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CAPILLARY ELECTROPHORESIS OF WHEAT GLIADINS

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Abstract

Identification of wheat varieties by their gliadin spectrum with acidic polyacrylamide gel electrophoresis has been in use for decades. Since the early 70s electrophoresis has been one of the most frequently used analytical methods for separation and characterisation of gluten proteins. Electrophoresis separates the analytes according to the difference in their charge distribution and/or Stoke's radii. Capillary electrophoresis (CE), the miniaturised instrumental version of electrophoresis, uses similar separation principle as the traditional technique, with several advantages. These are: high electric field, thus fast separation; low sample amount (nl); low buffer consumption (5 ml/day) thus low running costs; on-column detection, thus quantitative analysis; use of aqueous buffers thus no environmental wastes. Due to the several advantages capillary electrophoresis is gaining popularity in a number of fields, as opposed to the standard electrophoretic techniques.

In our study a capillary zone electrophoretic method has been developed to separate the gliadin fraction of wheat proteins. The effect of the buffer composition on the resolution of the separation is shown. Various wheat types have been analysed for their gliadin spectra using both the traditional and the capillary electrophoretic method. Comparing the gliadin spectra obtained by means of the two methods, capillary electrophoresis seems to be a suitable alternative to the traditional method for identification /quality control of wheat species according to their gliadin spectra.

Keywords: wheat, gliadin, capillary electrophoresis.

Introduction

There are several requirements wheat production has to fulfil (higher grain yield, good baking quality, resistant varieties, cold/heat resistance, etc.), therefore new wheat species are replacing old varieties to meet the changing needs of the market.

One of the most important end-user requiremen is good baking quality of wheat, which is determined mainly by the quality and quantity of gluten proteins. Gluten proteins of wheat can be divided into two fractions; gliadins and glutenins [1]. Gliadin, the alcohol soluble fraction of the wheat proteins, consists mostly of single polypeptide chains with intramolecu-

lar disulphide bridges. Their tertiary structure is mainly globular. They have relatively small proportion of basic amino acids, are rich in glutamic acid as well as in proline and other amino acids with hydrophobic side-chains [2]. They can be further divided according to the electrophoretic mobility values of the sub-fractions to α, β, γ, or ω fractions [3]. The composition of these characteristic subfractions is genetically determined and is not affected by the breeding conditions, therefore different wheat sorts can be identified by the electrophoretic pattern of their gliadins [4]. Moreover, the results obtained by the qualitative and quantitative analysis of gluten proteins can also be used for homogeneity and similarity testing [5].

Separation techniques have improved significantly since the first frontal electrophoretic separation of gliadins was performed [6]. Today more than 40 gliadin fractions can be distinguished by their different electrophoretic mobilities [7]. The gliadin spectra obtained by the polyacrylamide gel electrophoresis (PAGE) technique have been used for identification of wheat species routinely by the breeders in the past ten years. *Table 1* gives an overview of several PAGE conditions generally used for gliadin analysis.

In 1982 Jorgenson and Lukacs developed the instrumental version of electrophoresis [8]. In capillary electrophoresis the separation of the analytes is performed in fused-silica tubings with small inner diameter. Due to the small I.D. (generally 10 – 100 μm), high electric field strength can be applied during the run resulting in fast separation with extremely high efficiency. Using quartz capillaries enables on-column detection, therefore there is no need for the cumbersome fixation-, staining/destaining procedures generally used after traditional electrophoretic separations.

Capillary electrophoresis was first used for quantitative/qualitative analysis of wheat species by BIETZ in 1992 [9]. He compared complex extracts of different wheat samples by using micellar electrokinetic chromatography (MEKC). He claimed that the extracts were characteristic of the species like fingerprints. Since then, several CE methods have been developed for the characterisation and/or identification of wheat-, bean- and lupine samples ([10, 11, 12] respectively).

In this paper, a simple and fast CE method was developed to analyse the gliadin fraction of wheat proteins. The effect of buffer composition on the separation efficiency is also studied. A limited number of wheat samples were analysed according to their gliadin fractions, and the results were compared to their electrophoretic pattern obtained by traditional electrophoresis.

