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# Characterization of food proteins by capillary electrophoresis

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**Abstract**

A simple analytical method for characterization of food proteins by capillary electrophoresis (CE) has been developed. Major proteins in chicken eggs and cow's milk are characterized and can be quantitated by the CE technique. Egg white proteins are well resolved while the yolk shows a substantially more complex protein separation pattern than that in the egg white. Caseins and whey proteins in fresh milk were resolved by CE in a similar buffer system. Separation of both milk and egg proteins was performed reliably and reproducibly in an untreated fused-silica column, 25 cm × 20 μm I.D. Diluted egg or milk samples can be directly loaded on an automated instrument with on-line injection, detection and real-time data analysis in less than 10 min.

## 1. Introduction

Chicken eggs and cow's milk are the two major dairy products rich in protein content. They are also among the most important sources of the daily diet that contains all the essential amino acids. Despite the recent awareness of cholesterol diet intake in dairy products, both chicken's egg and low-fat and non-fat cow's milk are still the primary source of protein intake. Egg whites account for about 58% of the entire egg mass, with 10–12% of the mass being protein and the remainder of the mass being water. Among the proteins in egg whites, ovalbumin, ovomucoid, globulins and conalbumin are the major components constituting 54, 11, 10 and 13%, respectively, with lysozyme as the minor component of about 3.5% [1]. Proteins in the egg yolk, on the other hand, are substantially more complex than those in egg white and not as well characterized. The yolk comprises 32% of the mass of egg, and is about 49% water, 33% fat and 16% protein [2]. Cow's milk contains about 3.2% protein regardless of its fat content [3]. Protein in cow's milk contains mostly caseins, approximately 80%; the remainder being whey proteins.

Separation of both egg white and yolk proteins has been achieved by Shepard and Hottle [4] who used the Longsworth-modified free zone electrophoresis [5] of Tiselius apparatus, with the classical zone electrophoresis. Capillary electrophoresis (CE) has rapidly become the method of choice for most of the analytical applications [6–10]. The feasibility of performing a routine protein analysis by capillary zone electrophoresis in an untreated fused-silica capillary [11–13] was established. Using a high-phosphate-based buffer in conjunction with a capillary with I.D. of 20 μm, we have demonstrated a high-resolution protein analysis of complex mixtures such as human serum, urine and ascites fluid [14]. Addi-

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tion of urea to the high-phosphate buffer allowed us to characterize milk proteins with a reasonably good resolution [15]. This report is an attempt to establish the feasibility of rapid analysis of proteins in egg and milk with a simple borate-based buffer.

## 2. Experimental

### 2.1. CE procedures

A P/ACE 2100 automated CE system by Beckman Instruments (Palo Alto, CA, USA) was used with P/ACE system software controlled by an IBM PS/2 Model 55 SX computer. Post-run data analysis was performed on System Gold software by Beckman Instruments (Fullerton, CA, USA). Capillary columns of 25 cm length (18.5 cm to detector window) × 20 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA) were assembled in the P/ACE cartridge format (50 × 200 μm aperture). On-line detection of the P/ACE system was at 200 nm. During electrophoresis, the capillary was maintained at ambient temperature (usually 23°C) with circulating

