C), the acidic side of the pattern not being affected. This effect was likely to result from the flattening of the pH gradient at the cathodic side when DTE concentrations were increased from 5 mM to 50 mM (Fig. 4). This result is understandable by considering that DTE is a weak acid which is likely to lower the expected pH value at the cathodic side. This artificial flattening of the basic part of the theoretically linear gradient is likely to result in an improvement of the separation of basic proteins, such as LMWG, that focus around pH 7.5. Accordingly, the optimized IEF system that has been selected for routine analyses consisted of gels first polymerized with water, then dried and rehydrated with 8 M urea, 50 mM DTE and 2.6 % carrier ampholytes.

3.2 Identification and quantification of the LMWG components in durum wheats

A comparison of the IEF patterns of storage proteins from cvs. belonging either to the 'γ-45' or the 'γ-42' type showed that the most basic bands consisted of a strong triplet in the former (*e. g.* cv. Agathe; Fig. 5A) and a fainter quadruplet in the latter (*e. g.* cv. Kidur; Fig. 5B). A more specific identification of the two allelic LMWG types, and their assignment to those previously described in an SDS-PAGE system, was also obtained by two-dimensional analysis of proteins extracted by the water/acetonitrile/mercaptoethanol solvent after sequential removal of proteins by 0.5 M sodium chloride, water, and

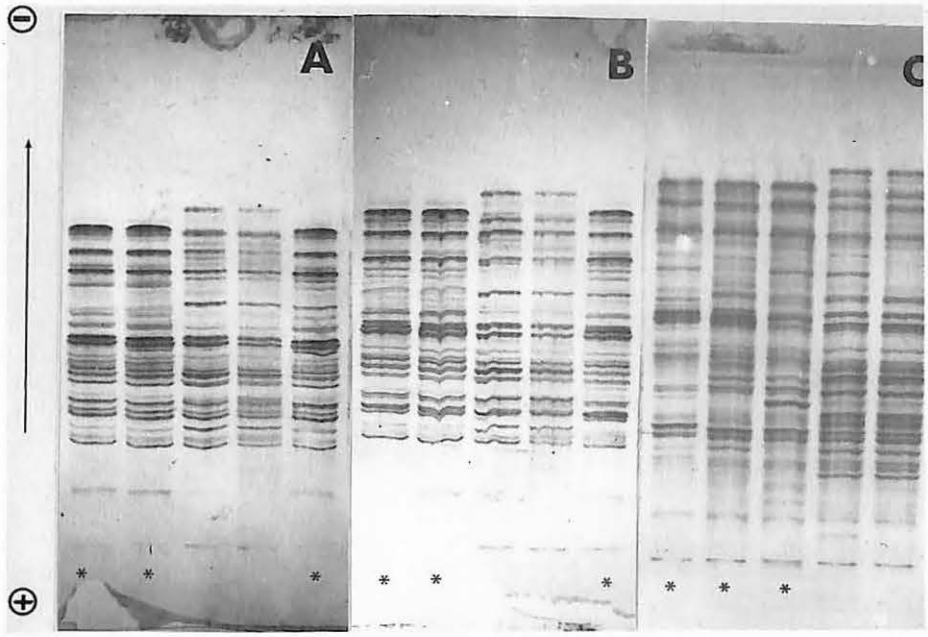

Figure 3. Effect of various DTE concentrations on band resolution of wheat storage proteins extracted by water/acetonitrile/mercaptoethanol from five durum genotypes. Dried gels were rehydrated with 8 M urea, 2.6 % carrier ampholytes, pH 4–9, and: (A) 5 mM DTE, (B) 50 mM DTE, (C) 100 mM DTE. Labeled (*) 'γ-45' wheat genotypes.