Table 1
Comparison of the traditional electrophoretic methods used for characterisation of wheat gliadins

Extraction	Gel composition	Buffer composition	Running time	Authors
70% EtOH + saccharose for 1 hour, room temp.	6% acrylamide 0.3% bis	8.8 mmol/l Al-lactate + lactic acid pH 3.1	6.5 hours + staining + destaining	Bushuk Zillman 1978
70% EtOH + saccharose for 1 hour room temp.	6%acrylamide 0.3% bis	3.5 mmol/l Na-lactate + lactic acid pH 3.1	5 hours + staining + destaining	Wrigley Autran Bushuk 1982
25% ethylene-chloro-hydrine + saccharose for 1 hour, room temp	6%acrylamide 0.3% bis	3.5 mmol/l Na-lactate + lactic acid pH 3.1	5 hours + staining + destaining	Autran 1979
1 mol/l urea, for 1 hour at room temperature	gradient, 2.5 – 13% acrylamide	4.25 mmol/l NaOH, lactic acid pH 3.1	2 hours + staining + destaining	Pharmacia du Cros Wrigley
70% EtOH + saccharose for 1 hour room temp.	6% acrylamide 0.3% bis	8.8 mmol/l Al-lactate + lactic acid pH 3.1	4 hours + staining + destaining	Sapirstein Bushuk 1982

Materials and Methods

Chemicals

All of the chemicals used in this study were of analytical grade and were purchased from Fluka Chemie (Buchs, Switzerland) unless mentioned otherwise. For the preparation of the electrolytes double distilled Milli-Q water (Waters, Milford, MA, USA) was used. Acrylamide, ammoniumpersulphate (APS) and Bis [N,N',methylene-bis-acrylamide] and urea were obtained from Bio Rad Laboratories (Richmond, CA.USA). Acetonitrile, methanol and ethanol were of HPLC grade and were purchased from Chemolab (Budapest, Hungary).

Preparation of Samples

A limited number of wheat samples (Kincso, Othalom, MV4, Chinese spring, Marquis) were chosen for the study. The samples were crushed, and extracted with 70% EtOH (3 μl/mg) overnight. The samples were then centrifuged (at 6000 g for 15 minutes) and the supernatant was subjected to analysis. For sample and buffer preparation a TH 22 centrifuge (VEB MLW MEDIZINTECHNIK, Germany), a UC 450 PJ1 ultrasonic bath (TESLA, Prague, Czech Republic), a pH meter OP-211/1 (Radelkis, Budapest, Hungary) and a magnetic stirrer OP 951 (Radelkis, Budapest, Hungary) were used.

Horizontal Electrophoresis

For the classical electrophoretic analyses a traditional horizontal PAGE (210×147×3 mm) system (Pharmacia, Sweden) was used. The gel solution contained 7.5% acrylamide, 0.4% bis-acrylamide, 0.1% ascorbic acid, 0.005% Fe_2SO_4, 1.07% acetic acid. The pH of the buffer was adjusted with glicine to 3.1. 1.4 ml APS (1.4%) was used to initiate the polymerisation. The electrode buffer was 1.0% acetic acid adjusted to pH = 3.1 with glicine. The separation conditions were as follows: sample load: 20 μl; separation temperature: 22°C; applied electric field: 400 V and running time: 5 hours. Following the runs, the gels were put in a fixing/staining solution containing Coomassie BB, R250, 12.5% TCA and 30% MeOH. The gels were stained overnight and then washed in distilled water.

Capillary Electrophoresis

The home-built capillary electrophoretic system consisted of a CZE 1000 PN 30 power supply (SPELLMAN High Voltage Corporation, Plainview, NY, USA), a Spectra 100 UV/VIS detector (Thermo-Separation Products, San Jose, California, USA) and a DTK Personal Computer (Parity Ltd. Budapest, Hungary) equipped with an analog to digital converter board (Data Translations, Framingham, MA, USA) and a data acquisition and analysis software (Caesar) (Analytical Devices Inc. Alameda, CA USA). All the analyses were performed at ambient temperature; the capillaries were cooled by using a laboratory fan.

