Advances in Cereal Science and Technology

Volume V

CHAPTER 5

IDENTIFICATION OF CEREAL VARIETIES BY GEL ELECTROPHORESIS OF THE GRAIN PROTEINS

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I. INTRODUCTION

The many factors that determine the price and suitability for processing of cereal grain can be divided into two groups: seasonal factors and inherited factors. Seasonal factors are largely determined by growing, harvesting, and storage conditions. These aspects of quality are readily tested and include moisture content, test weight, soundness of the grain, and the presence of contaminants.

On the other hand, many other aspects of quality are bred into the seed long before the grower receives it. These factors include milling yield, flour color, and the strength, stickiness, and extensibility of the dough in wheat, and malting quality in barley. The comprehensive testing of these characteristics upon receipt at the grain elevator would be impossible. Fortunately, such testing is not necessary, because these factors are largely defined by varietal specification. Some rapid tests, such as the Bolling test for nonbread wheats (Jonas, 1978), have been devised for on-the-spot identification of grain with undesirable quality. However, most wheat producing countries have adopted a system of assuring the quality characteristics of the grain they receive by restricting deliveries to certain suitable varieties.

Because the prices paid for grain often vary, an ability to identify the variety of

the grain samples is important. Although grain appearance is widely used for preliminary identification, electrophoresis of the gliadin proteins is being adopted in many countries as a routine laboratory procedure for positive identification.

Identification by protein electrophoresis is possible because the proteins are direct products of gene transcription and translation and therefore reflect the genotype and the history of the organism. Zuckerkandl and Pauling (1965) stated: "Of all natural systems, living matter is the one which, in the face of great transformations, preserves inscribed in its organization the largest amount of its own past history." These researchers divided the molecules of living organisms into three categories: semantides (sense-carrying molecules), episemantic molecules (molecules produced by enzymes), and asemantic molecules (not produced by the organism). Only semantides provide reliable information about the identity of the organism. Proteins, classed as tertiary semantides after the genes and mRNA, are thus documents containing information about the identity and history of the organism. This information can be "read" by characterizing the individual proteins with analytical methods such as gel electrophoresis.

Techniques capable of distinguishing among cereal cultivars have been available for more than twenty years (Coulson and Sim, 1964; Elton and Ewart, 1962; Jones et al, 1959). The biochemical methods for routine varietal identification have improved, especially during the past decade, and their development has been accompanied by the discovery that prolamin composition indicates genotype irrespective of growth environment (Lee and Ronalds, 1967; Wrigley, 1970; Zillman and Bushuk, 1979a), and by an increasing need for quality control in grain receipt and handling.

Although electrophoresis is very successful in varietal identification, it is only one of many other procedures, the results of which must be considered in combination with electrophoretic patterns. Often, electrophoresis may not be as suitable as other procedures, which include observation of grain morphology, the phenol and sodium hydroxide (NaOH) tests, growing the seed to observe plant characteristics, and determining specific resistances to plant pathogens.

II. WHEAT

A. Suitable Classes of Protein for Identification

GLIADIN

The three main protein fractions of wheat grain—albumin, gliadin, and glutenin—have been assessed in terms of their suitability for varietal identification. The gliadin proteins are clearly the best and most often used. They are readily extracted and fractionated, and the genetic control of their synthesis is well understood (Sozinov and Poperelya, 1979; Wrigley and Shepherd, 1973). Furthermore, the gliadin electrophoregram is not affected by the growth environment of the grain, by its protein content, by sprouting, dusting, or fumigation of the grain, or by heat treatment up to and beyond that required to destroy baking quality. Significant changes occur in the relative intensities of gliadin bands only when sulfur is severely deficient during growth (Wrigley et al, 1980), and this condition rarely, if ever, exists in commercial crops.

Various extracting solvents have been used satisfactorily for dissolving the gliadin proteins from the crushed grain. The solvent used in the classical Osborne fractionation of grain proteins, a water ethanol (1:2) mixture, is a good solvent for gliadin, but after extraction, its composition must be altered by dilution, and sucrose must be added to make it suitable for electrophoresis (Almgard and Clapham, 1977; Bushuk and Zillman, 1978). Several other satisfactory alternatives include 25% aqueous 2-chloroethanol (Autran, 1975c; Ellis and Beminster, 1977), 6% urea solution (du Cros and Wrigley, 1979), and acidic buffer (Ellis, 1971).

Solutions having a low pH or high concentration of urea (2*M* and more) tend to extract glutenin, which may cause streaking throughout the gliadin pattern. On the other hand, the extraction of albumin proteins with the gliadins is not a disadvantage, because they migrate at pH 3, far ahead of the gliadins.

The following procedures involve several extracting solutions, any of which is satisfactory.

ALBUMIN

The composition of the water-soluble and salt-soluble proteins (albumins and globulins) of common wheat (*Triticum aestivum*) differs little among varieties (Hussein and Stegemann, 1978; Nitsche and Belitz, 1976). The albumin and globulin proteins of the grain are thus poorly suited to varietal identification. However, this varietal uniformity makes the water-soluble grain proteins suitable for comparisons between species because of the obvious differences in composition at this taxonomic level. Electrophoretic analysis of this class of proteins has thus proved valuable in determining the presence of *T. aestivum* in durum wheat products (Cubadda and Resmini, 1970; Feillet and Kobrehel, 1972; Windemann et al, 1973). Specific staining of the electrophoretic gel for the enzymic activity of water-soluble proteins sometimes has provided useful distinctions between varieties (Almgard and Clapham, 1977; Hussein and Stegemann, 1978).

GLUTENIN

Glutenin, the least soluble portion of gluten, is less amenable to fractionation than gliadin, but suitable separation techniques have recently been devised, including sodium dodecyl sulfate (SDS) gel electrophoresis. This procedure, which fractionates the glutenin proteins as the reduced polypeptide subunits, provides useful distinction between varieties (du Cros et al, 1980; Hussein and Stegemann, 1978; Shewry et al, 1978a). Because the synthesis of glutenin is under different genetic control from that of gliadin (Lawrence and Shepherd, 1980), glutenin analysis should provide different information about genotype than gliadin analysis.

LEAF PROTEINS

Gel electrophoresis of the proteins extracted from plant leaves shows similar patterns for different varieties and species of wheat. The procedure is useful for comparisons at the genomic level between different cereals (Wrigley and Webster, 1966). Distinction has been made between wheat varieties by staining for esterase isozymes after gel electrophoresis of extracts of seedling leaves (Menke et al, 1973), but the need to wait approximately eight days for seedling growth is a disadvantage, compared with direct extraction of dry grain.

B. Sample Preparation

Because electrophoretic identification is basically a comparative technique, authentic samples of the varieties must be used. Standard samples from a central wheat collection should be compared to pedigreed seed in use locally. A routine practice is to group samples, so that those of the same declared variety are examined together on the same gel that has an authentic sample of the variety.

Examination of a wheatmeal sample is recommended for initial electrophoretic identification because the act of grinding serves to average the contributions of many seeds, thus providing an indication of purity as well as identity. The fineness of grinding is not critical, but if the sample is only coarsely ground, a portion larger than 1 g must be taken to avoid possible sampling error.

If the sample is heterogeneous, grains must be examined individually, and the results must be statistically analyzed. Each grain can be crushed with a hammer or a pair of pliers or cut into small pieces with a scalpel. Paulis and Wall (1979) described a small "mill" for single grains.

Knowledge of the phenol reaction of individual grains is helpful for preliminary identification. Phenol-treated grains should be soaked in water to remove excess phenol before they are extracted for electrophoresis (Wrigley and McCausland, 1975). Alternatively, the end or some of the bran layer from a dry grain should be cut off, and the cut surface of the piece should be placed on phenol-soaked paper (Wrigley, 1976a). The bulk of the grain is then available for electrophoretic analysis.

A major advantage of electrophoretic analysis is that it can be applied to milled products, to various processed foods (Wrigley, 1977a), and to grain that is pinched, immature, sprouted, fumigated, or discolored. Satisfactory results can be obtained with samples that are quite old (stored for as long as 125 years), but examination of archeological samples has not been successful (Zeven et al, 1975).

C. Starch Gel Electrophoresis of Gliadins

PRINCIPLE

Electrophoretic separation of gliadins produces a pattern of bands that is characteristic for the variety. Early investigators of gliadin fractionation demonstrated the potential value of starch gel electrophoresis (Bourdet et al, 1963; Coulson and Sim, 1964; Doekes, 1969; Elton and Ewart, 1962; Feillet and Bourdet, 1967; Graham, 1963; Lee and Wrigley, 1963). Ellis (1971) first proposed a systematic key based on starch gel electrophoregrams (electrophoretic patterns) and later suggested other tests (phenol test, kernel hardness, and coleoptile color). Identification required several days and could not be applied to samples containing a mixture of varieties. More recently, the potential of this work was translated into an effective procedure of varietal identification based on starch gel electrophoresis of gliadins alone (Autran, 1973; Autran and Bourdet, 1973, 1975a; Wrigley and Shepherd, 1974).

In the most commonly used methods, electrophoresis is done in acidic buffered starch gel, which acts as support medium. At such a pH, gliadin proteins are positively charged, and in the applied electric field they migrate towards the cathode and separate into individual bands according to the electric charge density and molecular size. After electrophoresis, the gel is stained to reveal the

location of the protein bands (Fig. 1). These patterns (electrophoregrams) are related to genetic constitution and are the "fingerprints" of varieties. Because most wheat varieties have unique gliadin patterns, the variety of an unknown sample can be identified by its gliadin electrophoregram. If a sample is suspected of being a mixture of varieties, the single kernel technique can be used to determine the varieties in the mixture and to obtain information about the composition (Autran and Bourdet, 1975b, 1975c; Wrigley and Baxter, 1974; Wrigley and McCausland, 1975).

The following procedure is used regularly in many European countries to check the adherence to the varietal specification of contracts, especially when discrepancies exist in technological tests.

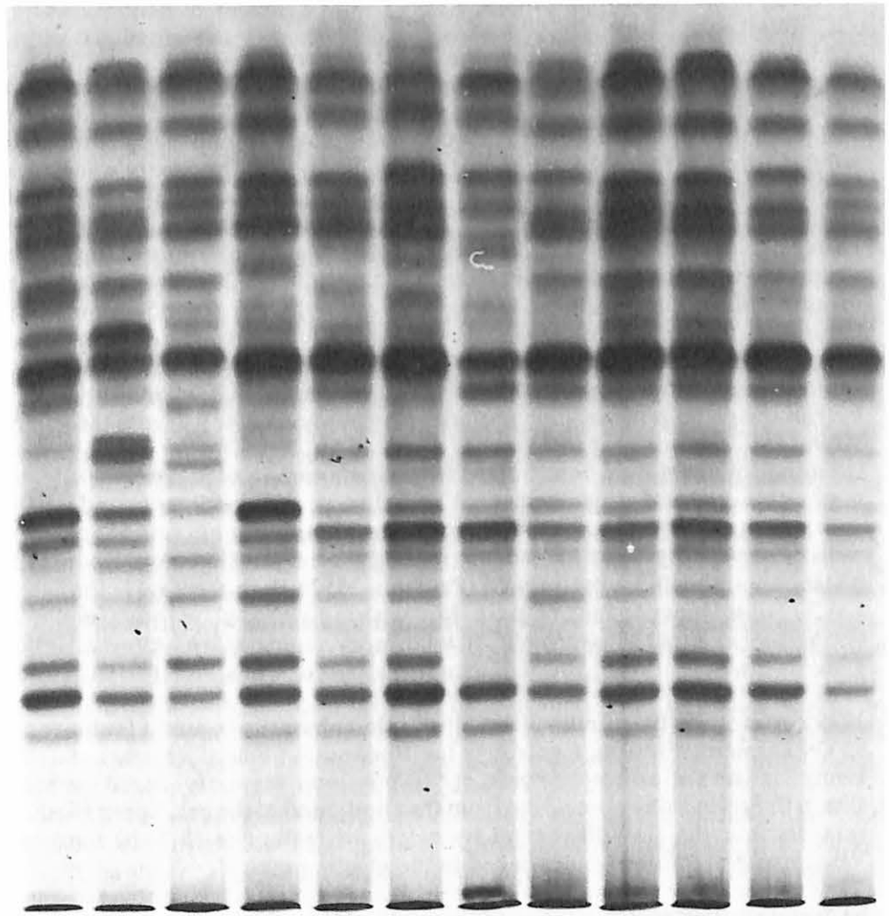


Figure 1. Electrophoregrams of gliadin proteins extracted with 2-chloroethanol 25% on 10% starch gel containing aluminum lactate (pH 3.20). Time of electrophoresis: five hours at 8 V/cm. Varieties are (from left): Valmy, Bocquiau, Rudi, Roazon, Capitole, Eloi, Rafa, Vilmorin 53, Wattines, Cappelle, Rémois, Top. (From Autran, 1979)

APPARATUS

The apparatus, which is made of acrylic sheet, comprises the gel compartment and two electrode buffer tanks. It is available from Apelex (92220 Bagneux, France) or from O.S.I. (75739 Paris Cedex 15, France).

In the routine procedure the gel is cast in the gel former (300 × 170 × 9 mm), the ends of which form bridges that cause the gel to be in direct contact with the buffer (Autran, 1979). At the recommended voltage, overheating does not occur, and the gel need not be cooled. However, control of gel temperatures is recommended to improve the quality of the electrophoregram. Other apparatuses are available that use cool water for higher voltages (Wrigley and McCausland, 1975).

The power supply should be capable of delivering 400 V and 100 mA.

PROCEDURE

The following procedure employs the Apelex apparatus.