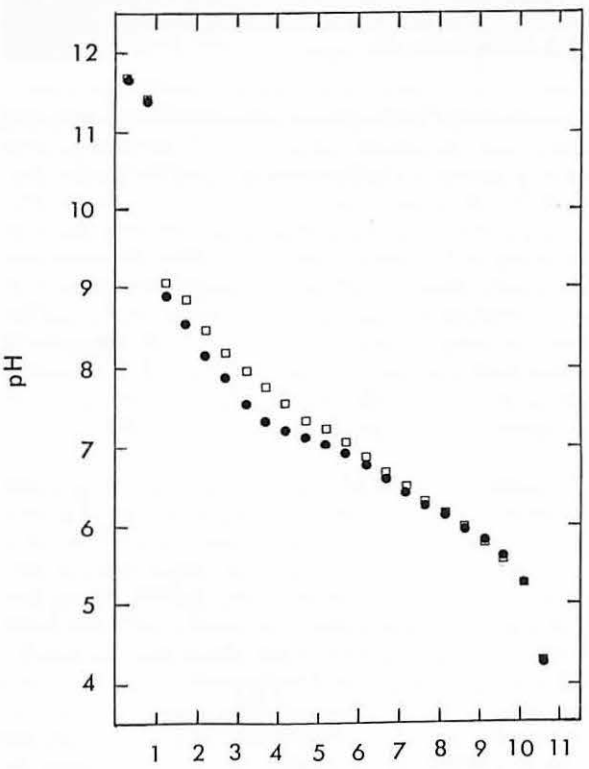

Figure 4. pH Gradient profile of gels (A) – □ – and (B) – ● – from Fig. 3.

70 % v/v ethanol [11]. The components (referred to as 750-806-847 in SDS-PAGE [11]) of the cv. 'Agathe' LMWG-2 allelic type gave three main spots and two fainter ones, the three main spots making up the basic IEF triplet in the two-dimensional map (Fig. 5A). Conversely, the components (referred to as 750-806-839 in SDS-PAGE) of the cv. 'Kidur' LMWG-1 allelic type gave four spots in the two-dimensional map (Fig. 5B). These four spots match with the most basic IEF quadruplet. Similarities between LMWG-1 and LMWG-2 components could be revealed by superimposition of the two-dimensional patterns of cvs. Agathe and Kidur, respectively (Fig. 6): LMWG-1 components essentially differ from LMWG-2 components by the presence of a specific subunit (No. 4b) and the lack of subunit No. 3.

Although these results demonstrate that the LMWG group corresponds to the most basic region of the IEF patterns, possible overlappings with other minor components had to be investigated prior to any densitometric scanning of the IEF gels. This was achieved by reconstituting the IEF pattern using a horizontal projection of the components separated in two-dimensional electrophoresis.

The patterns of total wheat proteins (extracted by the water/acetonitrile/mercaptoethanol) presented in Fig. 7 illustrate two different cases. In Fig. 7A, one HMWG subunit (referred to as No. '20' allelic type [23]) and one α-gliadin spot overlap with the region of the LMWG p*I*s, whilst in Fig. 7B there is an overlap with another HMWG subunit (No. '8'). These overlapping components induce additional peaks or shoulders that can be easily located on the basic IEF densitometric tracing (arrows in Fig. 8) and that should be subtracted from the total area under the curve if a more accurate and specific quantitation of LMWG was required.

Densitometric scanning of IEF gels was carried out from 20 durum wheat cvs. (10 of each 'γ42' and 'γ-45' genetic type; 6 repeats for each) and raw (uncorrected for overlaps) LMWG areas were quantified and their proportions estimated on the basis of the total area under the densitometer tracing (Fig. 8). Quantitative data (mean and standard deviation) are presented on Table 1. Considering each cv., the standard deviation of the proportion in LMWG fractions ranged between 1.4 % and 5.3 %. Mean values of the LMWG percentages evaluated from ten cvs. of each of the 'γ-42' and 'γ-45' genetic type varied according to the γ-gliadin type but also according to the HMWG type because of overlaps between the most basic bands. Independent of the HMWG allelic type, however, durum wheat of the 'γ-45' genotype presented a significantly greater proportion of LMWG with regard to 'γ-42' genotypes (see Section 4.2).