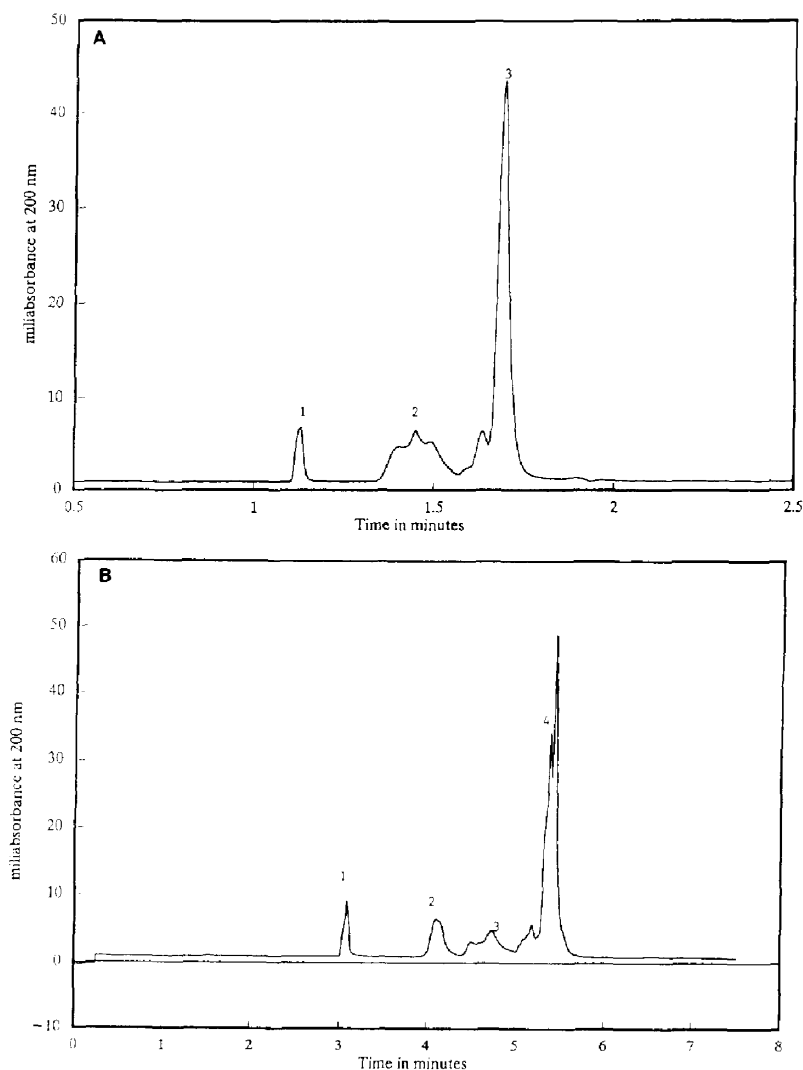


Fig. 1.

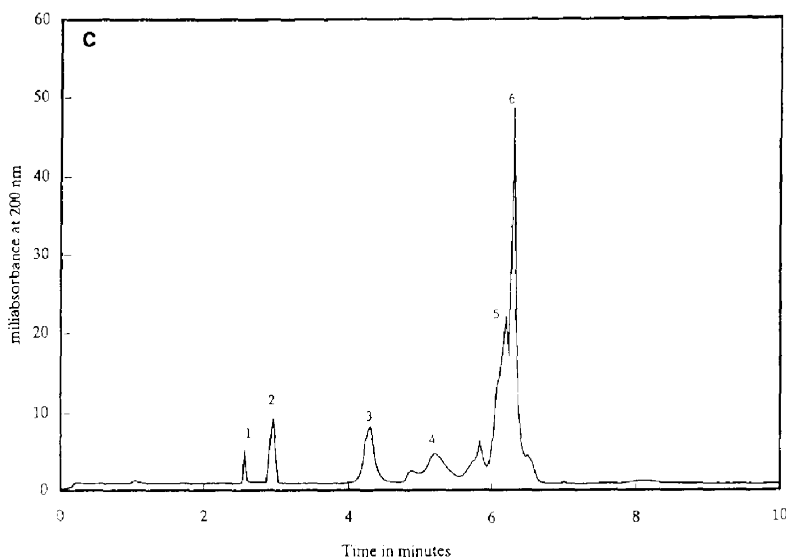


Fig. 1. Electropherograms of egg white proteins. (A) Conditions: untreated fused-silica capillary, 25 cm × 20 μm I.D.; applied potential, 20 kV/17 μA; buffer, 80 m*M* borate at pH 10.0; peaks: 1 = neutral marker (DMF); 2 = conalbumin and globulins; 3 = ovomucoid and ovalbumin. (B) Conditions: untreated fused-silica capillary, 25 cm × 20 μm I.D.; applied potential, 10 kV/17 μA; buffer, 200 m*M* borate at pH 10.0; peaks: 1 = neutral marker and lysozyme; 2 = conalbumin; 3 = globulins; 4 = ovomucoid and ovalbumin. (C) Conditions: untreated fused-silica capillary, 25 cm × 20 μm I.D.; applied potential, 12 kV/26 μA; buffer, 300 m*M* borate at pH 10.0; peaks: 1 = lysozyme; 2 = neutral marker; 3 = conalbumin; 4 = globulins; 5, 6 = ovomucoid and ovalbumin.

coolant surrounding the capillary. Diluted samples were introduced by pressure injection for 15–30 s and electrophoresis was performed at the voltage indicated on the electropherograms. Between runs, the capillary was sequentially washed with 1.0 *M* sodium hydroxide and water (12 s each, with pressure rinsing at 15 p.s.i.; 1 p.s.i. = 6894.76 Pa) followed by reconditioning with the borate buffer (60 s with pressure rinsing at 15 p.s.i.).