1. To prepare the gel former, close the lower openings to the bridges with adhesive tape. Insert a sheet of glass (285 × 169 × 2 mm) into the gel former and place the apparatus in a horizontal position. Aluminum lactate buffer (μ = 0.0045, 0.5 *M* urea, pH 3.20) is used to prepare the gel. Prepare two volumes of buffer solution. Heat the first one (360 ml) to boiling, and mix the second one (120 ml) carefully with 50 g of hydrolyzed starch (Connaught Laboratories, Toronto, Canada) in a 1-L beaker. Add the boiling buffer to the starch in suspension and mix vigorously in a blender for 30 seconds. Pour the resulting slurry into the gel former and cool the gel for 45 minutes at laboratory temperature or in a refrigerator. Finally, remove the adhesive tape and place the gel former horizontally on the electrode tanks filled with buffer solution, and cover the gel with plastic film to limit dehydration.
2. Prerun the gel to remove ionic impurities by applying 250 V across the gel (8 V/cm) for 75 minutes at a current of 35–40 mA.
3. Gliadin proteins should be extracted from single kernels to prevent the effects of possible contaminants. Place each kernel between folded paper and crush with a hammer or pliers. Transfer the crushed grain into microtubes or into wells of a microtiter plate and add extracting solvent (25% 2-chloroethanol in water containing 0.2% pyronin G) (Prolabo, 75526 Paris Cedex 11, France). Use 3 μl of solvent per 1 mg of grain. Mix with individual glass rods and allow extraction to proceed overnight at laboratory temperature. Alternatively, gliadins can be extracted with 1 *M* urea from flour or wheatmeal (du Cros and Wrigley, 1979). Extraction time can be reduced to one to two hours (Autran, 1979) or even to 10 minutes if an ultrasonic apparatus is used (Technicon, 95330 Domont, France). Centrifuge for 10 minutes to clarify extract.
4. Using a lancet and an acrylic guide, cut 10-mm-long, regularly spaced vertical slits in the gel in a line about 3 cm from the anodic end of the gel. Apply gliadin samples into the slots in the gel by means of rectangles (5 × 10 mm) of Whatman No. 3 filter paper soaked in the gliadin extracts.
5. Turn on the power supply (8 V/cm of gel) and continue the electrophoresis until the pyronin G dye marker has migrated 17 cm (about five hours).
6. Turn off the power supply. Release the gel from the gel former using a scalpel. Lift out the 2-mm glass plate and insert a 3-mm glass plate of identical size. Slice the gel horizontally with stainless or nylon wire. Discard the top and transfer the bottom portion of the gel that remains on the plate into a plastic container for staining. Submerge the gel in staining solution (500 ml of 0.05% nigrosine and 2% trichloroacetic acid in water) and leave overnight.

RECORDING RESULTS

Electrophoretic bands are clearly visible after overnight staining. Staining may be hastened by raising the temperature or dye concentration (Aragoncillo et al, 1975). After staining, excess nigrosine should be removed from the gel by transferring it to 40% ethanol solution. The electrophoregrams can be interpreted within one hour. Results can usually be assessed by direct examination of the gel, but if a permanent record is required, the gel can be photographed in reflected light or scanned in a recording densitometer using reflected light. The gel can also be stored for several weeks in 95% ethanol solution.

DISCUSSION

Starch gel was the first support medium that gave a satisfactory resolution of gliadins to make the procedure effective for varietal identification. The starch gel procedure has several other advantages. Simple equipment is used that does not require cooling; the fastest-moving gliadin bands have good resolution; and the support material (starch) is nontoxic.

However, starch gel also has many drawbacks (Autran et al, 1981). Resolution is influenced by stirring and heating conditions of the starch slurry, and resolution of the slowest-moving gliadin bands is poor. Uniform and reproducible gels are difficult to make because the consistency of commercial batches of starch varies; pre-electrophoresis is generally required for consistent results. Gels must be sliced before interpretation and therefore must be thicker, increasing the cost. Densitometer scans are of questionable accuracy. Application with paper rectangles requires very concentrated extracts. In spite of these disadvantages, however, the starch gel procedure, introduced in 1975, is still routinely used in many European laboratories for wheat variety identification. Interpretation of electrophoregrams derives from a scheme of varietal formulas (Autran and Bourdet, 1973) (Table I). The electrophoregram of each variety comprises about 20 bands. A total of 50 different gliadin bands have been identified among European wheat varieties.

A chemotaxonomic key, similar to that used for botanical flora, was developed for wheat. It is based on the presence or the absence of certain specific bands and, through a dichotomic approach, unambiguously identifies most of the varieties grown in European countries (Table II).

So far, the starch gel procedure has been used only on a small laboratory scale. At least one attempt has been made to scale up and automate the procedure using the "Gliaphore" apparatus (Technicon Company, 95330 Domont, France). The apparatus can analyze as many as 100 kernels per day, but because the electrophoregrams obtained by this procedure are significantly different from those obtained by the more commonly used procedure, it is more effective for identifying a small number of excluded varieties than for identifying a large number of varieties, using the published key.

TABLE I
Relative Intensitites[a] of the Starch Gel Electrophoregrams of Six French Wheat Varieties

Relative Mobilities[b]	Capitole	Top	Hardi	Roazon	Talent	Lutin
21						
22	+	+	+	+	+	+
25						
26	++	+++	+++	+++	++	++
28						
30	+	++	++	+	+	+
33						
34	trace	trace	trace	trace		
37						++
39	+	+	+	++		
41						trace
43				+		trace
44	+	+	+		+	
45				++		++
46	++	++	+++		++	
49	+	+	+	+++		+++
50						
52						
53	+	+	trace		trace	
55						trace
56	+	+	+		+	
57						trace
59						
60						
62	++	+	+	+	++	++
65	+++	+++	+++	+++	+++	+++
68	++	+	+	+	trace	
69						
71	+	+	+	+	+	+
72	+				trace	
74		++	++			++
75				++		
77	+	trace	++		+++	++
79	+++	+++	+	+++	++	trace
80						
81	++	++	+++	++	+++	+++
82						trace
83	++	++		+++		
85						
86						trace
88						
90		+++	++	+++	++	++
91	++				+	
93						
94						
96	+++	+++	+++	+++	+++	+++
98	trace	+	+	+	trace	
99						
100						
105						

[a] Absence, trace, or presence (+, ++, or +++).
[b] From 0 to 105 relative to an arbitrary mobility of 65 of a selected reference band.

D. Homogeneous Polyacrylamide Gel Electrophoresis of Gliadins

PRINCIPLE

One-dimensional electrophoresis on uniform polyacrylamide gel permits the separation of proteins, based on their difference in net charge, size, and shape. The polyacrylamide gel serves as the stabilizing support medium for the electrophoresis. The rectangular gel bed may be held in a horizontal or a vertical position, depending on the apparatus used. As in other one-dimensional systems, a single band may represent one protein or several different proteins of the same mobility, resulting from a particular combination of the three fundamental properties indicated above. Two-dimensional electrophoresis (Mecham et al, 1978) or a combination of electrophoresis and electrofocusing run in perpendicular directions (Wrigley, 1970) can be used to determine if a band represents one or more proteins, but this is seldom required for variety identification. This method (Bushuk and Zillman, 1978) will be referred to as uniform PAGE. This method was initially adopted over the starch gel method of Autran and Bourdet (1975a) because it was easier to prepare reproducible uniform gels from acrylamide than from starch.

TABLE II
Chemotaxonomic Key for Varietal Identification of the 15 Most Extensively Grown Bread Wheats in Europe[a,b]

Specific Electrophoretic Bands	Variety
Presence (+++) of component 60	Etoile de Choisy
Absence of 60	
Presence (++) of 37	Lutin
Absence of 37	
Presence (+ or ++) of 39	
Presence (+) of 33	Corin
Absence of 33	
Presence (+) of 53 and (++) of 56	
Presence (++) of 74	
Presence (++ or +++) of 81	
Presence (++ or +++) of 77	Hardi
Absence or trace of 77	Top
Absence of 81 with presence (++) of 80	Wattines
Absence of 74	Capitole
Absence of 53 and 56	
Presence of 75	Roazon
Absence of 75	Rudi
Absence of 39	
Presence (+++) of 49	Rivoli
Absence or trace of 49	
Presence (++) of 74	
Type 90 (++) − 96 (+++) for α-gliadins	
Presence (++) of 85	Maris Huntsman
Absence of 85	Champlein
Type 90 (+) − 1 (+) − 96 (+) − 98 (+) for α-gliadins	Castan
Absence of 74	Talent
	Castan

[a] Based on starch gel electrophoregrams presently used in France.
[b] From Autran (1975a, 1975b, 1975c).

APPARATUS

The electrophoresis chamber is constructed of rigid acrylic plastic according to the measurements shown in Fig. 2A and B. Electrodes (removable for cleaning) are made of stiff platinum wire (0.04 cm in diameter) attached to male "banana" plugs. The lead wires of the power supply were fitted with insulated banana jacks, thus facilitating their connection to the electrodes. Any suitable power supply can be used, but the one used by Bushuk and Zillman (1978) was the Model 500 supplied by Bio-Rad Laboratories. Temperature was controlled by a circulating water bath such as the Ultrathermostat from Colora. Besides being easy to construct from inexpensive materials, the apparatus incorporates several useful features, including a relatively long gel slab (241.3 mm) for improved resolution, efficient gel cooling, relatively small electrode buffer wells, and single-unit construction. Included as ancillary equipment are a sample grinder (e.g., Cyclone Sample Mill, Tecator, Inc., fitted with a 1-mm screen), a small mortar and pestle for grinding single seeds, a microsyringe (e.g., 50-μl Drummond disposable tip micropipet), a small bench-top centrifuge (e.g., Precision with 15-ml tubes), and a staining and destaining tray (e.g., 17 × 25 cm polyethylene Frig-O-Seal food saver).

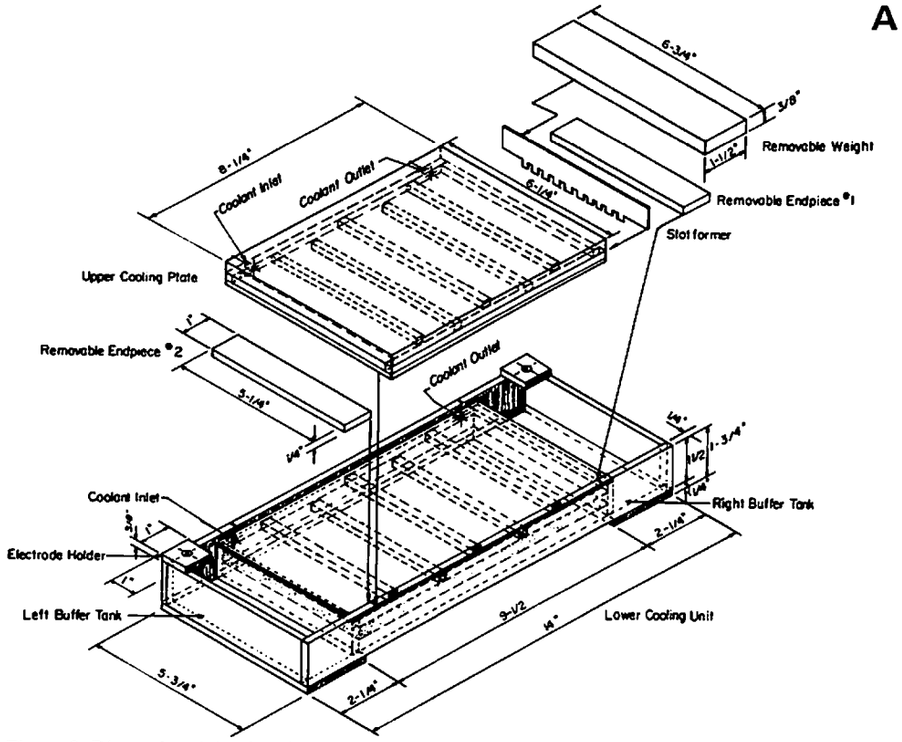


Figure 2. Dimensional details of the apparatus for variety identification by uniform polyacrylamide gel electrophoresis. All material is acrylic. Slot former is Teflon™. All joints are fused with solvent. All exposed edges should be buffed. A, whole apparatus; B, detail of upper cooling plate; C, detail of slot former; D, detail of lower cooling unit. (From Bushuk and Zillman, 1978)

REAGENTS

1. Gel solution: Dissolve 12.0 g of acrylamide, 0.6 g of *N,N'*-methylenebis-acrylamide, 0.2 g of ascorbic acid, 0.005 g of $FeSO_4$, and 0.3 g of 60% sodium lactate syrup in distilled water and adjust to pH 3.1 with lactic acid. Add distilled water to make 200 ml of solution. (The original procedure used 0.5 g of aluminum lactate instead of the sodium lactate.)
2. Catalyst solution: Aqueous 3% hydrogen peroxide solution.
3. Tank buffer: Dissolve 1.5 g of sodium lactate (60% syrup) in distilled water to make 1,000 ml of solution. Adjust pH to 3.1 with lactic acid.
4. Staining solution: Dissolve 0.1 g Coomassie Brilliant Blue R in 10 ml of 95%

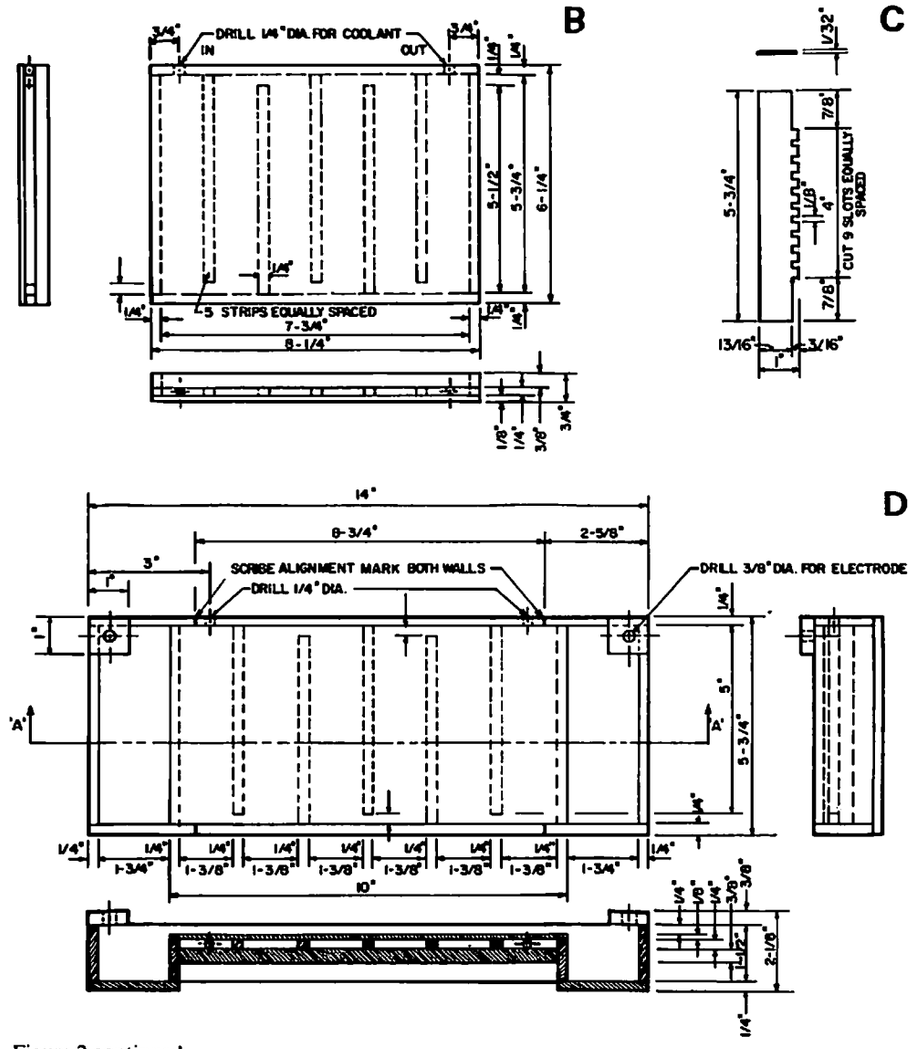


Figure 2 continued.

ethanol, filter through glass wool, and add to 250 ml of 12% trichloroacetic acid.
5. Destaining solution: 250 ml of 12% trichloroacetic acid.
6. Gliadin solvent: 70% aqueous ethanol.
7. Powdered sucrose.
8. Methyl green dye.