In our experiments fused-silica capillaries (Polymicro Technologies Phoenix, AZ, USA) with inner diameters of 50 and 75 μm were used. In some cases the capillaries were coated with linear polyacrylamide (LPA) using a modified coating procedure of Hjerten's [13, 14]. Capillaries were

preconditioned by subsequent washing with 20 column volumes (approx. 100 microliter) of 1 mol/l NaOH, water, 0.33 mol/l phosphoric acid, water, 0.1 mol/l NaOH, water, 0.33 mol/l phosphoric acid, water and the separation buffer when fused-silica capillaries were used. In case of LPA coated capillaries, the capillary washing procedure was simpler; 200 microliter electrolyte was used to flush the capillary before each run. The separation buffer for coated capillary (75 μm I.D.) consisted of 75% ethanol and 25% 50 m/mol/l phosphate buffer (pH 2.5). When fused silica capillaries (50 μm I.D.) were used, the separation buffer was 50 mmol/l phosphate buffer (pH 2.5) containing 0 – 30% acetonitrile, 0.05% hydroxypropylmethylcellulose and 1 – 3 mol/l urea to decrease solute-wall interaction and improve selectivity. Samples were injected in all cases in to the capillary by electrokinetic injection using 15000 V for 10 seconds.

Results and Discussion

Sample Preparation

At the beginning of the experiments the OSBORNE [1] fractionation was performed to be able to distinguish the albumin, globulin and gliadin fractions from each other.

Fig. 1 shows the electropherograms of the albumin/globulin fraction of three different wheat varieties. As it can be seen, the protein components of the various varieties have very similar electrophoretic mobilities suggesting similar structures. The difference is found mainly between the peak areas and their ratio. Since the albumin and globulin fractions have higher electrophoretic mobilities than the gliadines, the sample preparation was simplified to that described in the previous section. *Fig. 2* shows the electropherogram of a wheat extract obtained according to the simplified extraction procedure. As it can be seen, the albumin/globulin fractions do not interfere with the gliadin compounds.

Capillary-Wall Interactions

In the capillary electrophoresis of proteins, one of the major concerns is the possible interaction between the silanol groups of the capillary wall and the protein. The interaction can be either ionic or hydrophobic, depending on the conditions [15]. Using low pH buffers the ionisation of silanol groups can be minimised. Coating the capillaries either by dynamic coating or