### 2.2. Sample and buffer preparations

Chicken egg albumin, conalbumin, trypsin inhibitor and lysozyme, and milk caseins, α-lactalbumin and β-lactoglobulin A and B, were obtained from Sigma (St. Louis, MO, USA). Borate buffer at pH 10.0 was prepared by dissolving boric acid and adjusting the pH with the addition of sodium hydroxide solution. Fresh milk and chicken egg samples were purchased from a local supermarket. All protein standards were dissolved in buffer containing 75 m*M* sodium chloride, 20 m*M* potassium phosphate, 0.01% sodium azide, pH 7.0 (phosphate-buffered saline, PBS). Each protein concentration was about 0.2 to 0.5 mg/ml. Egg white was obtained from fresh egg and dissolved in PBS at 1:50 dilution. The egg yolk was separated from the egg white and dispersed in PBS at 1:40 dilution followed by delipidation with SeroClear (Calbiochem, San Diego, CA, USA). Dimethylformamide (DMF) as a neutral marker was added to the sample diluent, PBS, at final concentration of 0.01% (v/v).

## 3. Results and discussion

In the presence of 80 m*M* borate buffer at pH 10, egg white is resolved into two major fractions as shown in Fig. 1A. Conalbumin and globulins

appear as a broad band migrate between 1.3 and 1.55 min while ovomucoid and ovalbumin appear at 1.75 min. Lysozyme is absent, presumably due to adsorption to silica surface, at pH of 10. As the borate buffer increases to 200 m*M*, egg white proteins begin to separate into four zones, conalbumin, globulins, ovalbumin and ovomucoid, shown in Fig. 1B. Lysozyme is missing, presumably migrates along with the neutral marker, DMF. Addition of an authentic sample of lysozyme to the sample showed that lysozyme co-migrates with DMF. Further increase of borate buffer to 300 m*M*, results in better defined separation pattern as evident in Fig. 1C. Lysozyme clearly migrates at 2.6 min, ahead of neutral marker, at 2.9 min, consistent with its isoelectric point of 11.0, which is 1.0 pH unit above the buffer pH. A high salt buffer prevents