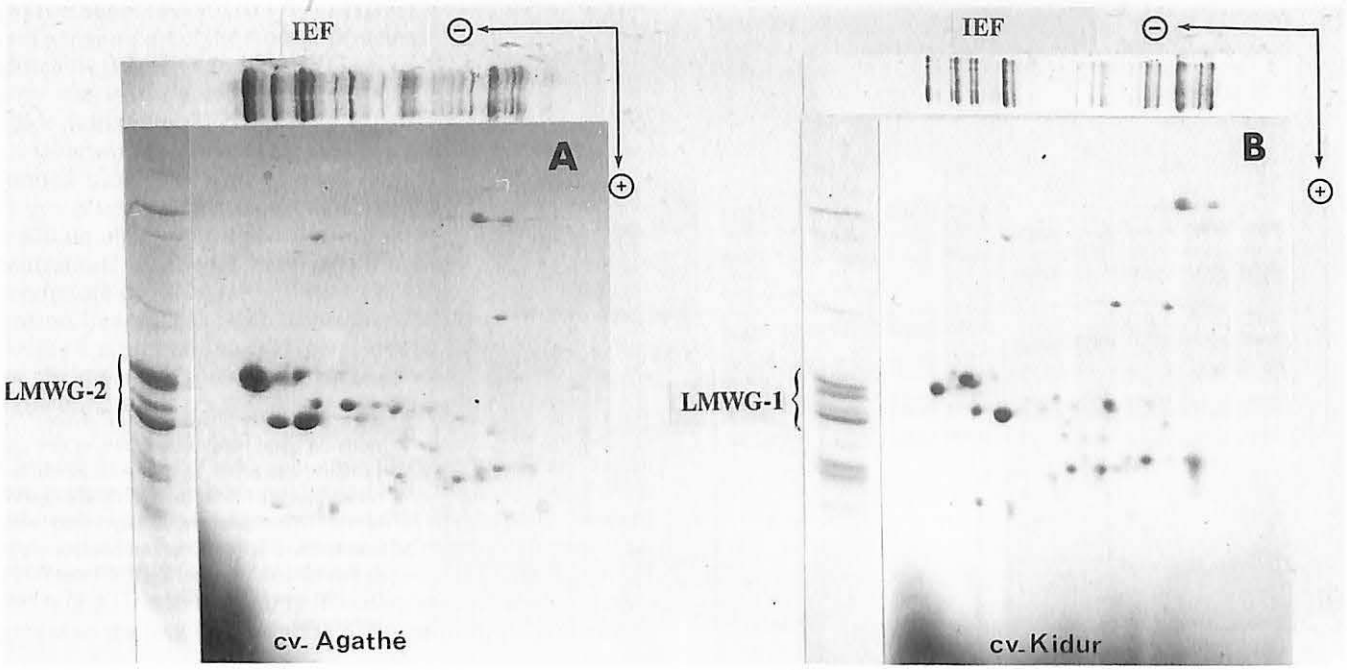

Figure 5. Characterization of LMWG subunits by two-dimensional (IEF × SDS-PAGE) electrophoretic patterns. Wheat semolina was sequentially extracted by 0.5 M sodium chloride, water, and 70 % v/v ethanol. Proteins were then extracted from the last pellet by water/acetonitrile/mercaptoethanol and submitted to isoelectric focusing. (A) cv. 'Agathe' (LMWG-2 allelic type) and (B) cv. 'Kidur' (LMWG-1 allelic type).