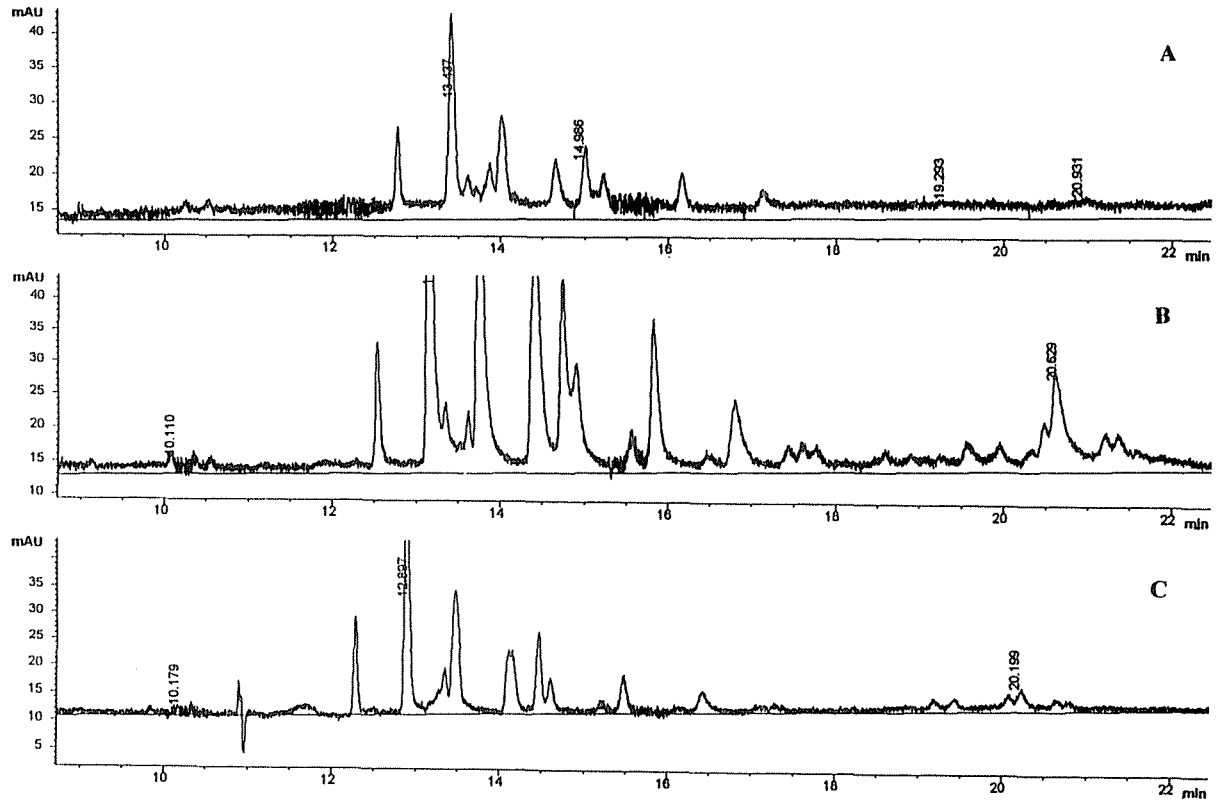

Fig. 1. Comparison of the albumin-globulin fractions of wheat varieties Conditions: Electrolyte: 50 mmol/l phosphate pH 2.5, 30% ACN, 1 mol/l urea, 0.01% HPMC, fused silica capillary (50 μm I.D.), with the effective length of 50 cm and total length of 75 cm. The applied electric field was 25 kV. Detection was performed at 200 nm. Samples are A) Kincso, B) Othalom, C) Chinese spring

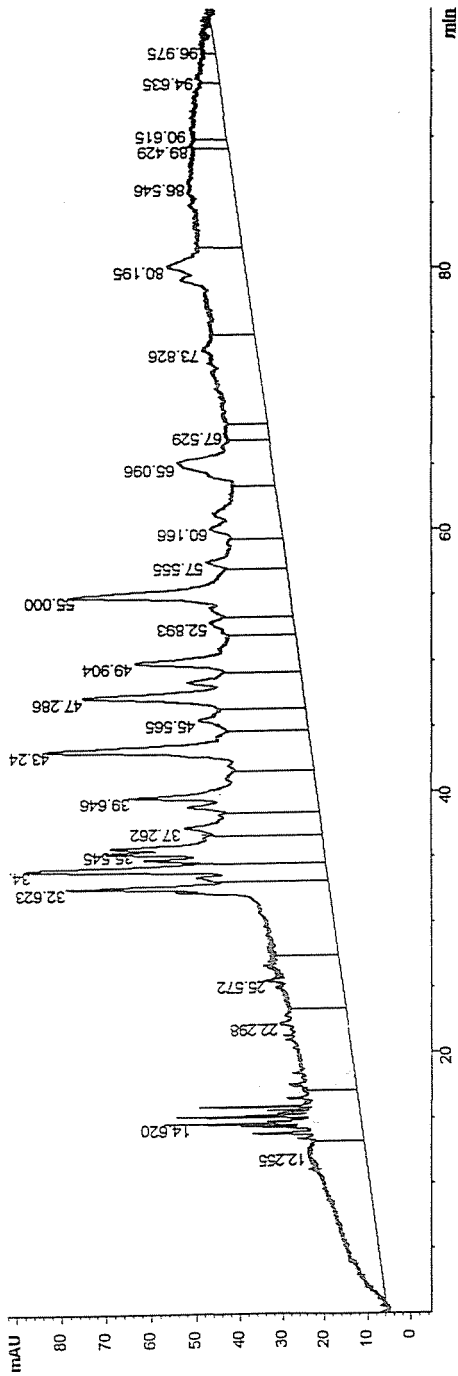

Fig. 2. Electropherogram of MV-9 sample using the simplified extraction procedure
Conditions: as in *Fig. 1*

