Meat Species Identification: Rapid Electrophoretic Methods and Staining Techniques

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Summary In this study a simple equipment for isoelectric focusing which doesn't require any cooling was tested for its suitability to meat species identification. Furthermore, a method is described in order to combine different staining techniques. Investigated materials were raw meat from slaughter animals and poultry. The extracted proteins were separated by isoelectric focusing in 0,5 mm polyacrylamide gels and visualized using Serva Violet 17 and specific stainings of myoglobin and esterases. The results show that the presented instrumentation is suitable for meat species identification within 2 to 3 hours depending on the staining procedure. Using a self made "triple electrode electrofocusing cell" up to 50 samples can be investigated in a single run. For increasing reliability and saving time a method was developed in order to combine both the staining of sarcoplasmic proteins and myoglobins.

Introduction: Electrophoretic methods and staining techniques usually used for meat species identification are time consuming and require a great deal of instrumentation. Most times the sarcoplasmic proteins as a whole are visualized after isoelctric focusing (IEF) by a protein dye (e.g. Coomassie Blue) exposing complicated pattern (TINBERGEN and OLSMAN, 1976; KAISER et al., 1980; BAUER, 1981). The development of the "myoglobin method" by HOFMANN and BLÜCHEL (1986) using the native red-brownish color of the myoglobin bands was a decisive step for meat species identification. BAUER and HOFMANN (1987a,b,c) further increased the sensitivity of this method by a specific staining of the myoglobins based on their pseudoperoxidase activity. However the identification of closely related animals like duck and goose or sheep and goat may be difficult or impossible. For solving this problem the time consuming visualization of the total sarcoplasmic pattern is necessary.

The objective of our investigation was to test a simple commercially available equipment for IEF which doesn't require any cooling and different staining methods for its suitability to identify meat species.

Materials and Methods

Materials: Pork, beef, mutton, horse-, rabbit-, chicken- and turkey-meat.

Sample preparation: One part of minced meat is extracted with four parts of water or with a solution of 0.001 M $K_3[Fe(CN)_6]$ under occasional stirring for at least 15 minutes, followed by filtration. If the extracts are cloudy they have to be cleared by membrane filtration (0.2 µm cellulose acetate). Press juices are prepared and treated as described by BAUER and HOFMANN (1987a).

Gel preparation: as described previously (BAUER and HOFMANN, 1987a; BAUER and KELNER, 1989).

Electrophesis: 3 µl of the samples are applied to the gel using a piece of filter paper (approx. 7x1mm) near the anode. The gel is layed upside down on the coal electrodes of the "Mini IEF CELL" (BIORAD, Richmond, California). The electrode distance is 5 cm.The electrophoresis is carried out 15 min at 100V, 15 min at 200 V and 60 min at 450 V.

Staining methods: Visualization of the whole sarcoplasmic proteins by Commassie Brilliant Blue (NEUHOFF et al., 1985) and SERVA VIOLET 17 (PATESTOS et al., 1988), the myoglobins (BAUER and HOFMANN, 1987a) and the esterases (BGA, 1988; BAUER and KELNER, 1989)

Diffusion Blot: Roll a sheet of wetted nitrocellulose on the electrofocusing gel followed by a some sheets of filter paper and weight it with a few glass plates for 15 min. Carry out the desired staining.

Results and Discussion

Using described isoelectric focusing cell meat species under investigation could be identified within 2 to 3 hours depending on the staining method. The electrophoretic procedure is finished after within 90 minutes. The specific staining of the myoglobines requires only 15 minutes but meat from animals with low myoglobin content and closely related animals e.g. chicken and turkey cannot be

Figure 1: IEF of different meat species using the "Mini IEF cell" Stainings: left - Serva Violet 17; middle - myoglobin staining; right - esterase staining by Fast Blue RR; cathode on the top.