Sodium lactate can be obtained from Fisher Chemicals, aluminum lactate can be obtained from Research Organic/Inorganic Chemical Corp. (Sun Valley, CA), and Coomassie Brilliant Blue R from Sigma Chemical Company (St. Louis, MO). The hydrogen peroxide was purchased locally. All remaining chemicals are of reagent grade and were obtained from Fisher Chemicals.

PROCEDURE

Preparation of Gliadin

1. Extract a sample (0.5 g) of wheat meal (1 mm sieve) or flour with three times its weight (1.5 ml) of 70% aqueous ethanol in a stoppered centrifuge tube. The mixture is vortexed periodically during the extraction period of 60 minutes at room temperature. If the grain sample may be a mixture of varieties, meal of single kernels should be extracted.
2. Centrifuge the contents for 10 minutes at 20,000 g at room temperature.
3. Decant the supernatant into a clean test tube and mix with two times its volume (2 ml) of tank buffer.
4. Dissolve powdered sucrose in the sample solution to a concentration of 30% w/v to increase the density and facilitate sample application.
5. Add methyl green (0.02 g) to serve as a tracking dye during electrophoresis.

Preparation of the Gel

1. To prepare the apparatus for gel polymerization, insert the two removable acrylic end pieces into each end of the lower cooling unit, thus forming a gel tray. Allow the coolant (water at 21°C) to circulate through both the upper and lower gel cooling plates for five minutes.
2. Chill 200 ml of gel solution in a 400 ml beaker to 1°C before adding the catalyst. Add one ml of peroxide catalyst, swirl the mixture briefly, and pour quickly into the gel tray.
3. Immediately place the upper cooling plate over the gel solution. To avoid trapping air bubbles under the cooling plate, bring one edge of the plate into contact with the edge of the gel solution, and then gradually lower the other end. Through this method, excess gel and air are forced out over the far edge of the gel tray.
4. Place the slot former (usually 10-place) against the end of the upper cooling plate so that the slot teeth protrude into the uncovered portion of the gel solution. Hold in place during polymerization with a heavy strip of acrylic that also covers the remaining end of the gel solution, thereby making the gel of overall uniform thickness.
5. During gel polymerization, which occurs quickly, do not disturb the apparatus for 5–10 minutes before removing the three removable pieces of acrylic and the slot-former. Remove these carefully and without disturbing the gel.
6. Position the electrodes in the buffer compartments so that they extend the entire width of the gel and are parallel to each end. Then fill both compartments with tank buffer to a level even with the upper surface of the gel.

Sample Application

1. Just before the start of electrophoresis, carefully deposit 20 μl of sample solution into each slot with a microsyringe. Sample application is easier if the slots are first filled with buffer so that the sample solution, which is more dense, settles uniformly into the slot, displacing the buffer.
2. The two outer slots are generally used for the reference sample (e.g., Marquis), while the remaining eight slots accommodate the test samples.

Electrophoresis

1. Connect the lead wires to the electrodes and to the power supply, making certain that the slot end of the gel is the anode.
2. Perform the electrophoresis at a constant current of 100–110 mA. This corresponds to a voltage of about 16 V/cm at the beginning of the run, which decreases to 14 V/cm by the end.
3. To maximize the separation of gliadin bands, continue electrophoresis for 30 minutes after the second dye band has migrated out of the gel. This usually results in a total run time of 5–5.5 hours.
4. At the conclusion of electrophoresis, remove the upper cooling plate, and transfer the gel to a staining tray. The transfer is best done with a thin sheet of acrylic cut to the same width as the gel and with one end bevelled. Carefully slide the acrylic sheet under the gel and lift to remove the gel.

Staining

1. To stain the protein bands, allow the gel to remain in the Coomassie Brilliant Blue solution for 48 hours. Cover the staining tray to prevent evaporation during this period. Gentle agitation during staining diminishes precipitation of the dye on the gel surface. Precipitate detracts from the appearance of the stained gel and subsequent photographs.
2. To maximize the clarity of the stained bands, destain the gel for 24 hours in 250 ml of 12% aqueous trichloroacetic acid. Use a cotton swab to remove precipitated dye adhering to the gel surface.

Photography

1. After destaining, transfer the gel from the tray onto a sheet of clear glass and rinse with water.
2. Illuminate the gel from below by means of a fluorescent light box and photograph on Kodak 5069 high-contrast copy film. Develop the film with D19 developer.
3. Print on Kodak Ektamatic SC type F paper. Conditions of printing vary among individual negatives. Figure 3 shows electrophoregrams obtained by this procedure for Canadian wheat varieties.

APPLICATION FOR VARIETY IDENTIFICATION

Visual Comparison of Electrophoregrams

For routine identification, the electrophoregram of the unknown sample is compared visually with the electrophoregrams of known varieties in the photographic library. With some experience, this comparison can be made fairly quickly. If necessary, the identity can be confirmed by running the extracts of the unknown and the known variety as adjoining electrophoregrams in a single gel. For a numerical record or for computer matching of the electrophoregram, the

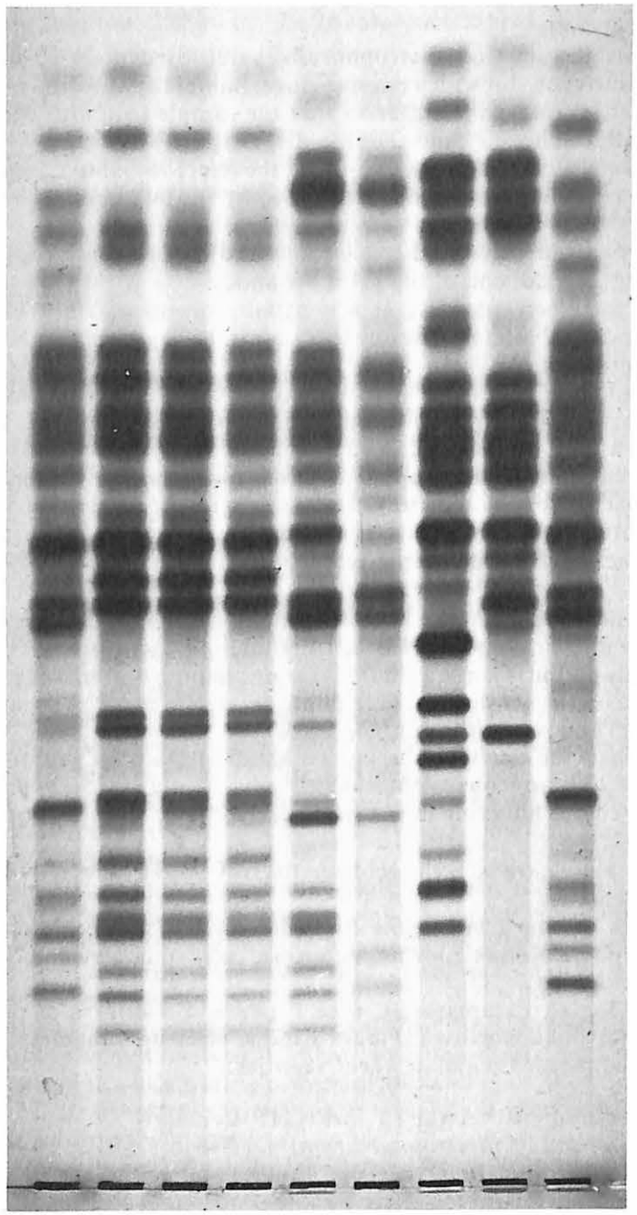


Figure 3. Typical gliadin electrophoregrams on 6.0% polyacrylamide gel containing sodium lactate (pH 3.1) for Canadian wheat varieties. From left to right: Marquis, hard red spring (HRS), Neepawa (HRS), Manitou (HRS), Thatcher (HRS), Glenlea (HRS), Fredrick (soft white winter), Wascana (durum), Wakooma (durum), and Marquis (HRS).

following variety formula procedure below should be used.

Derivation of the Variety Formula

The variety formula is a numerical representation (digital signature) of the electrophoregram. The formula facilitates recording of results and also makes possible the matching of electrophoregrams by means of a computer. In the system developed at the University of Manitoba (Bushuk et al, 1978), each band in the electrophoregram is represented by two numbers; the first represents the relative mobility, and the second represents relative intensity. Both numbers are derived by comparison with the values for an arbitrary reference band in the electrophoregram of the Canadian hard red spring wheat variety, Marquis. Two procedures have been developed for deriving the formula. Sapirstein (1981) described a procedure that uses densitometric measurements of band mobility and intensity. An explanation follows of the other procedure, which uses a manual measurement of the migration distance and a subjective measurement of band intensity. Computer analysis of the digital signature for the purpose of variety identification has been done successfully by Jones et al (1980) and Bushuk et al (1978). Wrigley et al (1981) also used the computer to match gliadin electrophoregrams (starch gel), but with a different nomenclature than the one described here.

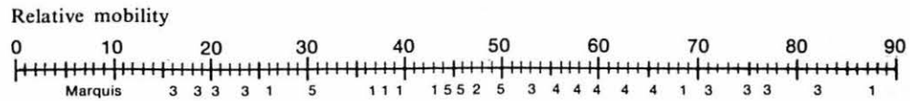
The derivation of the varietal formula for Marquis is shown in Fig. 4. The Marquis reference band, readily identified because it is followed by a distinct doublet, is assigned an arbitrary relative mobility (r.m.) of 50, and its migration distance is measured to the nearest millimeter. Similarly, the migration distances of all other visible bands are measured, and the relative mobility values are calculated to the nearest half unit (right). With an appropriate linear scale based on 50 for the reference band (left), the relative mobilities can be read directly.

Next, the band intensities are quantified subjectively by a number from one to five, with five representing the most intensely stained bands. This can be done by visual examination of the electrophoregram, with one "intensity reference" band chosen to represent each relative intensity (r.i.).

The varietal formula for Marquis can be written in two ways. In the first formula, the r.m. of each band is represented by the first number, and the r.i. band is shown in parenthesis. For computer handling of this formula, the decimal can be omitted.

16.0(3)	18.5(3)	20.5(3)	23.5(3)	26.0(1)	30.5(5)	36.5(1)
38.0(1)	39.5(1)	43.0(1)	44.5(5)	45.5(5)	47.5(2)	50.0(5)
53.0(3)	55.5(4)	58.0(4)	60.0(4)	63.0(4)	65.5(4)	68.5(1)
71.0(3)	75.0(3)	77.0(3)	82.0(3)	87.5(1)		

In the second formula, the position of each band is identified by locating the appropriate r.i. number under the appropriate r.m. number on the linear relative mobility scale (top). This arrangement is particularly suited for comparing a large number of varieties, which can be listed in tabular form under a single mobility scale.



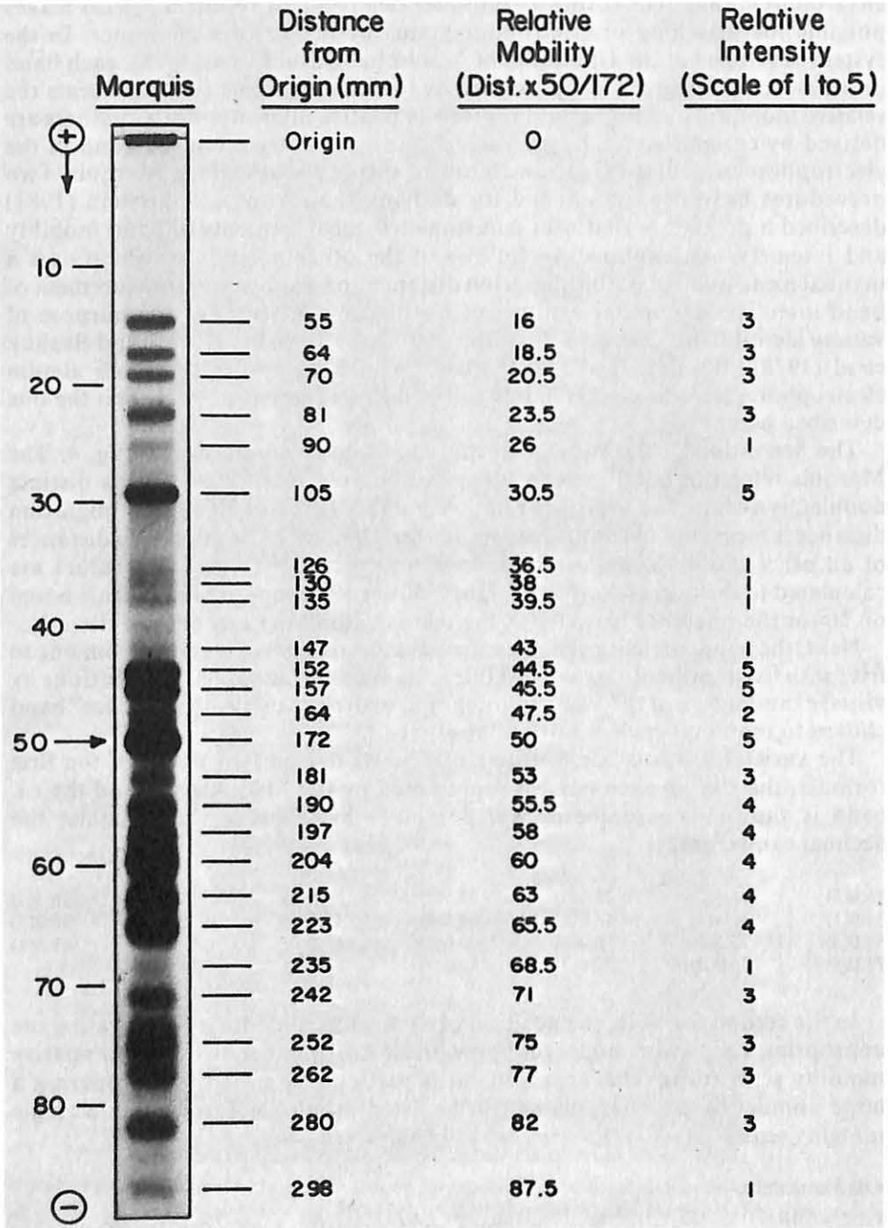


Figure 4. Derivation of the varietal formula from the gliadin electrophoregram for the variety Marquis. (From Bushuk and Zillman, 1978)

NOTES ON FLAT BED PAGE METHOD

1. Acrylamide monomer is a toxic chemical that is absorbed directly through the skin, so caution should be exercised when handling the crystalline powder, the solution, or the gel.
2. The hydrogen peroxide, which initiates gel polymerization, gradually decomposes during storage. Thus, freshly prepared peroxide solutions should be used routinely. Optimum concentration is approximately 3%. Lower and higher concentrations produce inferior gels that are soft and tend to swell extensively during staining, making them difficult to handle. The optimum concentration should be established for each source of hydrogen peroxide by trials with several different concentrations.
3. The formation of bubbles in the gel during polymerization is due to dissolved air coming out of solution and occurs when the gel solution is mixed too vigorously before pouring.
4. A common problem encountered in flatbed gel electrophoresis is band sloping. Band sloping can cause closely migrating bands to appear as one continuous band when viewed perpendicular to the gel surface. One method to minimize the effects of this problem is to minimize the volume of sample applied to the slot. In this way, the height of the column of sample is minimal, and the sloping is less apparent. Very little protein is actually required for the stained band to become visible.
5. Since the current, and hence the rate of protein mobility, is sensitive to changes of temperature, the coolant must be maintained at a constant temperature throughout electrophoresis.
6. The time required for electrophoresis to be completed at 110 mA varies somewhat with the voltage. The voltage drop at constant current is affected by the purity of the chemical components in the gel, especially in acrylamide monomer, which varies significantly in conductivity.
7. Excessive staining of the gel should be avoided because the dye, which penetrates deeply into the gel matrix, will not easily be removed by destaining, and faint bands can be masked. When destaining is completed the gel should be photographed as soon as possible in order to retain the faint bands, which fade after a few days of storage.
8. Transferring the gel during staining and destaining can be done using a sheet of flexible plastic cut slightly larger than the size of the gel.