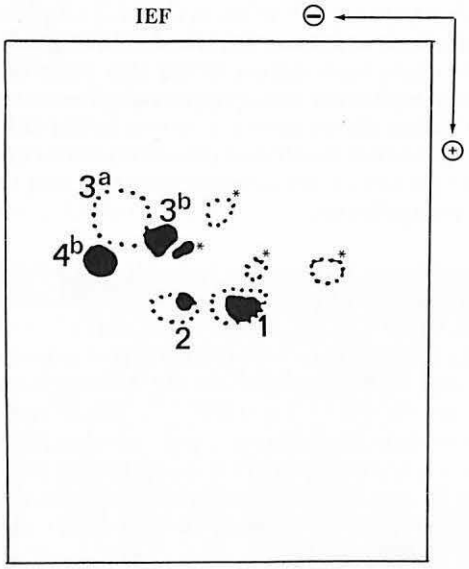

Figure 6. Similarities between LMWG-1 and LMWG-2 components shown by superimpositon of the two-dimensional patterns of cvs. 'Agathe' (LMWG-2, dotted spots) and 'Kidur' (LMWG-1, filled spots). Components No. 1 and 2 are assumed to be qualitatively similar between the two types of patterns. The strong component No. 3a is specific of LMWG-2 patterns, whilst components 3b and 4b are specific of LMWG-1 patterns. Labeled (*) spots are minor LMWG components.

4 Discussion

4.1 Optimizing composition of rehydrated ultrathin IEF gels to resolve LMWG fractions from wheat

Wheat storage proteins are unique among cereal and other plant proteins in their capacity to form a dough with viscoelastic properties ideally suited to make bread or pasta. Although the detailed molecular basis of these properties is not clearly understood, it is likely to be related to the unique physicochemical and functional properties of specific protein fractions (*e. g.* gliadin and glutenin) making up the gluten complex. LMWGs, the least characterized group of gluten proteins, are especially involved in these phenomena because of their sensitivity to oxidation, high aggregating behavior and tendency to form large complexes, making them insoluble in the absence of strong denaturing agent. This has prevented the use of many techniques such as native PAGE or conventional IEF and has made it necessary to develop a new IEF technique derived from the one described by Radola [16] and adapted to a sample sensitive to oxidation by Altland [18, 24].

The IEF technique reported in this study limits gradient drift and inhibits protein oxidation owing to the combination of several favorable factors. Ultrathin gels are used, making it easy to wash out catalyst residues and unpolymerized monomer. These gels are dehydrated and can be rehydrated with freshly prepared (and therefore weakly carbamylated) urea solutions containing DTE. Also, this system permits the application of high voltages without any heating problem, and allows an optimal IEF of wheat glutenins to be reached within 2 h. Our results demonstrate that such IEF conditions are likely to keep LMWG in a reduced form, preventing their reaggregation. They permit migration of the most basic LMWG as discrete and reproducible bands with very little background. They allow, therefore, a more accurate quantitation of these proteins. In contrast to Altland's reports [18, 24] on oxidation of human globins during focusing in immobilized pH gradient gels in the presence of 50 mM DTE and 8 M urea, no splitting of LMWG bands was observed in our IEF system, as demonstrated also by two-dimensional results. According to Altland [24], the occurrence of an oxidizing activity would result from drying immobilized pH gradient gels and its effect would increase with migration time. An oxidizing activity in our dried gels cannot be ruled out, however, because a gradual loss of resolution with the occurrence of streaks was noticed