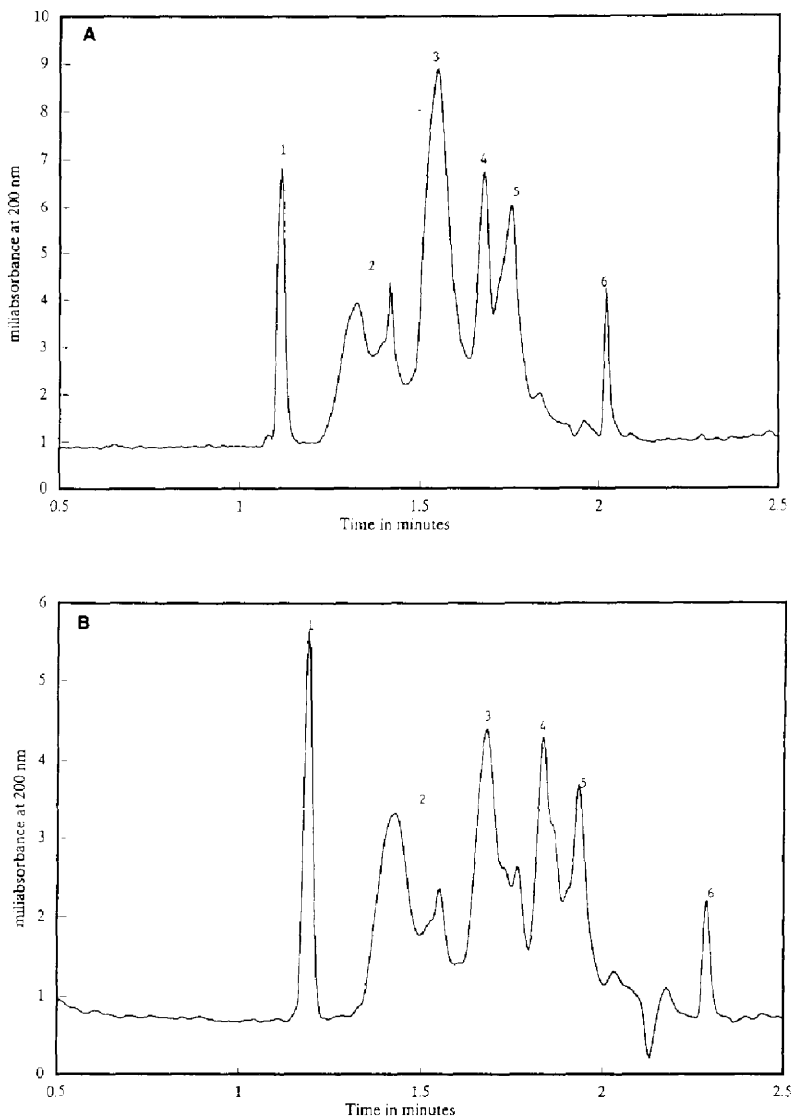


Fig. 2.

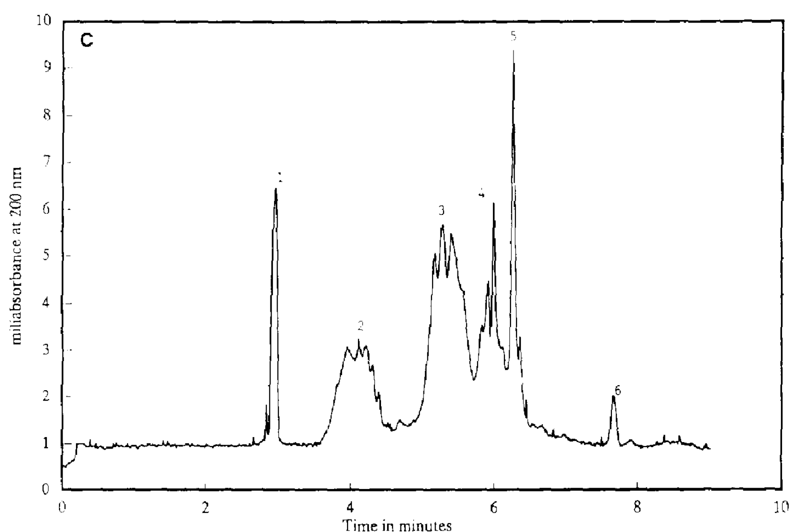


Fig. 2. Electropherograms of egg yolk proteins. (A) Conditions: untreated fused-silica capillary, 25 cm × 20 μm I.D.; applied potential, 20 kV/17 μA; buffer, 80 m*M* borate at pH 10.0. (B) The lipid portion in yolk was extracted with SeroClear; conditions as for (A). (C) Conditions: untreated fused-silica capillary, 25 cm × 20 μm I.D.; applied potential, 10 kV/21 μA; buffer 300 m*M* borate at pH 10.0. Peaks: 1 = neutral marker; 2 = yolk immunoglobulins; 3 = yolk lipoproteins.

lysozyme, a very basic protein, from adsorption on the silica surface [16]. The remainder of the proteins migrate in order according to their isoelectric point. Ovomucoid (p*I* 4.0) and ovalbumin (p*I* 4.6) appear between 6 and 6.6 min, globulin (p*I* 5.5–5.8) migrates as two broad peaks at 5.2 and 5.35 min, and conalbumin (p*I* 6.6), at 4.3 min; they are calculated to be 64, 10 and 14%, respectively, while lysozyme was less than 3.5%.

Egg yolk when dispersed into saline, exhibits a cloudy solution. In the presence of 80 m*M* borate buffer, pH 10.0, it shows primarily six fractions shown in Fig. 2A. Extraction of the cloudy solution with SeroClear, a fluorocarbon, results in a clear solution that exhibits an electropherogram in Fig. 2B, similar to that of Fig. 2A. Protein peaks between 1.5 and 1.8 min appear to be reduced in Fig. 2B in comparison with that in Fig. 2A. Presumably, these are lipid associated species in egg yolk that was extracted into the SeroClear. Each of the four fraction in egg yolk proteins can be resolved substantially in the presence of 300 m*M* borate buffer shown in Fig. 2C. Separation pattern of egg white and yolk proteins by the present method appears to be similar to that obtained by Shepard and Hottle [4] who used the Longsworth-modified free zone electrophoresis [5] of Tiselius apparatus.