E. Gradient Gel Electrophoresis of Gliadins

PRINCIPLE

The use of a commercially available precast polyacrylamide gel is considerably advantageous in routine examination of large numbers of samples by staff without extensive laboratory experience. The 14-sample gel is provided ready for use in cassettes, so the handling of the toxic acrylamide monomer is avoided, and gel preparation time is eliminated. These gels, used in many branches of biochemistry, are available in a standard size (about 8 cm square) from a number of suppliers. Prices differ considerably. Figure 5 shows the gliadin electrophoregrams for representative wheat varieties.

In the gradient gel, the concentration of polyacrylamide increases from the top point of sample application to the bottom (Margolis and Wrigley, 1975). This

provides the advantage of optimal gel porosity for all sizes of proteins in a mixture. As the protein molecules move down a gradient gel, their mobilities decrease progressively as the increasing gel concentration restricts migration. The protein zones are consequently sharpened, and resolution is enhanced.

Whereas charge differences are accentuated in the early stages of electrophoresis, differences in molecular size become the basis of separation when migration becomes restricted by the pore size of the gel after prolonged electrophoresis. This can result in many zones being closely spaced in a mixture such as gliadin with many components of similar size. A linear gradient of 2.5–13% gel (Fig. 5) (Wrigley, 1980) is optimal for the gliadins, in combination with electrophoresis for about 400 V hours (one hour at 400 V). This gel is available

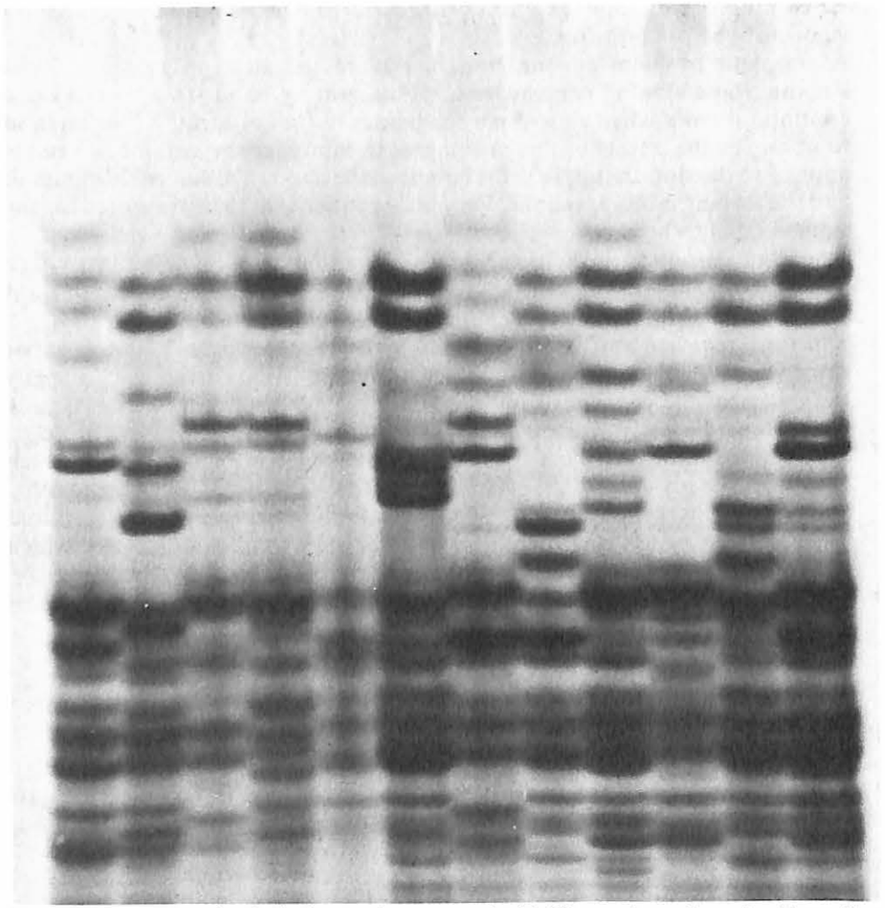


Figure 5. Electrophoresis of gliadin proteins, extracted with 6% urea, in a polyacrylamide gradient gel (2.5–13%) containing sodium lactate, pH 3. The origin and anode are at the top. Time of electrophoresis is two hours at 30 V/cm. Varieties (from left) are Scout 66, Inia 66R, Capitole, Diplomat, Marquis, Chinese Spring, Eagle (Australian), Halberd, Millewa, Olympic, Jabiru, and Lance.

from Gradient Pty. Ltd. (Pyrmont, N.S.W., 2009, Australia) and from Universal Scientific Ltd. (London, U.K.), and is similar to the 2–16% gel from Pharmacia AB (Uppsala, Sweden).

In the following procedure, sodium lactate buffer system gave better resolution than aluminum lactate, which traditionally has been used for gliadin electrophoresis. Furthermore, gel destaining was omitted by using a staining procedure based on that of Diezel et al (1972).

The entire procedure is comprised of four main steps: the prerun of the gel (to incorporate the desired buffer), application of samples, electrophoresis, and overnight staining.

APPARATUS

In this routine procedure, the gel slab (72 × 72 × 3 mm) is held between two glass plates (82 × 82 × 1 mm) with a spacing strip (82 × 5 × 3 mm) down each side. A plastic sample spacer is inserted between the glass plates on top of the soft end of the gel. This whole gel cassette is held vertically by a rubber gasket so that the gel slab has contact at top and bottom with the respective electrode solutions. Suitable apparatuses are available from Gradient Pty. Ltd., Pharmacia AB, Universal Scientific, and Isolab Inc. (Akron, Ohio). Facilities for longer and wider gels are also available. The power supply should be capable of delivering 400 V and about 50 mA for each gel cassette.

PROCEDURE

1. With the sample spacer in place, insert the gel cassette into the rubber gasket of the upper electrode compartment. Prevent the entry of air bubbles between the gel and the glass plates by moistening the bottom of the cassette, if necessary. Fill the electrode compartments with sodium lactate buffer (0.017% sodium hydroxide adjusted to pH 3.1 with lactic acid), and apply 200 V for about one hour (or 400 V for 30 minutes) with the lower electrode negative (cathode).
2. After gel and buffer have been equilibrated in the prerun, apply 10 μl of gliadin extract in each of the 14 sample positions across the top of the gel (low concentration end). (Prepare these extracts by mixing 50 mg wheatmeal or flour with 0.3 ml of 6% urea and centrifuging to clear the extract.)
3. Apply 400 V for one hour (or 200 V for two hours) at 25°C. These times may have to be reduced appropriately if the temperature of the buffer increases significantly.
4. After electrophoresis, remove the gel cassette, peel the tape off one side, and pry the two glass plates apart. Mark the position of the first slot by cutting a corner off the gel, and place the gel into a tray containing 95 ml of 12% trichloroacetic acid solution. Mix in 5 ml of 0.25% Coomassie Blue G250 solution (most of it precipitates) and leave overnight for staining.

RECORDING RESULTS

Electrophoretic bands are visible within approximately one hour. Staining may be hastened by raising the temperature. Results can often be assessed by direct examination of the gel, but if a permanent record is required, the following procedure should be followed. Store the wet gel in a sealed plastic bag. Air-dry the gel (loosely held between sheets of plastic mesh) after soaking for one hour in

acetic acid-water (1:12), for one hour in acetic acid-methanol-water (1:5:14), and overnight in glycerol-methanol-water (1:11:8). Photograph the gel by transmitted light, using a red filter to enhance contrast, or scan the gel, using a recording densitometer.

DISCUSSION

The main advantages of the gradient gel method are its convenience and suitability for routine checking of many samples. Although the small gel size does not give large separation of band zones, it does facilitate handling and storage, and shortens the time of electrophoresis. The cost of buying ready-made gels adds to the overall cost of analysis, but it must be balanced against the time and cost of gel preparation.

Variations in relative mobilities are expected in gradient gel electrophoresis, because of the progressive increase in molecular sieving during the run. However, this effect is not serious in a 2.5–13% gel, for which the average variation in relative mobility from 400 to 450 V hours was less than 1%. Good reproducibility of patterns has been obtained irrespective of minor variations in the time of electrophoresis.

Gradient gel electrophoregrams for the systematic identification of Australian wheats have been published by du Cros et al (1980). For identifying these varieties, gradient gels were preferred over starch or uniform-pore polyacrylamide gels (du Cros and Wrigley, 1978, 1979), but Redman et al (1980) preferred 6% polyacrylamide to gradient gels for distinguishing between English wheats. Gradient gel electrophoresis satisfactorily identified Californian wheats (Qualset and Wrigley, 1979).

F. Gel Isoelectric Focusing of Grain Proteins

PRINCIPLE

Whereas methods of gliadin fractionation have involved adapting a technique originally devised for proteins of another type, much of the early development of gel isoelectric focusing was done using grain proteins from wheat (Wrigley, 1968) and from soybean (Catsimpoolas, 1968). Its potential for distinguishing between wheat varieties was realized at this early stage (Wrigley and Moss, 1968). It has since been applied to a wide range of seed proteins (Wrigley, 1977c) as well as throughout biochemistry, either alone or in combination with gel electrophoresis (Wrigley, 1970).

Gel electrofocusing was one of the first electrophoretic methods to be adopted as an official AOAC method of analysis (Lundstrom, 1980). In this application (identification of fish species), the method is said to be particularly "forgiving" of small errors or variations in experimental conditions (Lundstrom and Roderick, 1979).

The information about protein composition provided by gel isoelectric focusing is likely to differ from that obtained with gel electrophoresis because the principles of separation are different. Whereas gel electrophoresis involves the migration of proteins at uniform pH with consequent separation according to size and charge, the protein molecules in isoelectric focusing are focused within a pH gradient to the point at which each protein loses its overall charge, that is,

where the pH of the support medium equals the isoelectric point of the protein. A two-dimensional combination of the two methods has thus proved useful, particularly for distinguishing between closely related varieties (du Cros and Wrigley, 1979; Hussein and Stegemann, 1978; Qualset and Wrigley, 1979).

The pH gradient in which isoelectric focusing occurs is formed by applying an electric field to a mixture of carrier ampholytes in a stabilizing medium such as polyacrylamide gel. The ampholytes used here have a spectrum of isoelectric points from three to 10. Thus, as they focus in the electric field, they form a continuous range of pH from three at the positive end of the gel to 10 at the cathodic end. The pH gradient is completed with paper strips containing a strong acid (1*M* phosphoric acid) at the anode and a strong base (1*M* sodium hydroxide) at the cathode. The cost of carrier ampholytes restricts the routine use of this method, though it can be overcome by synthesizing them in the laboratory (Vinogradov et al, 1973).

APPARATUS

Gel isoelectric focusing is generally performed in a flat thin layer of polyacrylamide, and samples are applied on the surface of the gel. Alternatively, vertical tubes may be used in a disc-gel electrophoresis apparatus. The brief details that follow apply to the former type of gel, but procedures for both have been published by Leaback and Wrigley (1976). Suitable apparatuses may be obtained from a range of suppliers. In addition, preformed gels are available from LKB Produkter, Bromma, Sweden.

PROCEDURE

1. Prepare the gel-forming cell to receive the gel reagents premixed in the following order:

Urea	3.6 g
Water	16.9 ml
Carrier ampholytes (pH 3.5–10.4)	1.5 ml
Acrylamide stock solution	7.5 ml
Riboflavin (0.01%)	1.5 ml

This provides 30 ml of 7.5% gel containing 2*M* urea. Pour the gel mixture into the gel-forming cell and illuminate it with a fluorescent tube about 10 cm from the cell for about 30 minutes. Alternatively, the gel may be chemically polymerized (without the need of illumination) by replacing the riboflavin solution with an equal volume of potassium persulfate (10 mg/ml). The acrylamide stock solution contains 30 mg acrylamide (pure grade) and 1 g *N,N'*-methylenebisacrylamide per 100 ml solution.

2. Place the gel on the cooling plate of the apparatus, preferably on a film template imprinted with positions to apply samples (du Cros and Wrigley, 1979). Protein zones are focused from any part of the pH gradient, so the position of their application is not critical; they may be applied anywhere between electrodes. To apply, place a piece of filter paper soaked with gliadin extract on the surface of the gel. Apply other samples in sequence across the gel, parallel to the positions of the electrode strips.
3. Place strips of filter paper, soaked in either 1*M* sodium hydroxide or 1*M*

phosphoric acid, across the ends of the gel. Place the electrodes on these (cathode and anode, respectively), and apply an electric field, progressively increasing the voltage to about 50 V/cm. After about 2.5 hours, remove the gel and place it in a solution of 3.5% perchloric acid containing 0.06% Coomassie Blue for 1.5 hours, preferably after a preliminary soak of 39–60 minutes in 12% trichloroacetic acid. Destaining is not necessary.

4. Alternatively, if the isoelectrically focused gel is to be used for further fractionation by two-dimensional analysis (Fig. 6), do not fix or stain it, but place the appropriate strip of gel in the sample position of a starch or acrylamide gel for electrophoresis (Wrigley, 1976b). If SDS gel electrophoresis is to be used in the second dimension, soak the isoelectric gel for 20–30 minutes in the electrode buffer, containing 4% SDS, before electrophoresis.

RECORDING THE PATTERN

Techniques for photography and drying of polyacrylamide also apply to isoelectric gels.

DISCUSSION

Gel isoelectric focusing has not proved popular for routine identification of wheat cultivars, even though it is capable of making such distinctions (Almgard and Clapham, 1977; Wrigley, 1968). This is partly because differences in gliadin composition tend to be obscured by the presence of the water-soluble proteins that are extracted with the gliadins in the simpler extraction procedures.