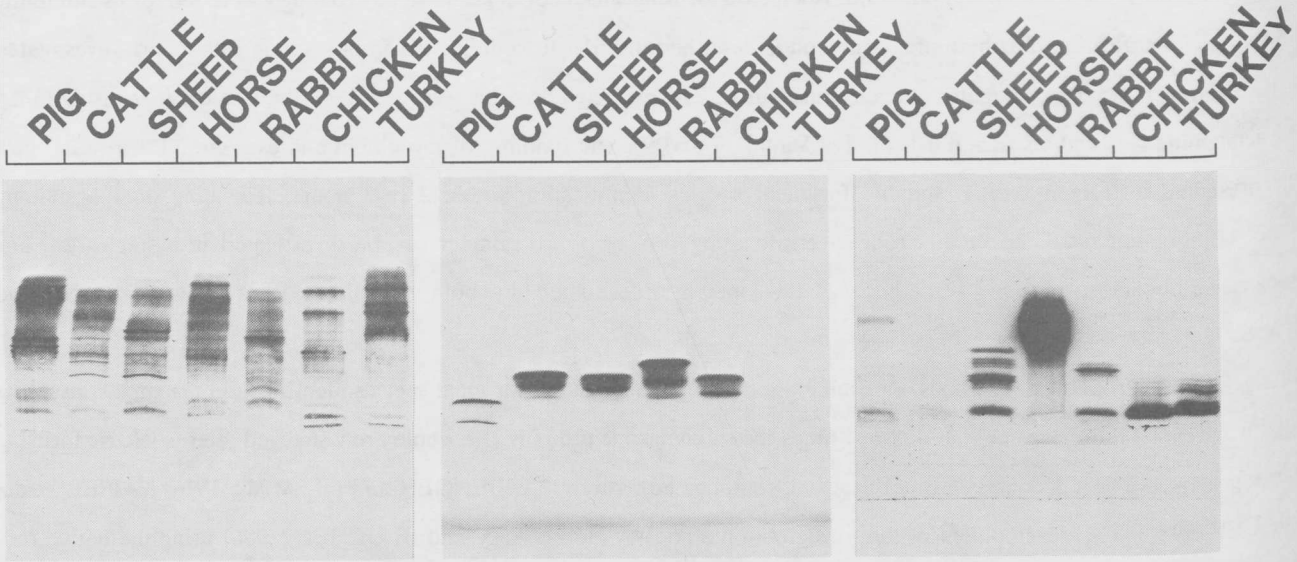


Figure 2: IEF of different meat species using the "Mini IEF cell" stained by Serva Violet 17 (left) and "incomplete diffusion blot" (right); myoglobin staining; cathode on the top.