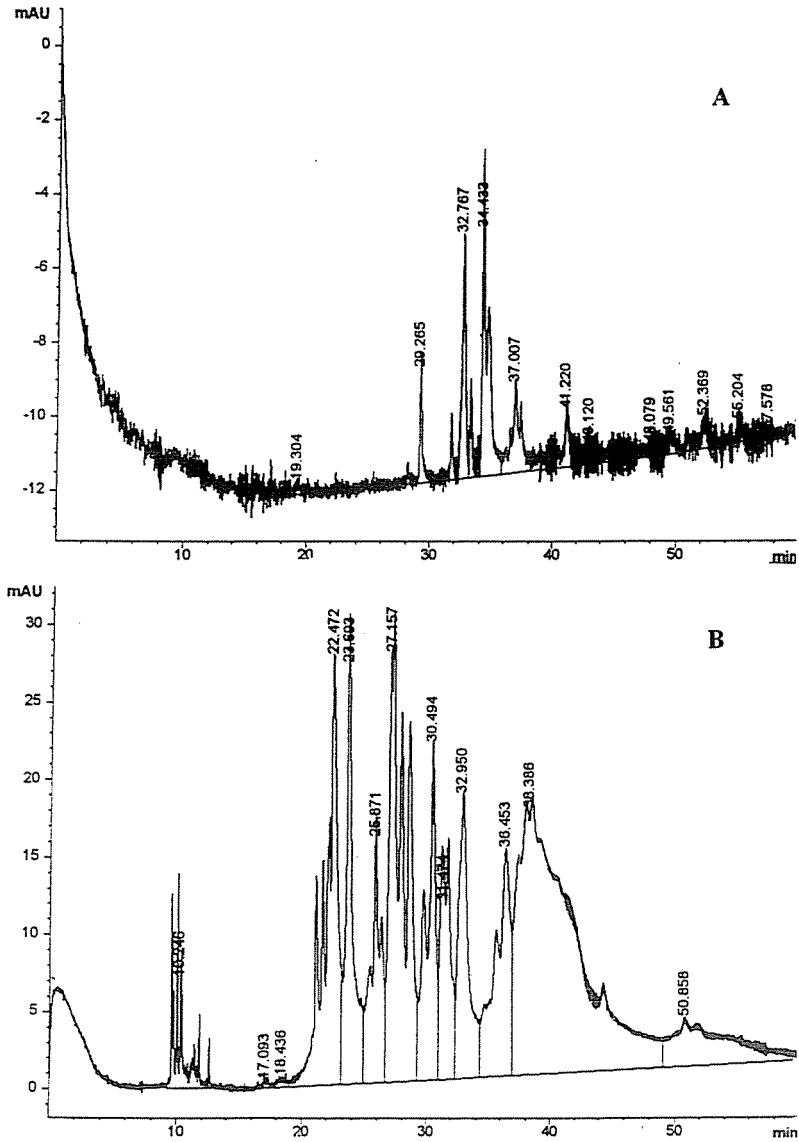

Fig. 3. Effect of capillary coating on the separation efficiency
Conditions: Electrolyte: 50 mmol/l phosphate pH 2.5, 10% ACN, fused silica capillary (50 μm I.D.), with the effective length of 50 cm and total length of 75 cm. The applied electric field was 25 kV. Detection was performed at 200 nm. Sample was Kincso A) separation buffer without HPMCE and B) with 0.01% HPMCE