On the other hand, the combination of isoelectric focusing with gel

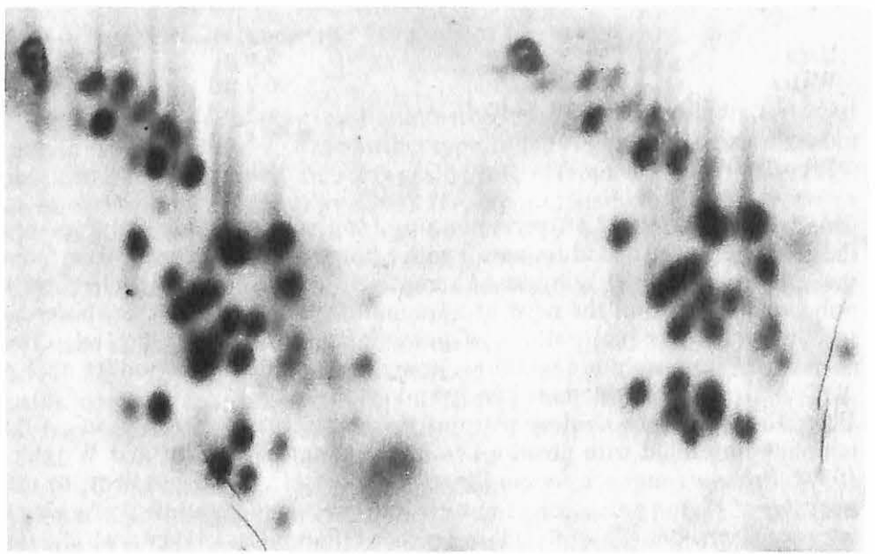


Figure 6. Distinction between cultivars (Shasta, left; and Inia 66R, right) with similar gliadin composition by two-dimensional analysis involving gel isoelectric focusing (pH 5, left; pH 9, right) followed by starch gel electrophoresis at pH 3 (origin at top). (From Qualset and Wrigley, 1979)

electrophoresis is useful in distinguishing between closely related wheats (du Cros and Wrigley, 1979; Hussein and Stegemann, 1978; Qualset and Wrigley, 1979; Wrigley, 1976b). In this application (Fig. 6), isoelectric focusing has revealed differences in gliadin composition that would be masked during electrophoresis in only one dimension by other components of similar electrophoretic mobility.

Gel isoelectric focusing (alone or with electrophoresis) is useful as a secondary procedure for supplementing routine gel electrophoresis when discrimination between varieties needs to be increased. It also appears promising in this role in combination with specific staining for esterase, acid phosphatase, or amylase isozymes (Almgard and Clapham, 1977; Nakai, 1979; Nishikawa and Nobuhara, 1971).

G. Electrophoresis of Albumins

PRINCIPLE

Electrophoretic separation of water-soluble proteins produces a pattern of bands that is characteristic of the species (Coulson and Sim, 1964; Doekes, 1969; Elton and Ewart, 1962; Feillet, 1965; Feillet and Bourdet, 1967), and sometimes of the varieties (Nitsche and Belitz, 1976; Ohms, 1980; Silano et al, 1969).

APPARATUS

The electrophoretic apparatus is constructed from acrylic plastic (Kobrehel and Feillet, 1975). It comprises a lower cooling unit, two buffer tanks, and an upper cooling unit. The gel is cast in a gel former (300 × 175 × 6 mm) that includes a slot-former lid (10 slots; 10 × 5 × 1 mm).

Alternatively, two other apparatuses can be used—the horizontal type of Bushuk and Zillman (1978), or the vertical type, such as the GE 4 apparatus from Pharmacia or the Havana from Desaga (Velizy, France).

The power supply should be capable of delivering 400 V and about 50 mA for each gel to be run.

PROCEDURE

1. Dissolve 17.53 g of acrylamide and 0.47 g of *N,N'*-methylenebisacrylamide in 300 ml of distilled water, and filter. Add 1 ml of dimethyl-aminopropionitrile and 0.3 g of ammonium persulfate. Homogenize thoroughly, pour into the gel former and cover with the slot-former lid. After two hours of polymerization, release the gel and soak in 3 L of tris buffer, pH 8.6 (4 g of tris, 0.4 g of EDTA, and 0.3 g of boric acid, per liter). The gel is ready for use after overnight storage.
2. Suspend one gram of wheat flour or meal in 3 ml of tris buffer, pH 8.6. After one hour at laboratory temperature, centrifuge 20 minutes at 35,000 g at 4° C. Use supernatant as sample extract without further purification.
3. Apply extracts to the slots (50 μl). Cover the gel with the upper cooling unit. Run at 5 V/cm (about 400 mA per gel) for 4½ hours.
4. Turn off the power supply. Transfer the gel to a plastic tray and submerge for 30 minutes in staining solution (1% amido black 10B in methanol-acetic acid-water, 45:45:10, V/V). To destain wash continuously with 5% acetic acid solution with circulation through activated charcoal (Feillet and Kobrehel,

1972). Alternatively, destaining can be done by electrophoretic removal of the dye from the gel.

RECORDING RESULTS

Electrophoretic bands are visible after two hours. Results can be assessed by direct examination of the gel. If a permanent record is required, the gel can be photographed or scanned on a recording densitometer with transmitted light.

DISCUSSION

Electrophoresis of albumins is a simple and rapid method that can help varietal identification in some special cases. For example, Silano et al (1969) distinguished four groups of bread wheats and two groups of durum wheats on the basis of albumin electrophoregrams. Nitsche and Belitz (1976) described an albumin fractionation that permitted the distinction of the undesirable variety, Kranich, from nine acceptable varieties. After reduction by 2-mercaptoethanol of soluble proteins, Ohms (1980) succeeded in characterizing a group of German cultivars with good baking quality.

In general, however, the electrophoretic composition of soluble proteins differs little from one variety to another. On the other hand, such varietal uniformity makes the albumins more useful for comparisons between species than between varieties. In addition to methods based on enzyme patterns (Kobrehel and Feillet, 1976), albumin electrophoregrams have been extensively used for determining the presence of common wheat in durum wheat products either through polyacrylamide gel electrophoresis (Fig. 7) (Cubadda and Resmini, 1970; Feillet and Kobrehel, 1972; Garcia-Faure et al, 1969; Resmini,

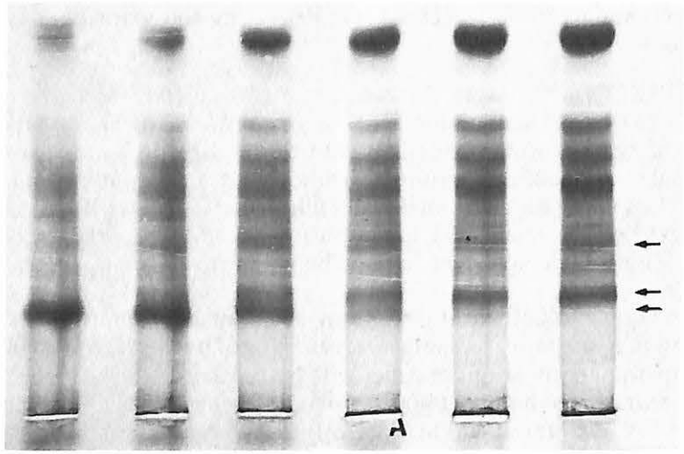


Figure 7. Electrophoresis of soluble proteins, extracted with tris buffer, pH 8.6, in a 6% polyacrylamide gel containing tris buffer. Time of electrophoresis is four hours, 30 minutes at 5V/cm. Samples used are durum wheat pasta containing variable amounts of *T. aestivum*: (from left) 100%, 80%, 60%, 40%, 20%, 0%. (From Feillet and Kobrehel, 1972)

1968; Silano et al, 1967, 1968) or through isoelectric focusing (Resmini and de Bernardi, 1976). Alternative methods based on immunochemical comparisons of antigenic albumins of durum and bread wheats have also been successful (Cantagalli et al, 1969; Hamauzu et al, 1967; Nimmo and O'Sullivan, 1967; Piazzi and Cantagalli, 1969; Piazzi et al, 1972). Common wheats can be identified by a specific albumin component called 0.19 by the Italian scientists and described by Feillet and Nimmo (1970) as albumin 13. More recently, Konarev (1978) showed that the antigenically specific component of common wheat differed from the 0.19 component.

An improved specificity of soluble protein electrophoregrams was obtained by using special staining. Silano and Pocchiari (1968) and Minetti et al (1971) observed that albumins, globulins, and gliadins stained with aniline blue-black gave blue, green, and red bands, respectively. Caldwell and Kasarda (1978) took advantage of differential dye binding of soluble proteins to distinguish varieties and species of wheats by viewing the Coomassie Blue-stained gels through an orange filter.

H. SDS Electrophoresis of Glutenin Subunits

PRINCIPLE

Although the gliadin proteins are generally examined in electrophoretic identification of wheat varieties, the information so obtained can be usefully complemented by analysis of glutenin-subunit composition, since synthesis of the two groups of proteins is under separate genetic control (Lawrence and Shepherd, 1980). Extraction of the glutenin subunits, gliadin, and albumin proteins is obtained by using 2-mercaptoethanol to break disulfide bonds and the detergent sodium dodecyl sulfate (SDS) to disrupt various noncovalent bonds. During gel electrophoresis, the presence of SDS maintains solubility. In addition, it provides fractionation that is mainly based on size differences because the presence of SDS largely cancels out differences in the charge of the proteins themselves. The group of components most appropriate for study (bracketed in Fig. 8) are those of lowest mobility (largest size) or the high molecular weight glutenin subunits. Useful information is also provided by the components of higher mobility.

APPARATUS

Several commercial units are available for SDS electrophoresis in tubes or in a horizontal or vertical slab of gel. A flat gel offers better opportunity for close comparison between samples than separate tubes do. A recent laboratory manual (du Cros et al, 1980) gives detailed instructions for performing SDS gel electrophoresis in apparatuses normally used for gradient gel electrophoresis.

PROCEDURE

Use of a discontinuous buffer system is recommended, together with separate stacking (upper) and separating gels. In addition, the use of a gradient gel (7.5–16% polyacrylamide) improves resolution for smaller polypeptides. The following procedure, however, involves a uniform 8.4% separating gel (du Cros et al, 1980) and is based on the method of King and Laemmli (1971).

1. Extract flour or wheatmeal (8 μl/mg sample) with 0.06*M* tris hydroxymethyl

amino methane (tris) buffer (pH 6.8) containing 4% SDS, 10% glycerol, 0.001% bromophenol blue, and 1.5% 2-mercaptoethanol. Centrifuge at sufficiently high speed to ensure clarity of the extract and absence of streaking in the electrophoretic pattern.

2. Set the lower separating gel in most of the space available for gel formation. It

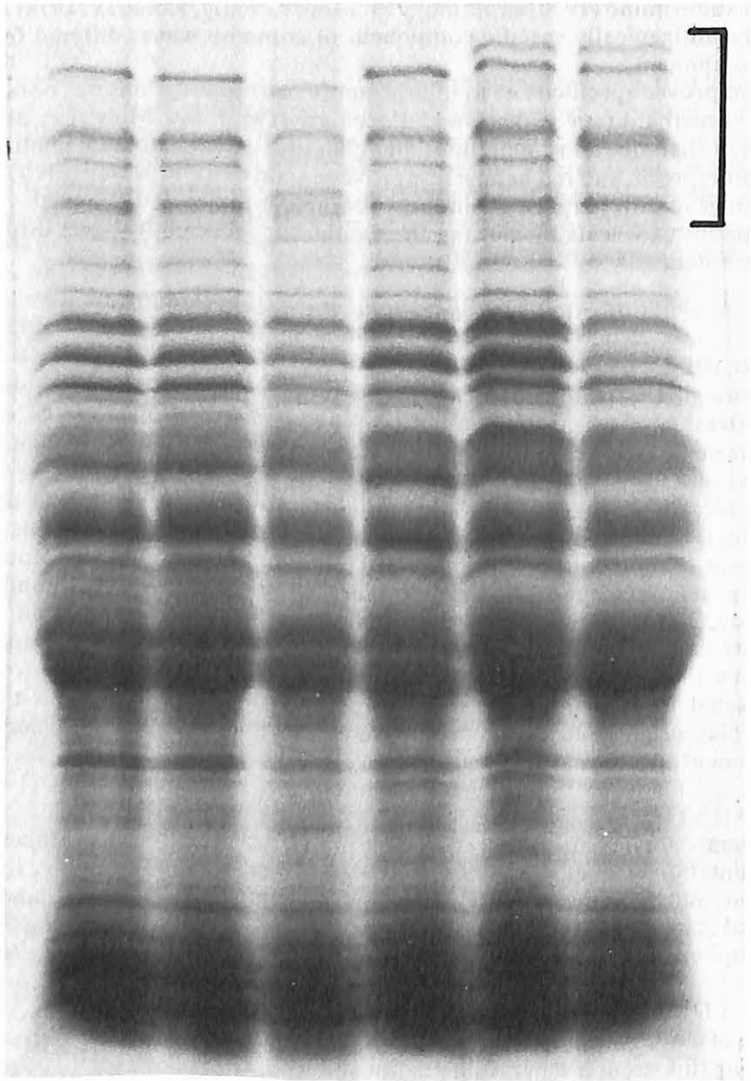


Figure 8. Fractionation of endosperm proteins from the wheat varieties Condor B (left), Condor A, Egret, Oxley, Shortim, and Timson by SDS-electrophoresis in a gradient gel (7.5% polyacrylamide at the top (origin, cathode) to 16%). The high-molecular-weight glutenin subunits are bracketed. Electrophoresis was performed for 3.5 hours at 12 V/cm. (From Wrigley, 1981)

is chemically polymerized from a mixture containing 0.38*M* tris, adjusted to pH 8.8 with HCl, 8.4% acrylamide, 0.7% *N,N'*-methylenebisacrylamide (BIS), 0.1% SDS, 0.1% *N,N,N'N'*-tetramethyl ethylenediamine (TEMED) and 0.013% ammonium persulfate. Form a layer (about 1 cm) of stacking gel on top of the separating gel. Stacking gel comprises 0.13*M* tris, adjusted to pH 6.8 with HCl, 3.0% acrylamide, 0.08% BIS, 0.1% SDS, 0.1% TEMED, and 0.02% ammonium persulfate. Leave the gel overnight before use. Minimize the amount of ammonium persulfate because an excess will modify the electrophoretic pattern.

3. Mount the gel in the apparatus so that the sample proteins migrate into the gel towards the anode (positive). The electrode buffer contains 0.025*M* tris, adjusted to pH 8.3 with glycine, and 0.1% SDS. Apply samples (10 μl for a 2 $\times$ 8 mm area). Turn on the power and continue electrophoresis until the dye front reaches the end of the gel.
4. Remove the gel. Mark the position of the first sample. Soak the gel overnight in 0.025% Coomassie Blue in 5.8% trichloroacetic acid, 18% methanol, and 6% acetic acid. Destain in acetic acid-methanol-water (1:6:13).