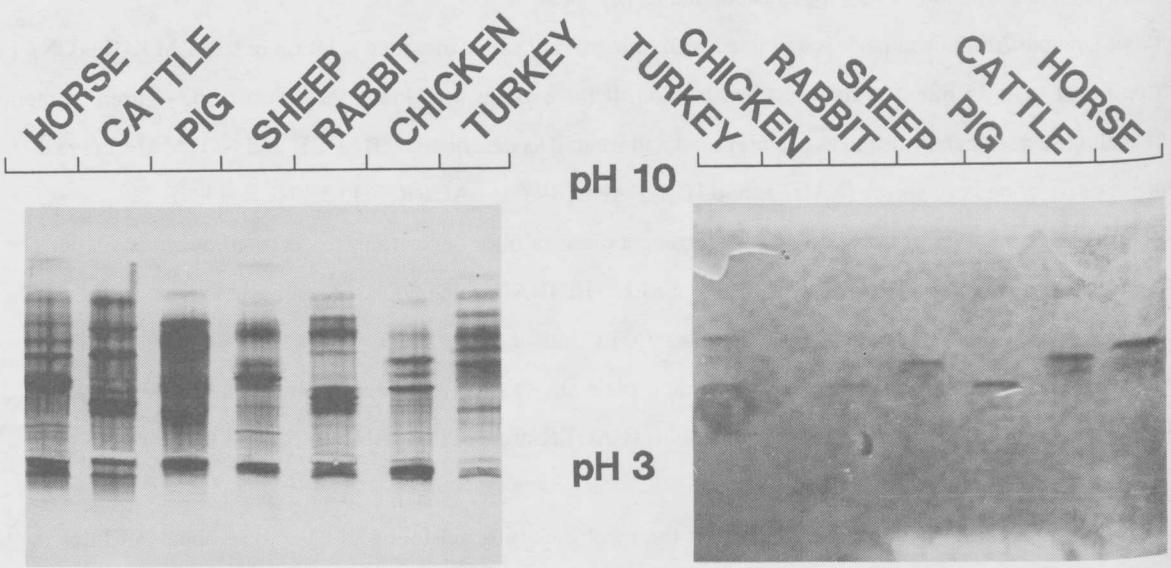
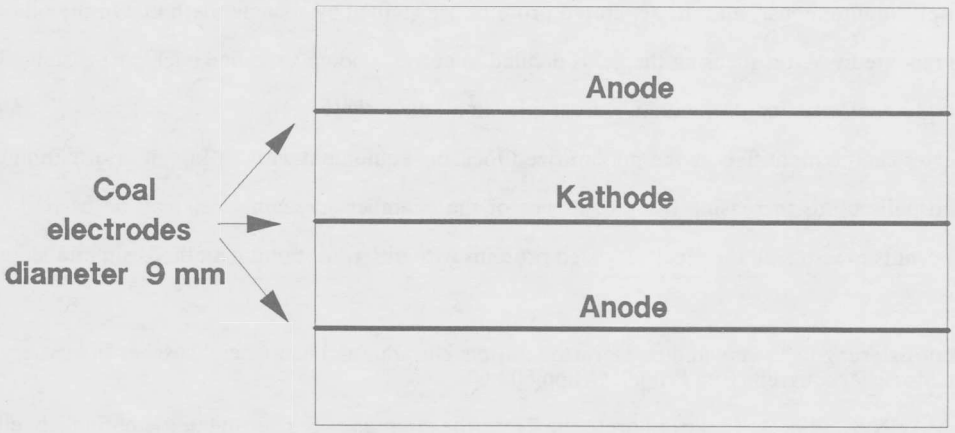