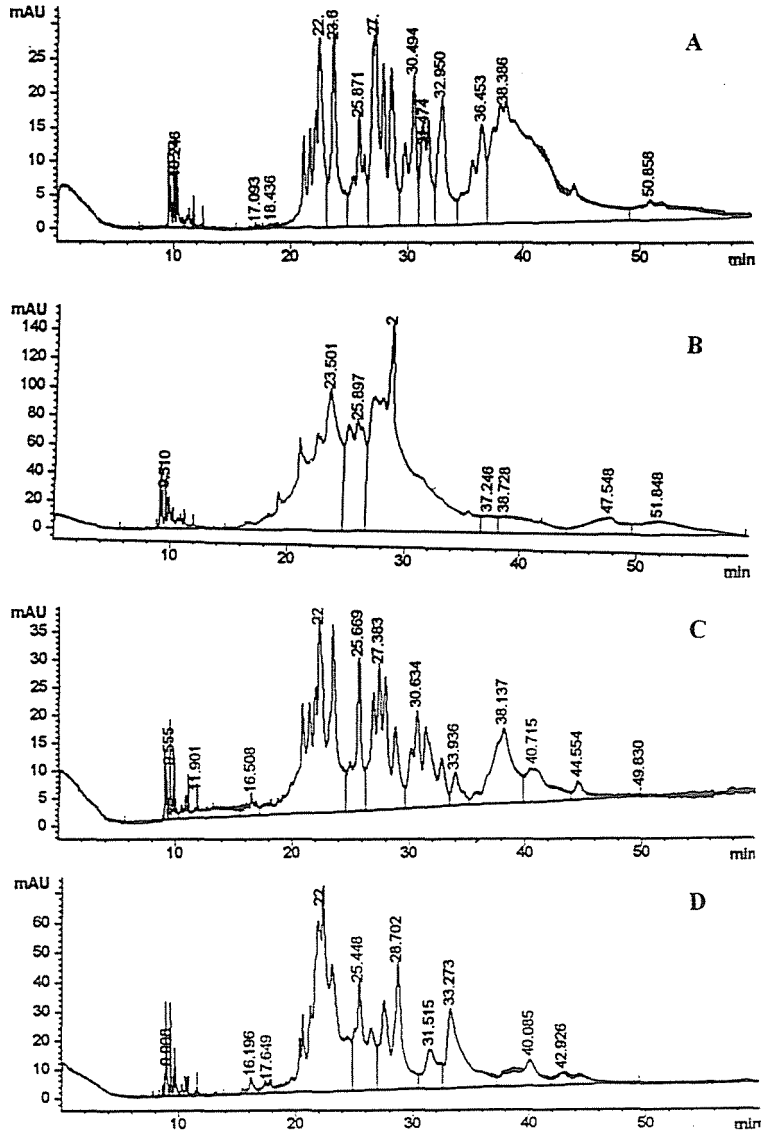

Fig. 4. Comparison of the gliadin spectra of wheat varieties using the optimised CE method

Conditions: Electrolyte: 50 mmol/l phosphate pH 2.5, 30% ACN, 3 mol/l urea, 0.01% HPMC, fused silica capillary (50 μm I.D.), with the effective length of 50 cm and total length of 75 cm. The applied electric field was 25 kV. Detection was performed at 200 nm. Samples are A) Kincso, B) Othalom, C) MV9 D) Amadeus

static coating techniques can also help in minimising the interaction between the solute and the capillary wall. The above coating approaches were compared in our experiments, namely the static coating by covalent binding linear polyacrylamide to the capillary wall and the dynamic coating by adding hydroxypropylmethylcellulose to the background electrolyte. Both coating techniques resulted good separation of the gliadins, however, dynamic coating was preferred due to its simplicity. *Fig. 3* shows the effect of capillary coating on the separation efficiency. As it is seen even when a low pH buffer is used, the proteins interact with the capillary wall if no coating is applied. The interaction can be somewhat decreased by adding urea to the buffer, but not completely eliminated (data not shown).

Effect of Various Organic Additives on the Separation

Further improvement in the separation can be obtained by increasing the amount of the acetonitrile and urea in the separation buffer. *Fig. 4* shows the electropherogram of two different wheat varieties using the optimal conditions. As it is seen, slight differences in the gliadin content can be well distinguished.

The CE electropherograms of the samples were always compared to those obtained by the horizontal PAGE, and good agreement in the major patterns was found in all cases (data not shown). Comparing the spectra obtained by means of the two methods, capillary electrophoresis seems to be a suitable alternative to the traditional method for identification of wheat species according to their gliadin spectra. With the developed capillary electrophoretic technique protein fractions present only in small concentration were discovered and quantitatively analysed. The electrophoretic pattern of these fractions seems to be also characteristic each wheat variety.

In our further investigations we will increase the number of the different species involved in the study.

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