RECORDING THE PATTERN

Photograph or scan the gel when it is destained. Contrast between bands and background may be enhanced by overnight storage in a plastic bag at 4° C.

DISCUSSION

The SDS electrophoregrams of the high molecular weight glutenin subunits were listed for 65 Australian wheat varieties (du Cros et al, 1980). The method provided clear distinctions between some varieties with similar gliadin composition. In other cases, a group of varieties showed the same glutenin pattern. The procedure is therefore recommended for verification of identity for cases in which it is more discriminating than gliadin electrophoresis. SDS electrophoregrams and methods have been published for European wheats by Shewry et al (1978a) and by Hussein and Stegemann (1978).

I. Systematic Evaluation of Identity

A systematic approach to identification is important to minimize its cost and to ensure efficiency and accuracy. Such a system is suggested in Fig. 9 (du Cros et al, 1980). Section A of the flowchart is designed to answer the question, "Is the sample true-to-label?" In many cases, no more information than this is needed, but when the answer is negative, there is the inevitable further question, "Then what is it?" Sections B and C of Fig. 9 address themselves to the second question, for pure and for heterogeneous samples, respectively. The approach emphasizes the use of complementary test methods and of electrophoresis of wheat meal samples to minimize the time and expense of unnecessary electrophoretic analyses.

J. Complementary Testing Methods

VISUAL IDENTIFICATION

A trained inspector can provide a reasonably definite identification by

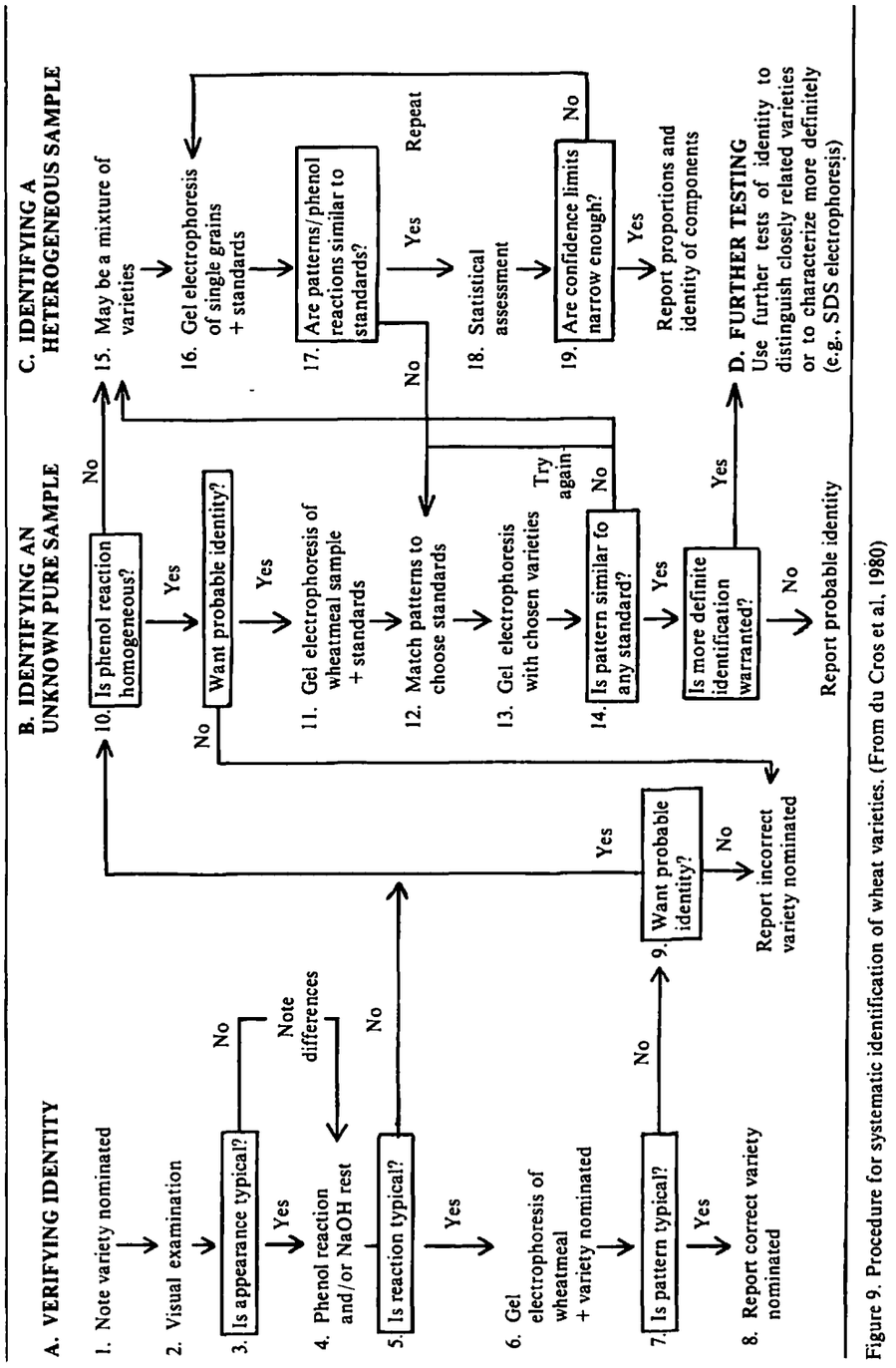


Figure 9. Procedure for systematic identification of wheat varieties. (From du Cros et al, 1980)

examining the shape, size, and morphological features of the grain (Fig. 9, Step 2). Further information may be obtained by examining glumes and awn fragments present in all but the best cleaned samples. Descriptions of varieties to assist in gaining experience are available for wheats of most countries (Bolling and Meyer, 1976; Ferns et al, 1975, 1978; Owen and Ainslie, 1971; Senser, 1976). Grain hardness should be particularly noted for individual grains and for the whole sample, on which quantitative tests of hardness may be applied.

PHENOL TEST

Another procedure for checking the identity of many samples is the phenol test (Fig. 9, Step 4). The test can also indicate purity, because the reactions of grains are shown individually. Furthermore, single grains of known phenol reaction can be chosen for subsequent electrophoretic analysis, following a few hours soak in water to remove excess phenol. However, the distinguishing ability of the test is limited to only three or four categories of color reaction. Its suitability thus depends on the range of reactions of the specific varieties being considered.

Two procedures are available—the rapid test (Wrigley, 1977b) and the traditional test (Anonymous, 1966). For the traditional method, soak grains overnight (or for at least four hours) in water, blot off excess water, and spread them on absorbent paper (e.g., Whatman No. 1 filter paper) moistened with 1% phenol solution. Cover the container and await color development for four hours at 25°C, 2.5 hours at 40°C, or 1.5 hours at 50°C. In the rapid test, soak dry grains in 1% phenol solution for about three minutes, and spread the grains on absorbent paper moistened with 1% phenol solution containing 0.25% ammonia. Major differences in reaction are evident after 15 minutes at 25°C (or 10 minutes at 40°C); full coloration develops after 30–40 minutes at 25°C (or 20–30 minutes at 40°C).

Other alternatives are available, including assay of polyphenol oxidase activity (Lamkin and Miller, 1981) and a test paper incorporating catechol (Wrigley, 1976a). The brown coloration typical of the phenol reaction develops in the outer layers of the grain (maternal tissue). The factors responsible are under the control of genes on chromosomes of homoeologous group 2 (Wrigley and McIntosh, 1975). The test indicates genotype irrespective of growth conditions, except that it may not be reliable with very immature grain. Additional information can be obtained from the phenol reaction of the glumes (chaff) (Wrigley and Shepherd, 1974).

GRAIN-COLOR TEST

Red-grained varieties usually can be distinguished from white wheats by their appearance, but the sodium hydroxide test provides a further basis for making this distinction in difficult cases (Coles and Wrigley, 1976; Kimber, 1971). The test is intended to remove interference caused by differences in grain texture and to enhance the red coloration of the bran. The grains must be soaked in 5% sodium hydroxide solution. After 20–30 minutes, grains of red wheats appear brick-red, whereas those of white wheats are straw yellow. The soaking time is reduced to five minutes if the test is performed at 55°C (Lamkin and Miller, 1980). In systematic identification (Fig. 9), the NaOH test serves a similar function (Step 4) to the phenol test in the verification of identity and the identification of contaminating grains. Such grains may be further examined by

electrophoresis if they are rinsed in water before extraction. Alternatively, the washed grains may be dried and stored for later electrophoretic analysis.

FURTHER ELECTROPHORETIC TESTS

As indicated in Fig. 9, exhaustive electrophoretic analysis of gliadin composition may not always be adequate for definite identification. Several methods may fill this role, depending on the specific varieties being considered. These include staining for specific isozymes after gel electrophoresis, or isoelectric focusing of extracts of dry or soaked grain or of seedlings (Almgard and Clapham, 1977; Hussein and Stegemann, 1978). The following enzyme systems are useful for this purpose: acidic phosphatases (Auriau et al, 1976; Nakai, 1973); amylases (Joudrier, 1974; Joudrier and Bernard, 1977; Nishikawa and Nobuhara, 1971); esterases (Cubadda and Quattrucci, 1974; Menke et al, 1973; Nakai, 1979); and peroxidases (Alexandrescu et al, 1979; Kobrehel and Gautier, 1974). The types of β-amylase and peroxidase isozyme patterns encountered in French wheat varieties are shown in Fig. 10 and Table III. A combination of the two isozyme patterns can be used to distinguish some otherwise indistinguishable varieties. For example, Axel and Fleurus have the same β-amylase type but different peroxidase type.

K. Authenticity of Standard Samples

All of the described methods are comparative; that is, the electrophoretic and the complementary tests compare the results for the sample being tested with those for an authentic sample of each variety being considered. The reliability of the identification thus depends on how genuine the standards are. These should

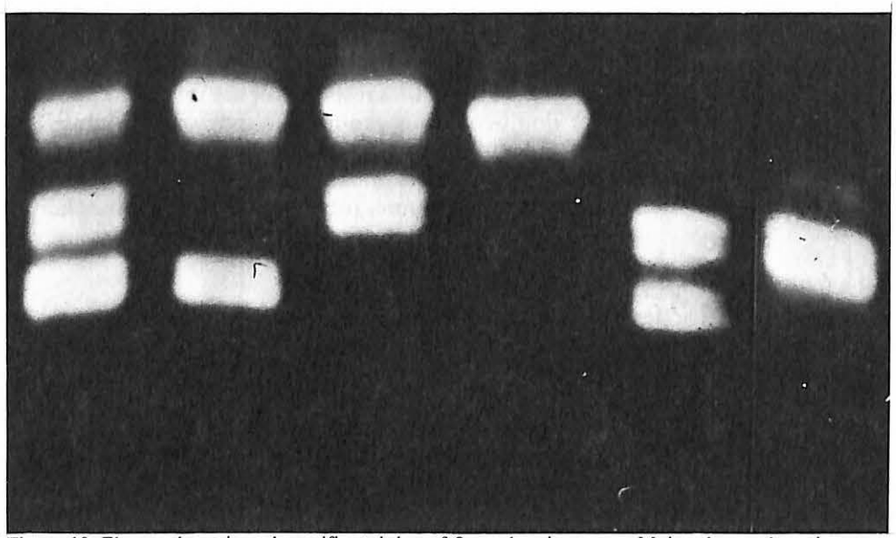


Figure 10. Electrophoresis and specific staining of β-amylase isozymes. Major electrophoretic types discovered among bread wheat varieties (from left): Ciano (type I), Roazon (type II), Heima (type III), Blason (type IV), Azteca (type V), Yaktana (type VI). (From Joudrier, 1974)

preferably be obtained from a central official cultivar collection, but familiarity with local certified seed should also be established.

Certain cultivars are polymorphic for electrophoretic pattern and/or phenol reaction), ie, different kernels give different patterns or phenol reactions, yet all can be shown to be of one cultivar and to have come from the same cross (Appleyard et al, 1979). Multiple biotypes of a cultivar may appear as the result of the selection methods used in their breeding, or they may be caused by the release of a different selection under the same name (Wrigley, 1976c). As many as four biotypes have been reported for a cultivar (du Cros and Wrigley, 1979), though in this case only two biotypes are present as major components. It is important, however, to examine the grains of a standard sample one by one to determine whether the variety is polymorphic, because this information is vital to its identification.

L. Evaluation of Electrophoregrams

In its simplest form, electrophoretic identification involves comparing the electrophoregrams of test samples with that of an authentic sample. A lack of correspondence between the respective patterns may indicate that the sample is not true-to-label or at least that it is contaminated with a variety having a different electrophoregram. Sections B and C of Fig. 9 recommend procedures for determining the true identity in such cases. These suggestions rely on the availability of a catalog of electrophoregrams of the varieties likely to be encountered. If reproducibility of electrophoresis can be assured, matching the pattern of the unknown to those in the catalog may be sufficient for identification. Alternatively, Step 12 would at least indicate a suitable set of

TABLE III
Use of Combined Specific Isoenzyme Electrophoretic Patterns in Distinguishing Closely Related Varieties with Identical Gliadin Patterns

Groups of Varieties with Identical Gliadin Patterns	β-Amylase Type[a]	Peroxidase Type[b]
Axel	IV	A
Fleurus	IV	B
Hardi	II	A
Noroit	IV	B
Top	II	B
Cappelle	IV	A
Ouest	II	A
Blason	IV	B
Capitole	II	A
Moisson	II	B
Splendeur	IV	A
Heima	III	A
Roazon	II	B

[a] From Joudrier (1974).
[b] From Kobrehel and Gautier (1974).

varieties for direct electrophoretic comparison with the unknown (Step 13).

Recent studies (Autran et al, 1979) and the current activities of Study Group 6 of the International Association of Cereal Chemistry (A-2320 Schwechat, Schmidgasse 3-7, Austria) will lead toward establishment of a uniform procedure for electrophoretic identification. Achievement of this aim would open the way for a world-wide catalog of electrophoregrams to be compiled for wheat. On the other hand, the task of pattern-matching with such a catalog, or with any reasonably extensive one, is difficult. This task has been facilitated in published catalogs by arranging the electrophoregrams in the form of a key, so that the presence of certain bands is considered before others (Autran and Bourdet, 1975a; du Cros et al, 1980; Ellis and Bemister, 1977; Wrigley and Shepherd, 1974; Zillman and Bushuk, 1979b).

Alternatively, such a task might be performed using computer-based methods of analysis. The initial step in both cases is transforming the arrangement of stained protein zones in an electrophoretic gel to form a suitable for manual or computerized comparison. This step usually has involved scoring for the presence or intensity of protein zones in a series of predetermined band positions. In addition, densitometric scanning of a photographic transparency has been interfaced to a computer to avoid the subjective assessment of band position and intensity (Bushuk et al, 1978). Autran and Bourdet (1975a) proposed an index of dissimilarity for comparing electrophoregrams in pairs. The computer programs used by Wrigley et al (1981) can select patterns, resembling those of an unknown sample, from a computer-stored catalog of electrophoregrams; a quantitative estimate of the degree of similarity compares the pattern of the unknown sample to each electrophoregram retrieved. Related programs may be used to examine relationships between the electrophoretic bands and other attributes, such as grain quality (Wrigley, 1981).