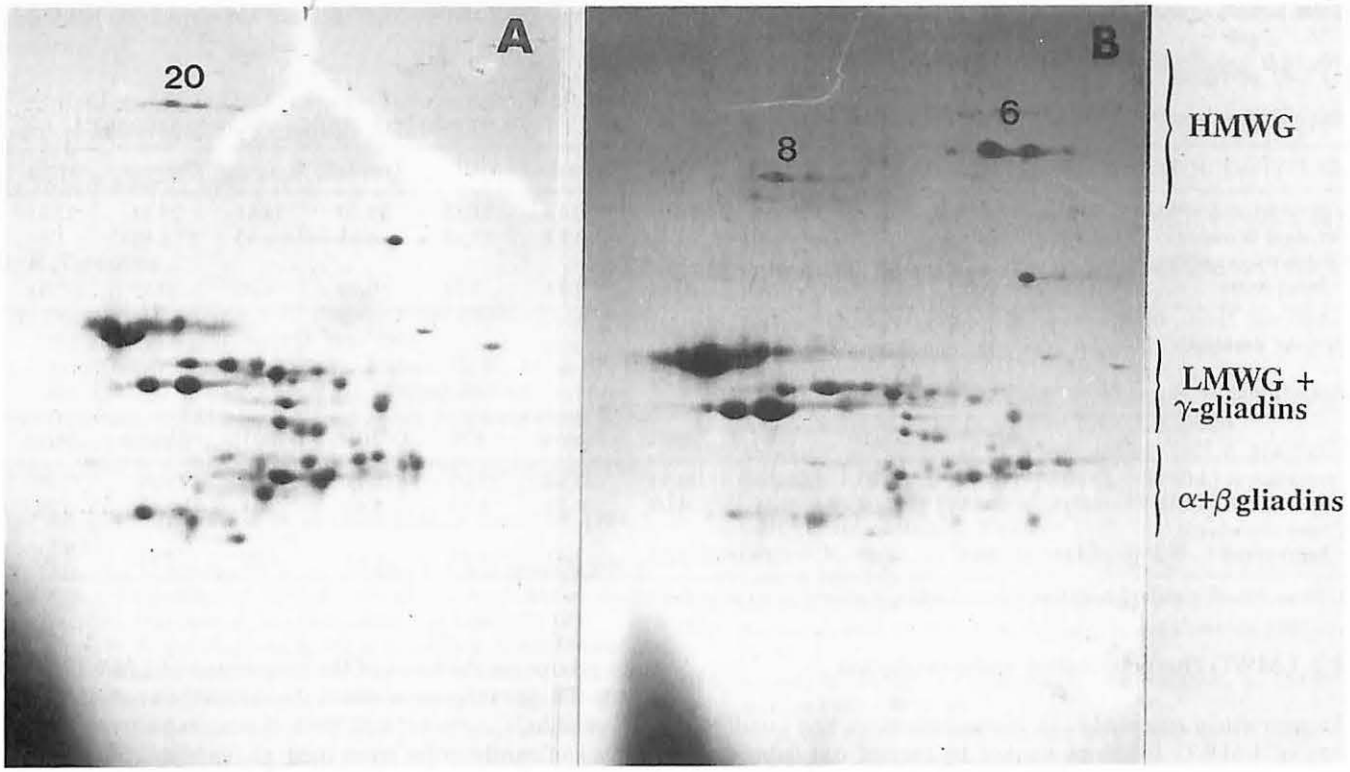

Figure 7. Two-dimensional (IEF × SDS-PAGE) patterns of wheat storage proteins extracted by water/acetonitrile/mercaptoethanol from two durum cvs. belonging to 'γ-45' allelic type, but differing in HMWG allelic type: (A) cv. 'Agathe' (HMWG '6+8' type) and (B) cv. 'Amidur' (HMWG '20' type).