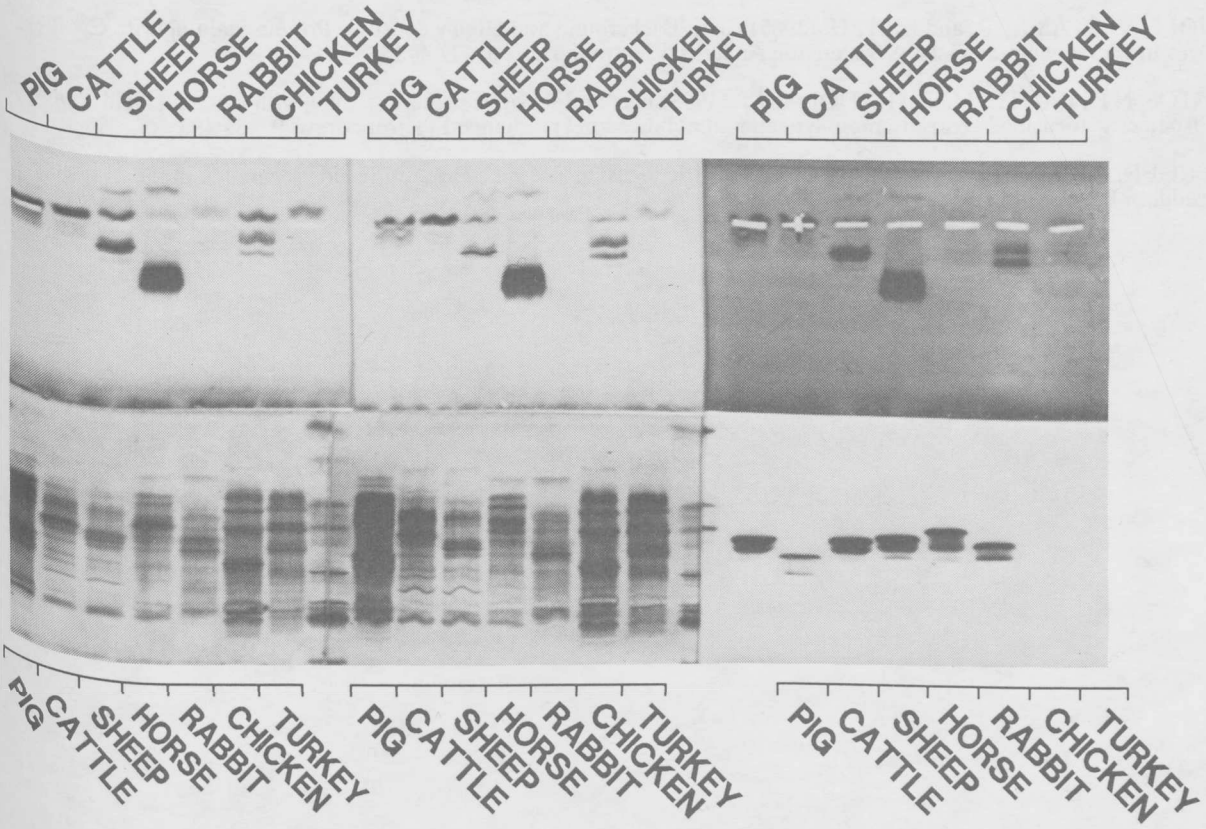
Figure 3: Triple IEF Chamber; electrode distance 5 cm.



distinguished by this method. This problem can be solved easily by staining esterases specifically. As a rapid method for staining the total sarcoplasmic proteins the protein dye "SERVA VIOLET 17" is suitable. In comparison to conventional IEF chambers the sharpness of the bands could be improved and furthermore the occurence of wavy bands in the acidic pH-region is minimized (Fig.1).

The separated proteins can be visualized by two different staining methods using the "incomplete diffusion blot" . So, the protein bands in the gel were stained by SERVA VIOLET 17 whereas only the myoglobins were visualized in the diffusion blot (Fig.2). This procedure combines the advantages of these two staining methods namely the possibility of the identification of closely related animals by the sacroplasmic protein patterns and the stability of the myoglobins against influences as storage or spoilage. By that means the duration of the investigation can be increased simultaneously with an improvement of relialibility.

Figure 4: IEF of different meat species using the "Triple electrode focusing cell". Stainings: top - esterase staining by Fast Blue B (left), Fast Blue RR (middle) and Fast Black K (right); bottom - staining of sarcoplasmic proteins by Serva Violet 17 (left), Coomassie Brilliant Blue R-250 (middle) and myoglobin staining (left); cathode on the top.



The commercially available IEF chamber without cooling is made for gels up to a length of 10 cm. Especially for screening purposes a "triple electrode focusing cell" has been constructed by ourselves as depicted in Fig.3. Using this chamber up to 50 sample can be electrofocused simultaneously and the separated proteins are stained by a single method. On the other hand a series of samples can be applied repeatedly. After focusing the gel is divided in corresponding parts and each part is stained by a different method in order to get complete information of the unknown sample simultaneously (Fig.4).

Conclusions: Meat species can be identified by the miniaturized focusing equipment without loss of information compared with electrophoresis chambers usually used. Increasing the dimensions of the chamber screening tests can be carried out easily. The incomplete diffusion blot permits to visualize the electrofocused proteins with different staining methods simultaneously.

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