M. Statistical Evaluation of Results

If a grain sample is a mixture of varieties, electrophoresis of a ground sample provides a limited indication of its composition, and kernel-by-kernel analysis is generally necessary (Fig. 9, Step 16). These results are then analyzed statistically (Wrigley and Baxter, 1974) whether they are obtained by electrophoretic analysis or by phenol or NaOH testing. This involves determining the confidence limits for the proportion of each component. For example, the identification of one kernel of A in a total of 10 kernels examined (10% of A found) indicates that A occurs in the whole sample between 0 and 45% (confidence limits, using a confidence coefficient of 95%). If, on further analysis, 20 grains of A are identified in 200 grains (still 10% of A found), the confidence limits on the proportion of A are reduced to 6–14%. It is thus advisable to use 200–500 grains routinely for phenol or NaOH testing.

Confidence limits for the proportion of a component can be determined for total grain numbers up to 100 by reference to tables of binomial distribution (Beyer, 1968). For larger numbers of grains, the confidence limits are determined from the formula $P \pm 2[P(100-P)/n]^{1/2}$, where P is the percentage of the component found in a total of n kernels examined. After determining the confidence limits for analysis of a set of kernels, further testing may be needed if the limits are too wide for the purpose (Fig. 9, Step 19).

Table IV provides a practical example of the effect of progressive electrophoretic identification on the statistical assessment of analyses of four samples taken from a truckload of grain for forensic purposes. Whole meal was examined by electrophoresis to provide an initial indication of identity and heterogeneity. The results indicated that Timgalen predominated, with Kite as a significant contaminant. Application of the phenol test was not helpful in this case, because nearly all grains gave the same (dark brown) reaction.

Electrophoretic identification of the first nine kernels from the center of the load might have suggested that Kite and Timgalen were present in equal proportions, without also considering the wide confidence limits (8–70% for each) at this preliminary stage. In contrast, the next nine grains examined were all Timgalen, for which the confidence limits changed markedly. Even after identifying 35 grains from this part of the load, the confidence limits were still wide, and a 95% probability existed that varieties present at up to 10% were still undetected.

Identification of a similar number of kernels from other parts of the truckload gave a more complete indication of its composition. Variations in the actual proportions of varieties from one point of sampling to another suggested that the truckload was not homogeneous, but this conclusion is not definitely supported

TABLE IV

Progressive Identification of Samples from One Truckload of Wheat[a]

Sample	Number of Grains Examined	Varieties					
		Timgalen	Gatcher	Kite	Winglen	Condor	Other
Center of truck[b]							
	First 9	33	11	33	0	22	0
		8–70	0–48	8–70	0–33	3–60	0–33
	First 18	67	6	17	0	11	0
		41–87	0–27	4–41	0–19	1–35	0–19
	35	63	9	20	0	9	0
		45–79	2–23	8–37	0–10	2–23	0–10
Rear of truck[c]							
	First 12	67	0	17	0	17	0
		35–90	0–27	2–48	0–27	2–48	0–27
	34	70	6	9	9	6	0
		53–85	1–20	2–24	2–24	1–20	0–10
Front of truck	35	80	9	3	6	3	0
		63–92	2–23	0–15	1–19	0–15	0–10
Preliminary	36	78	11	0	0	3	8
		61–90	3–25	0–10	0–10	1–10	0–10
Total	140	73	9	8	4	5	2
		65–80	4–13	3–13	1–7	1–9	0–5

[a] The first entry for each variety shows its percentage of the number of grains examined by electrophoresis. The confidence limits (based on a confidence coefficient of 0.95) appear immediately below as percentages. (From tables in Beyer, 1968)

[b] Examination of meal showed mainly Timgalen and significant contamination by Kite.

[c] Examination of meal showed mainly Timgalen and some Kite.

when the confidence limits are examined for each sample, as shown by comparing Kite center (20%, 8–37) with Kite preliminary (0%, 0–10).

III. OTHER GRAINS

A. Rye and Triticale

The electrophoretic techniques described for wheat grain identification can be applied to rye and triticale without any modification (du Cros and Wrigley, 1979). For example, Fig. 11 compares the prolamin electrophoregrams of varieties of wheat, rye, and triticale. Although differences are evident between

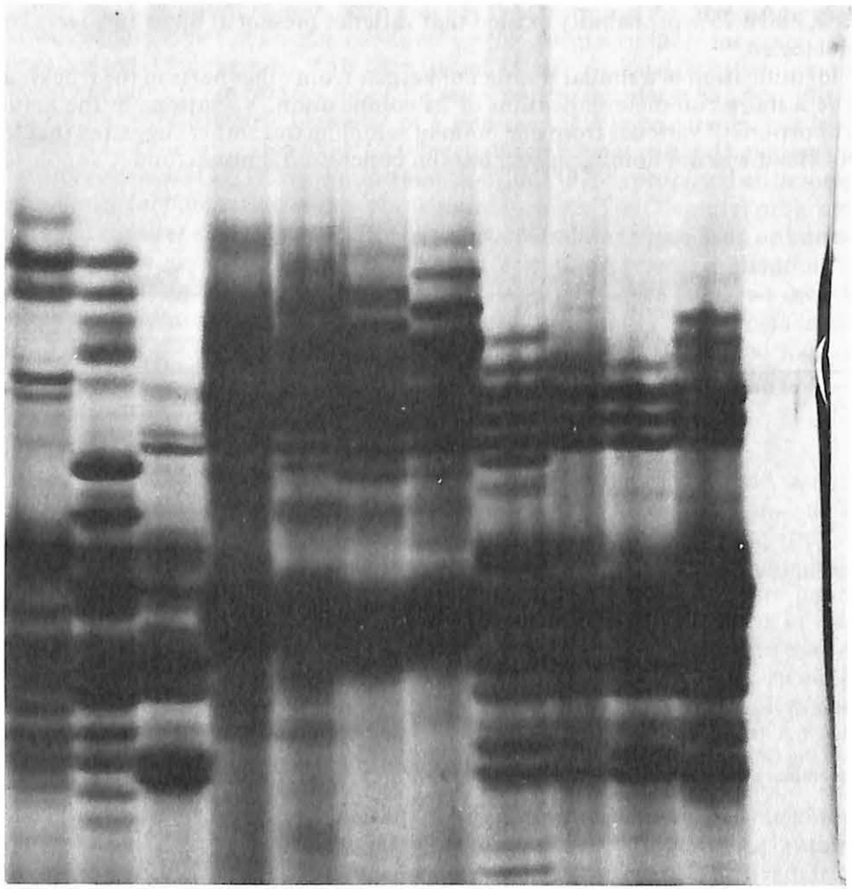


Figure 11. Gradient gel electrophoresis (pH 3) of prolamine proteins extracted from crushed grain samples with 6% urea solution for varieties of wheat (Marquis (left), Diplomat, Halberd, and Duramba), rye (Black Winter Rye, Prolific, Gazelle, and Puma) and triticale (Satu, Coorong, Tyalla, and Siskiyou). The gel contains a linear gradient by concentration of polyacrylamide from 2.5% (top, origin) to 13%.

varieties within each genus, general characteristics distinguish the electrophoregrams of each genus from one another. Such distinctions may be useful, for example, in distinguishing wheat from rye products after processing has removed more obvious distinguishing characteristics. Densitometric scanning of electrophoregrams has been used to determine the proportions of wheat and rye in mixtures (McCausland and Wrigley, 1976).

The phenol reaction of rye grain is controlled by genes on chromosome 2R (Wrigley and McIntosh, 1975). Although a dark phenol reaction is given by grains of many rye and triticale varieties, the test may sometimes make useful distinctions. For example, the Australian triticale Grow-Quick gives a light brown reaction, in contrast to the dark phenol reaction of other triticales grown in Australia (du Cros et at, 1980).

B. Barley

Both the prolamin (hordein) and albumin proteins of the barley grain are useful for varietal identification by electrophoresis. The described electrophoretic and isoelectric focusing methods may be applied to barley. The following modifications are recommended (du Cros and Wrigley, 1979). Add a reducing agent (1% 2-mercaptoethanol) to the extracting solution to promote the extraction of hordein. Reduce the time of electrophoresis by about 50% to retain the albumin proteins on the gel, and slightly decrease the proportion of liquid used for extraction to accommodate the lower protein content usual in barley. Figure 12 illustrates the use of gel isoelectric focusing to distinguish between barley varieties. The full range of electrophoretic methods has been applied to barley identification, including the added step of staining for enzymic activity (Almgard and Landegren, 1974; Autran and Scriban, 1977; Baxter and Wainwright, 1978; du Cros and Wrigley, 1979; Gavrilyuk and Diaguileva, 1975; Gunzel and Fischbeck, 1979; Konarev et al, 1976; McCausland and Wrigley, 1977; Mesrob and Ivanov, 1970; Neyreneuf and Bourdet, 1979; Scriban and Strobbel, 1978; Scriban et al, 1979; Shewry et al, 1978b, 1978c, 1980; Van Lonkhuysen and Marseille, 1978a).

C. Oats

For the identification of oat varieties (or species), grain proteins can be extracted with 2.5*M* urea (6 μl/mg crushed grain) for gel isoelectric focusing (Fig. 13) or for electrophoresis in a 2.5–27% gradient of polyacrylamide (sodium lactate, pH 3) for about 2.3 hours at 200 V (for standard 70 × 70 × 3 mm gels). Figure 13 illustrates the use of gel isoelectric focusing to compare the protein composition of wild-oat grains, fatuoid off-types, and the cultivated oat with which the off-types were growing. Gel electrophoresis of isozymes from grain and from seedlings has also been useful for characterizing varieties and species of oat (Almgard and Clapham, 1975; Dass, 1972; Singh et al, 1973; Smith and Bennett, 1974; Williamson et al, 1968).

D. Rice

Procedures for identifying rice varieties by their grain protein composition

were published by Park and Stegemann (1979) and by du Cros et al (1979). Both groups investigated both gradient gel electrophoresis and isoelectric focusing. Park and Stegemann (1979) found SDS electrophoresis unsuitable for varietal identification, preferring gradient (5–30%) gel electrophoresis (pH 9.9) of the albumin proteins (extracted with 0.02% sodium azide, and stained for protein or esterases). du Cros et al (1979) extracted the grain proteins with 3*M* urea containing 1% 2-mercaptoethanol (4 μl/mg crushed grain). The clarified extract was analyzed by gradient gel electrophoresis or by gel isoelectric focusing. According to Juliano (1980), the proteins being examined in these cases are albumins and globulins, rather than prolamins.

E. Corn

Gel electrophoretic and isoelectric focusing procedures were published for fractionation of maize seed proteins; these appear to be suited to varietal identification in this crop (Bietz, 1979; Drawert and Gorg, 1975; Paulis and Wall, 1979; Righetti et al, 1977; Soave et al, 1977; Stegemann, 1977).

F. Other Grains

Methods of varietal identification by electrophoresis have not been applied so

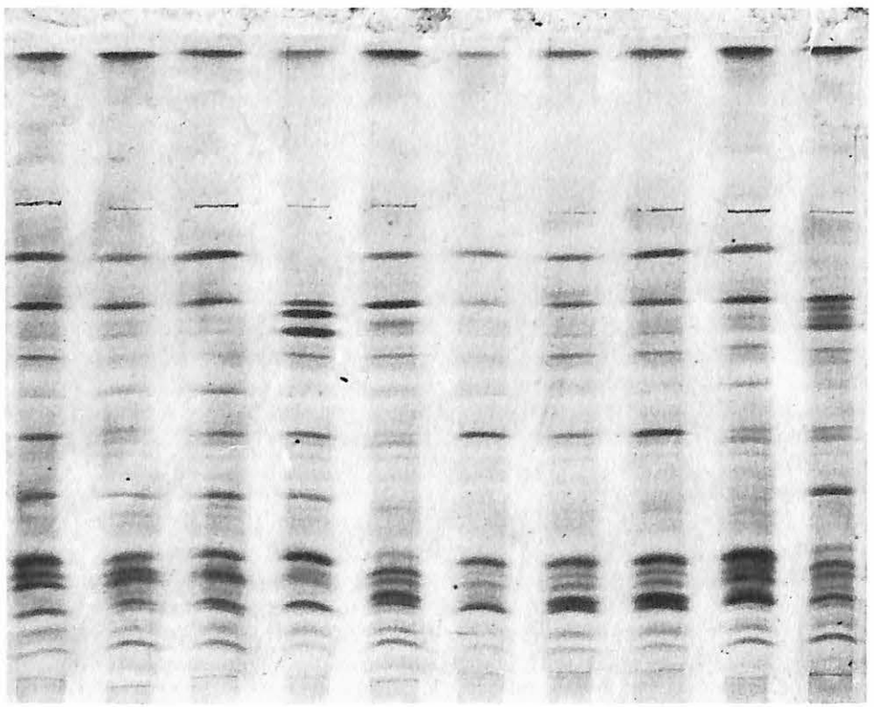


Figure 12. Gel isoelectric focusing (pH 3.5–10) of barley grain proteins, extracted with 6% urea containing 1% 2-mercaptoethanol, of a range of Australian varieties. (From du Cros and Wrigley, 1979)

extensively to other grains, but procedures are available for many, including sorghum (Suh et al, 1977), rape seed (Finlayson et al, 1969; Yadava et al, 1979), peanut (Ory et al, 1979), and soy bean (Llewellyn and Flaherty, 1976).

IV. CURRENT APPLICATIONS

A. European Economic Community

In recent years in Western Europe, several high-yielding wheat varieties totally unsuitable for bread baking—varieties such as Maris Huntsman, Clement, Kranich, Benno, or Anouska—have become increasingly popular. Therefore, the milling and baking industries have taken an increased interest in the quality of wheat varieties. Although variety is not the only factor influencing wheat quality, millers and bakers have become more insistent that the wheat grain they accept be of the claimed variety and free of other, unsuitable varieties. Varietal criteria seemed the best to guarantee a given level of technological quality. For example, they can differentiate bread wheats from nonbread wheats, so that the latter can be segregated after the harvest and excluded from the top grade.