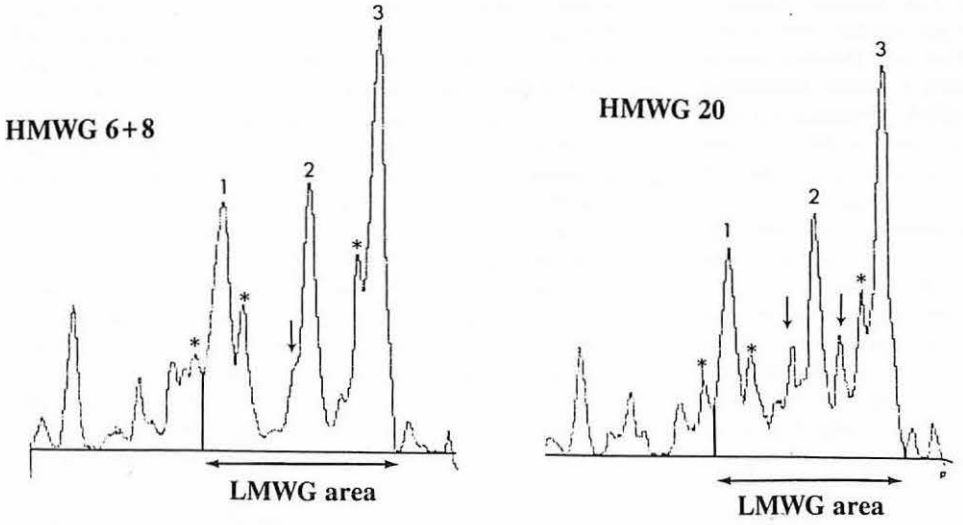

Figure 8. Densitometric profile of the basic region of IEF patterns containing LMWG bands for two durum cvs. belonging to 'γ-45' allelic type, but differing in HMWG allelic type: (A) cv. 'Agathe' (HMWG type '6+8') and (B) cv. 'Amidur' (HMWG type '20'). Major peaks correspond to major LMWG spots in two-dimensional separations. Labeled (*) peaks correspond to the minor LMWG components indicated in Fig. 6. Arrows indicate HMWG contaminants.

upon room temperature storage of the gels, preventing the application of high voltages after one week of storage. No oxidation effects were observed when the gels were used the day after their preparation and the migration time remained short (2 h, compared to 5 h in Altland's study). In addition, when 8 M urea concentrations were used, dried and then rehydrated gels were more convenient and reliable than standard gels, the latter frequently presenting polymerization faults such as a lack of adhesion to polyester supports and pronounced pH drifts.

In addition, the ultrathin layer IEF gels on polyester backings can be used as the first dimension of two-dimensional separations and, as previously reported by Görg *et al.* [25], seem more reliable as far as reproducibility of band positions along the IEF axis is concerned (better resistance to stretching), and are easier to handle than fragile tube gels. In the case of wheat proteins extracted by the water/acetonitrile/mercaptoethanol solvent, because the most basic components enter the gel after loading the sample at the anode, the two-dimensional patterns obtained by using our IEF system in the first dimension are expected to resolve all groups of storage proteins. These maps are obtained easily, without alkylating the proteins and avoiding the two different first dimensions as used in the studies of Holt *et al.* [12].

Table 1. Estimation of the proportion of LMWG fraction in wheat storage proteins, based on densitometric scanning of IEF gels[a)]

A) 'γ-42' genotypes

HMWG type	<---------- '6 + 8' ---------->						<---------- '20' ---------->			
cv.	WA 6291-1	Kidur-1	Cando-1	WA 6291-2	Kidur-2	Cando-2	97	Tomclair	Cargivox	Cargitoro	Mean
Proportion of LMWG	20.96	21.34	20.03	20.70	23.55	22.18	21.55	25.20	24.45	24.24	22.41
Standard deviation	3.30	1.89	2.27	4.20	3.70	1.83	3.38	5.23	1.40	2.68	1.81
Gluten viscoelastic index (mm)	0.65	0.85	1.19	0.89	0.60	1.34	1.21	0.90	1.00	0.55	0.92

B) 'γ-45' genotypes

HMWG type	<---------- '6 + 8' ---------->				<---------- '20' ---------->						
cv.	Agathe-1	Agathe-2	Mondur	76	Alpidur	Pastour	878	D78035-1	D78035-2	Amidur	Mean
Proportion of LMWG	27.30	28.62	29.27	29.50	33.95	33.48	35.03	35.43	35.00	33.09	32.07
Standard deviation	1.63	2.53	2.04	2.41	4.07	3.21	5.30	3.88	3.34	2.66	3.06
Gluten viscoelastic index (mm)	1.45	1.44	1.69	1.59	1.57	1.43	1.57	1.55	1.50	1.47	1.53