Milk proteins were previously analyzed by urea–phosphate buffer that could resolve α- and β-casein, while whey proteins were evident from the electropherogram [15]. In the absence of urea–phosphate buffer whey proteins can be resolved, but caseins migrate as a broad peak, appear to be aggregates [15]. In simplified borate buffer, 0.25 *M* at pH 10.0, the electropherogram of non-fat fresh milk in Fig. 3A shows that β-casein is well separated from α-casein. α-Lactalbumin exhibits as a sharp peak migrating in front of α-casein. β-Lactoglobulin B and A emerge with α-casein. Powdered milk, however, shows broad poorly resolved peaks presumably due to extensive thermal denaturation during the manufacturing process (spray drying process). The absence of the α-lactalbumin and/or β-lactoglobulin in powdered milk shown in Fig. 3B may be used as an indicator for the quantitation of the adulteration in fresh milk.

The utility of egg and milk proteins analysis by

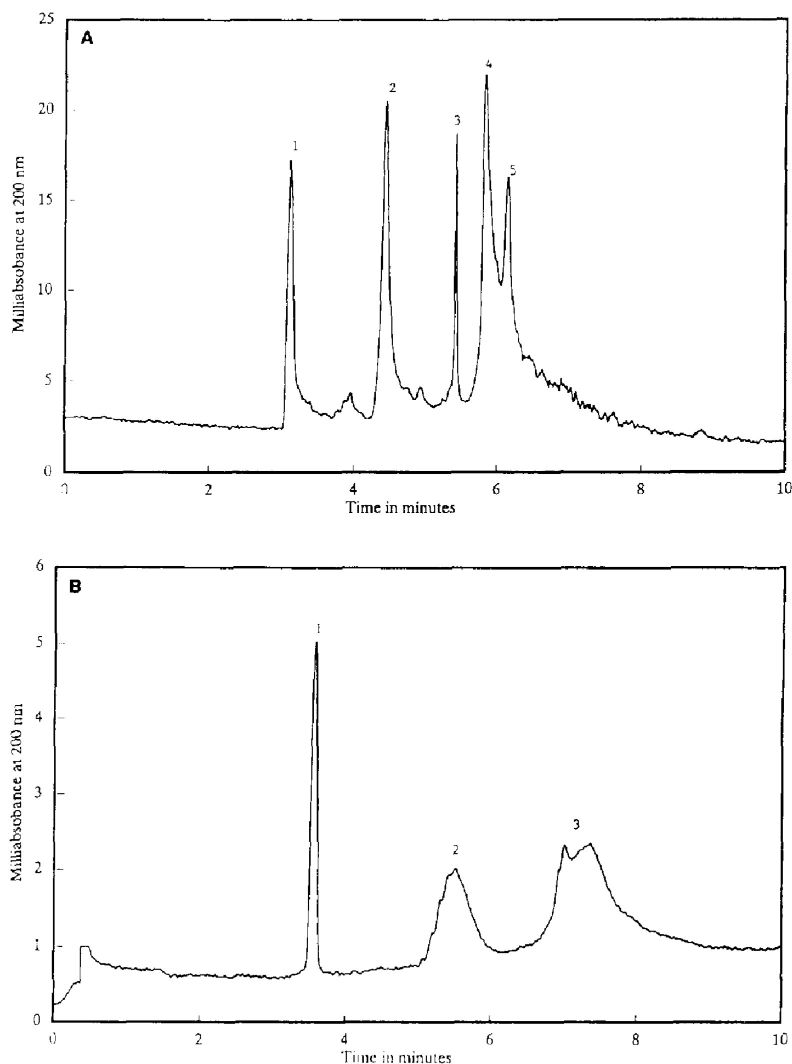


Fig. 3. (A) Electropherogram of the fresh non-fat milk proteins; conditions: untreated fused-silica capillary, 25 cm × 20 $\mu$m I.D.; applied potential, 10 kV/15 $\mu$A; buffer, 250 m$M$ borate at pH 10.0; peaks: 1 = neutral marker; 2 = $\beta$-casein; 3 = $\alpha$-lactalbumin; 4 = $\alpha$-casein; 5 = $\beta$-lactoglobulin. (B) Electropherogram of the powder milk proteins; conditions as for (A); peaks: 1 = neutral marker; 2 = denatured $\beta$-casein aggregates; 3 = denatured $\alpha$-casein aggregates.

CE in an untreated fused-silica column is demonstrated using borate buffer at pH of about 10.0. Increasing the buffer salt concentration results in a better resolution of proteins in both egg and milk proteins. Aggregation of proteins, milk caseins in particular is an analytical problem in which addition of urea to the buffer system is a common practice. The present buffer system with high buffer salt and high pH not only eliminates the casein aggregate problem but also increases the separation efficiency.

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