Until 1975–1976, grain and agricultural merchants lacked positive proof of varietal identity, and correct varietal description could only be checked by trained crop inspectors who examined the growing crop before harvest. Great reliance was placed on a methodical approach by contract growers and seed

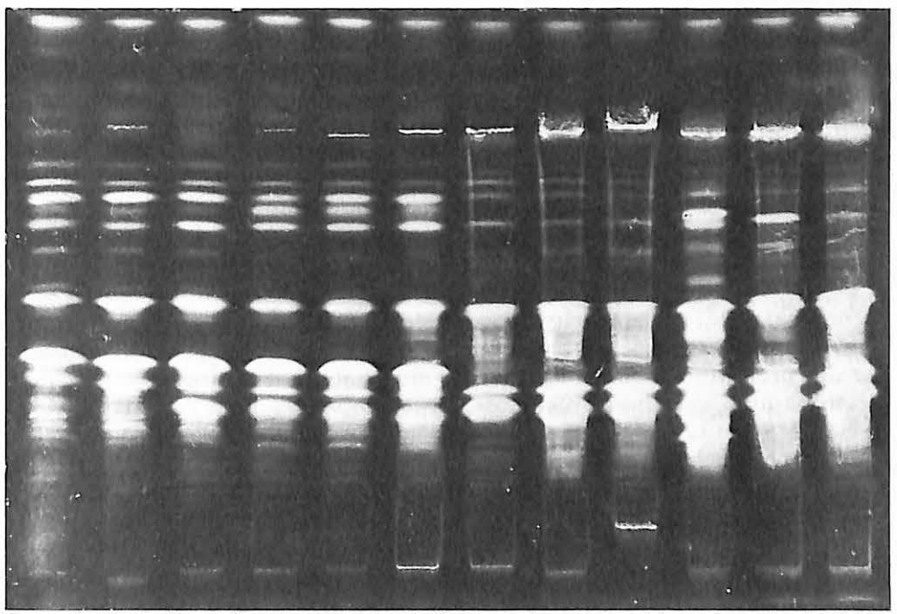


Figure 13. Gel isoelectric focusing (pH 3.5–10) of oat proteins, extracted from the grain with 2.5*M* urea. Each pattern represents a different grain or sample. Electrophoregrams 1–3 (from left) are grains of the variety Cassia; 4–6 are Cassia off-types; 7–9 are *Avena fatua*; and 10–12 are *Avena sterilis* subspecies *ludoviciana*. Conditions: 600 V across an 85 mm gel for 3.5 hours.

processors. Subsamples of seed lots sold had to be retained and grown in control plots to ensure the reliability of the harvesting, delivery, and processing routines. Needless to say, an error discovered at this late stage could not be rectified.

As soon as electrophoresis had proved valid for distinguishing European varieties of wheat and giving positive proof of varietal identity, people accepted the idea of a grain trade based upon variety specification, and two price levels were brought into play. Employees of many European grain companies were trained to carry out electrophoretic tests in their laboratories according to the starch gel procedure of Autran (1975b). In all countries, scientists were made aware of this new tool for improvement of varietal control (Almgard and Clapham, 1977; Bourdet, 1976; Bourdet and Autran, 1976; Colas, 1979; Dal Belin Peruffo, 1980; Ellis, 1976; Ellis and Beminster, 1977; Ewart, 1975; Godon and Autran, 1976; Gunzel, 1976a, 1976b, 1976c; Konarev et al, 1975, 1977; Kuchumova et al, 1978; Maier and Wagner, 1980; Meyer and Nierle, 1977; Nierle, 1976, 1977; Nitsche and Belitz, 1976; Ohms, 1980; Shewry et al, 1978a; Van Lonkuysen and Marseille, 1978a).

The "Groupement des Associations Meunières des Pays de la C.E.E." accepted starch gel electrophoresis of gliadins as the standard method for determining variety and recently submitted it to the European Economic Community.

Actually, grain consumers (millers, semolina manufacturers, and bakers) and seed producers (plant breeders and grain merchants) alike have benefited from electrophoretic tests.

Two types of contracts exist at the consumer level: exclusion of some varieties (such as Maris Huntsman or Clement in bread wheats, and Durtal, Rikita, or Tomclair in durum wheats), and a restrictive list of admitted varieties.

The declaration of identity of grain is made by the seller. This declaration is checked by technological tests, including baking tests. Starch gel electrophoresis, which is still an expensive and time-consuming method (50 kernels must be checked in a mixture of varieties), is not used as a routine test but is used every time abnormal behavior is found in technological tests.

The key for identification (catalog) of the varieties, which recently seemed inaccessible to control laboratories, is now commonly used. In France, this key is updated every year. To ensure proper use of the key, monthly collaborative tests are performed.

During seed production, electrophoresis can give additional information before harvest on the purity of a field. Several varieties, which may be morphologically similar, are electrophoretically distinct. Plant breeders can also use this procedure to advantage. Where morphological distinctness is lacking, electrophoresis can show differences between breeding lines that are new and valuable. Since 1977, the record cards of the new varieties registered in France include the starch electrophoregram.

Although it is less developed, electrophoresis is also useful in identifying varieties in other cereals. Malt and beer manufacturers are beginning to take interest in electrophoresis for identification of barley varieties, especially by analysis of malt hordein (Autran and Scriban, 1977; Konarev et al, 1976; Scriban and Strobbel, 1978; Scriban et al, 1979; Shewry et al, 1978b, 1980; Van Lonkhuysen and Marseille, 1978b) or peroxidase (Neyreneuf and Bourdet, 1979) patterns. In France and Italy, where regulations prohibit the use of cereals other than durum wheat in pasta making, electrophoresis is used extensively to check

for the presence of bread wheats through albumin (Resmini and De Bernardi, 1976) or peroxidase (Kobrehel and Feillet, 1976) patterns. Admixtures of barley can also be detected (Joudrier et al, 1981).

B. North America

In Canada, the statutory grade specifications for red spring and durum wheats and barley require that the two top grades be equal in quality to named standard varieties. The implication of this specification is that varieties that are not equal in quality to the standard must be readily identifiable by visual means so that grain of these varieties can be readily segregated in the grain handling system. Grain breeders are encountering difficulties in their attempts to breed in morphological characteristics that will facilitate visual identification in grading. This has generated much interest in a practical, accurate test for variety identification. The electrophoresis test is being actively investigated for this purpose. Further interest in a specific variety identification test has been generated by the proposal by Canadian Government of Plant Breeders Rights legislation currently before Parliament.

The electrophoresis test has no official status within the Canadian grain handling system. Unofficially, the Canadian Grain Commission, the government agency that regulates handling and grading of grain, uses the electrophoresis test (uniform PAGE) to identify suspected "unlicensed" varieties that are not readily distinguishable visually. The test has been extremely useful in this regard. A catalog of varietal formulas for all the Canadian licensed wheat varieties was published by Zillman and Bushuk (1979b).

The electrophoresis test has been used extensively for identification of varieties (or samples presumed to be particular varieties) in wheat breeding programs. The test is beginning to be used effectively for selecting potential parents for crossing purposes. Certain bands (or blocks of bands) appear to be linked to specific agronomic or quality traits (Sozinov and Poperelya, 1979). In durum wheat, the gliadin electrophoregram is being used for selecting varieties with superior spaghetti cooking quality (Kosmolak et al, 1980; Zillman and Bushuk, 1979b). The test is also used occasionally for identifying varieties of grain in breeding programs before compositing the samples for quality testing. With the large numbers of samples that are generated by breeding programs, it is easy to mix up varieties. The electrophoresis test has been particularly useful in distinguishing between them.

In the United States, there is no official requirement for identification of grain varieties as part of the commercial grading of the grain. However, in the past few years, the electrophoresis test has attracted some interest for variety identification in the official registration of new varieties. Private plant breeders are exploring the possibility of including the gliadin electrophoregram as evidence of variety uniqueness. A catalog of the electrophoregram formulas of the 80 most commonly grown U.S. wheat varieties was compiled by Jones et al (1980), using the Bushuk and Zillman (1978) nomenclature.

As in Canada, the electrophoresis test is now used quite widely in the United States to identify varieties (or samples of grain) in grain breeding programs. In the durum variety development program at North Dakota State University, the

gliadin electrophoregram is used to identify strong gluten varieties, as suggested by Damidaux et al (1978) and Zillman and Bushuk (1979b).

C. Union of Soviet Socialist Republics

As far as we know, the electrophoresis test is used only to a limited extent in the U.S.S.R. to identify grain varieties in commercial grain production, handling, and processing. However, the test is used more extensively than in any other country in genetic studies, plant breeding, and variety registration. Two different methods and nomenclatures have been developed and are being applied.

In Leningrad, Konarev et al (1975) have made extensive use of the electrophoresis test in their studies of the origin and evolution of wheat and related species and inheritance of gliadin bands. In addition to the genetic studies, the Konarev group has published extensive catalogs of gliadin electrophoregram formulae of Soviet hard (Gubareva et al, 1975) and durum (Gubareva et al, 1979) wheats.

Konarev et al (1972) used polyacrylamide gel cylinders (7.5% acrylamide) and 0.013 *N* acetic acid of pH 3.1 as the buffer system. To facilitate processing the extensive data, the Soviet workers developed an elaborate system of nomenclature based on the Greek letter-arabic numeral system introduced by Woychik et al (1961). On the basis of the electrophoregrams of the putative progenitors of each of the three genomes of hexaploid (common) wheats, they developed a composite electrophoregram having the formula: α, 1–7; β, 1–5; γ, 1–5; and ω, 1–12. The numbers identify bands within zones identified by Greek letters. In the Konarev formula, the numbers representing bands that stain intensively are underlined, those that stain weakly are written in parentheses, and doublets are identified with two dots above the band number. A minor deviation in mobility (not considered as a different component) of a β or an ω band is indicated by a subscript 1 if the displacement is in the direction of the faster neighboring band, and by a subscript 2 if the deviation is in the direction of the slower neighboring band. An example of the formula generated by this procedure for the variety Kharkovskaya 46 is

$$\alpha 5(6)7\ \beta(2)3_2 45\ \gamma 25\ \omega 3\ddot{4}\ddot{6}7$$

This nomenclature has been used extensively by Konarev and his co-workers, but it apparently has not been duplicated in any other laboratory. The published catalogs of varietal formulae of Russian wheat varieties use this nomenclature.

The second approach to the application of gliadin electrophoresis to genetic studies of wheat in the Soviet Union is that of Sozinov and Poperelya (1971) in Odessa. These workers use a method based on starch gel cylinders. By analysis of gliadin electrophoregrams of monosomic and segregating lines, Sozinov and Poperelya (1979) observed that gliadin bands were inherited in linked groups or blocks controlled by genes on specific chromosomes. When electrophoregrams of different varieties were examined, it was noted the same chromosome could give rise to different blocks, called allelic blocks. Each allelic block is identified by the chromosome and the block number. This approach has been developed to the extent that the varietal electrophoregram can be represented by an allelic block formula. Allelic blocks for five varieties studied by Sozinov and Poperelya (1979) are given in Table V. On the basis of the allelic block analysis, varietal formulae for the first two varieties in Table V can be written as

Bezostaya 1: 1A4, 1B1, 1D1, 6A1, 6B1, 6D1
Mironovskaya 808: 1A3, 1B3, 1D5, 6A3, 6D1, 6D2

The procedure of Sozinov and Poperelya (1979) is too complex for routine variety identification in commercial applications. However, it has great potential as a selection criterion in plant breeding programs, especially since the demonstration by the Soviet workers that certain gliadin blocks are linked to technological quality such as sedimentation value, and agronomic and phenotypic traits such as low temperature tolerance, disease resistance, and spike color. Further research is needed to fully exploit the potential of this approach.

D. Australia

Since the introduction of premium payments for selected varieties, a need has existed for varietal identification of Australian wheats. The segregation of New South Wales Strong White was an early exercise of this type in 1911. Variety-controlling legislation was recently extended to cover the receipt of all wheat grain. A reduced payment is made for grain that is not one of the varieties approved for receipt at the silo where it is offered (Wrigley, 1980). The main aim of the scheme is to improve the quality and homogeneity of the grain harvest. Additional aims are to encourage the growing of wheats that are most suitable agronomically in each district, to discourage the use of disease-susceptible varieties, and to provide for the release of high-yielding lines having quality characteristics that are satisfactory only in specific areas.

A declaration of the identity of this grain is made by the wheat grower. This declaration is checked by visual examination (Ferns et al, 1975, 1978) of the grain at delivery. Gradient gel electrophoresis (du Cros et al, 1980) is used to verify identity in cases of dispute and in screening randomly chosen samples. This procedure is being adopted as an Official Testing Method by the Cereal Chemistry Division of the Royal Australian Chemical Institute. Additional information on the application of this and other laboratory procedures to Australian wheats is provided by Wrigley and Shepherd (1974) and Wrigley and McCausland (1975, 1977).

Barley varieties are segregated according to their suitability for malting, food, or feed. Procedures are available for their identification by appearance

TABLE V
Gliadin Electrophoregrams Represented by Allelic Blocks of Sozinov and Poperelya (1979)

Variety	Chromosome					
	1A	1B	1D	6A	6B	6D
Bezostaya 1	4	1	1	1	1	1
Mironovskaya 808	3	3	5	3	1	2
Odesskaya 16	1 + 2	1	5	3	2	1
Odesskaya 51	2 + 4	1	1 + 5	3	2	1
Dnieprovskaya 521	1	2	1	1	1	1

(Fitzsimmons and Wrigley, 1979) and by laboratory methods (McCausland and Wrigley, 1977; Wrigley and McCausland, 1977).

Electrophoretic techniques are also useful for analysis of cereal products in foods (Wrigley, 1977a), for investigation of pedigrees (Wrigley and Shepherd, 1977), and for characterizing off-types in pure-seed production (Appleyard et al, 1979).

V. PROSPECTS FOR FURTHER DEVELOPMENTS

Electrophoretic identification of varieties is one example of the practical application of basic research. The described procedures are based on research conducted 15 to 20 years ago, which had no more practical aim than to investigate the composition of endosperm protein. This link with basic research will continue to influence the development of improved techniques for grain segregation.

In the future, international agreement on a reference procedure for electrophoretic identification may open the possibility of identification from a catalog of wheats from many countries, instead of the local basis of identification currently used. However, the likely introduction of multiline varieties and recommendations to sow varietal mixtures will greatly complicate the task of identification. So that the implications of such recommendations can be assessed, information on problems of identification should be provided to assist in making decisions. This information may also be required to accompany applications for registration of new varieties, to ensure that varieties of different quality types can be distinguished.

"Dry" methods of analysis are obviously preferred to "wet" chemical analysis. A prime example of a preferred dry method, near-infrared reflectance spectroscopy, may itself soon permit distinction between quality types. Pyrolysis-based methods being applied to the identification of microbes (Maugh, 1976) may provide a basis for rapid "dry" varietal identification. Gas-liquid chromatography of steryl esters has been used to distinguish durum from common wheats (Hsieh and Watson, 1977).

However, proteins and nucleic acids still hold the best potential for characterization of genotype. The rapidly advancing field of high-pressure liquid chromatography (Regnier and Gooding, 1980) promises to provide a procedure for analyzing a small volume of grain extract by ion exchange chromatography. The resulting profile might be computer matched to provide identification within minutes. The cereal chemist is challenged to make further research advances for the solution of a very important problem in our food industry.

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