a) Mean values, standard deviation, relation with viscoelastic index of gluten

4.2 LMWG characterization and quantitation

As previously reported (11), characterization and quantitation of LMWG fractions cannot be carried out from densitometric scanning of SDS-PAGE patterns because of overlaps between LMWG and various major γ- or ω-gliadin bands, as illustrated by two-dimensional separations. IEF patterns are more suitable because of the very basic character of LMWG subunits. So far, however, no satisfactory IEF pattern, showing a well-resolved LMWG area, has been reported. Following Holt *et al.* [12], most of the studies were based on two-dimensional NEPHGE × SDS-PAGE fractionations, with a first dimension in tubes, making it almost impossible to characterize and compare the LMWG components. Our system, therefore, permits for the first time LMWG subunits to be accurately characterized from a large series of samples and from an easy single-step experiment. IEF patterns also offer an interesting possibility of quantitation since only one HMWG subunit (No. '8' or No. '20') and one faint α-gliadin band may contaminate the LMWG region, making estimation of the relative proportions of LMWG much more reliable than from SDS-PAGE gels. In this study (Table 1), the proportions of total wheat proteins accounted for by LMWG fractions are: 22.4 % ± 1.8 % for genotypes 'γ-42' and 32.1 % ± 3.1 % for genotypes 'γ-45'. If the cvs. belonging to HMWG '6+8' or HMWG '20' allelic type are considered separately, the proportions are the following: 21.5 % ± 1.1 % (genotypes 'γ-42' + HMWG '6+8'); 24.6 % ± 0.5 ('γ-42' + HMWG '20'); 28.7 % ± 1.0 % ('γ-45' + HMWG '6+8'); 34.3 % ± 1.0 % ('γ-45' + HMWG '20'). These results essentially confirm for a larger number of genotypes the proportions previously calculated [11] from densitometric scanning of purified fractions (ion-exchange chromatography) containing variable amounts of LMWG and separated in an SDS-PAGE gel: 14 % and 27 % for two cvs. belonging to 'γ-42' and 'γ-45' types, respectively, both containing the same HMWG '6+8' allelic type.

The results in Table 1 indicate that, independently of whether HMWG contaminant is taken into account of not, densitometric scanning of IEF patterns permits a highly significant discrimination of durum wheat cvs. and lines into two distinct groups on the basis of the proportion of LMWG basic bands. These two groups match those based on native PAGE of γ-gliadins, *i. e.*. 'γ-42' and 'γ-45' types, respectively, which also significantly differ from their gluten viscoelastic index: 0.92 ± 0.26 mm (types 'γ-42') and 1.53 ± 0.08 mm (types 'γ-45'). This develops further the concept that the difference in the level of gluten viscoelasticity between the two main durum wheats types might be primarily determined by the amount of LMWG fraction in the wheat kernel or semolina. However, when considering separately the subgroups of cvs. belonging to the 'γ-42' or 'γ-45' type, respectively, it is no longer possible to relate the variation in viscoelastic index to different proportions of LMWG. For instance, considering γ-42 genotypes, in which there is a wide range of viscoelastic properties (from 0.55–1.34 mm), the relative proportions in the LMWG-1 group range from 20.03–23.55 % among HMWG '6+8' types and from 24.24–25.20 % among HMWG '20' types, but no correlation can be found with the viscoelastic index (mean values: 0.96 mm and 0.84 mm, respectively). The same is true in 'γ-45' genotypes, in which the variation in viscoelastic index is even narrower (from 1.43 mm to 1.69 mm). This suggests that quantitative (but also qualitative) differences in LMWG fraction clearly permit differentiation between the two main groups of durum wheat cvs., and that the rate of expression of the relevant genes is likely to explain the difference in quality expression, evaluated by the gluten viscoelastic index. Within these groups, however, variation of the total ratio of LMWG synthesized during grain development does not seem to modulate significantly the quality expression.

It is possible that qualitative (structural) differences between LMWG-1 and LMWG-2 protein fractions (or between some of their components) could account for differences in the quality potential between genotypes 42 and 45 and that the composition of minor components (including HMWG allelic types) might explain quality differences within the 'γ-42' and 'γ-45' groups. However, the small variations in composition of expression rate of the various LMWG subunits must be further investigated. The preliminary analysis of two-dimensional patterns has indicated that such variations do occur, so that a better understanding of the function of LMWG in the expression of quality is now in progress, based on two-dimen-

sional electrophoresis and image analysis of two-dimensional patterns.

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