COMPARSION OF DIFFERENT ELECTROPHORETIC TECHNIQUES AND STAINING METHODS FOR MEAT SPECIES IDENTIFICATION

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INTRODUCTION

Besides serological methods also various electrophoretic techniques have been used for the identification of meat species (RIGHETTI & BOSISIO, 1981; HITCHCHOCK & CRIMES, 1985; PATTERSON, 1985; KAISER & KRAUSE, 1985). The following electrophoretic methods are applied: the continous zone electrophoresis (EBERMANN & BARNA, 1972; SPELL, 1974; HEINERT & KLINGER, 1978 and 1980; HEINERT et al., 1988; SIBOUR et al., 1988) and the isoelectric focusing in polyacrylamide and agarose gels (TINBERGEN & OLSMAN, 1976; FRÜHLING & GERSONDE, 1980; KAISER et al., 1980 a,b and 1981; BAUER, 1982; KING, 1984). The protein bands in the gels were usually stained with Amido Black (EBERMANN & BARNA, 1972) or Coomassie Blue (SPELL, 1974; TINBERGEN & OLSMAN, 1976; HEINERT & KLINGER, 1978; FRÜHLING & GERSONDE, 1980; KAISER et al., 1980 a,b and 1981; BAUER, 1981; GRUNDHÖFER, 1982), in some cases special enzymes such as esterases (HEINERT & KLINGER, 1980; HEINERT et al., 1988), lactatdehydrogenase (SIBOUR et al., 1988), phosphogluconatedehydrogenase (KING & KURTH, 1982) or adenylate- and creatinekinase (KING, 1984) were made visible. Furthermore, myoglobins which can be made out because of their specific colour (SINCLAIR & SLATTEREE, 1982; HOFMANN, 1986 a,b; HOFMANN & BLÜCHEL, 1986) or, more sensitive, can be made visible because of their pseudoperoxidase activity (BAUER & HOFMANN, 1987 a-c), are used for the identification of meat species. Isoelectric focusing and continous zone electrophoresis have been included into the range of official methods of examination §35 LMBG as reference methods in the F.R.G.(BGA, 1988). The purpose of this investigestions was to compare the described methods of meat species identification and to check further electrophoretic and staining techniques for their suitabilty.

MATERIAL 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 & HOFMANN (1987a). For the application of the zone electrophoresis technique the extracts are mixed with bromphenolblue and in the case of vertical technique moreover with glycerol (final concentration approx. 0.1% bromphenolblue (w/v) and 20% glycerol (w/v)).

Gel preparation and electrophesis condition for isoelectric focusing (IEF)

0.5 mm thick polyacrylamide gels (5% T and 3% C, gel dimension 125x260 mm) containing 0.375 M Tris/HCl buffer pH 8.9 are prepared on polyester supports (Gel Fix from SERVA) using the capillary technique (SERVA information sheet Nr. 116). The gels are washed twice for at least 30 minutes with deionized water and then put into a solution of 3% sorbitol (w/v) and 2% polyvinylpyrrolidone (M.W. approx 10 000) (w/v) for at least 30 minutes. The gels are dried at room temperature. The dried gels are rehydrated by the mould technique (GÖRG et al., 1979). The solution for rehydration contains 3% carrier ampholytes (w/v) and 10% glycerol (v/v) in water. The rehydration takes at least 3 hours.

Pre-electrophoresis is carried out at 2 W constant power for 150 Volthours,

then the IEF is interrupted and the samples (10µl each) are applied using a silicon applicator strip put on the gel in a distance of 2-3 cm from the anode. Afterwards the voltage is set up to 400 V for 10 minutes. The electrophoresis is carried out at 20 W constant power and a final voltage of 1700 V for 2000 Vh.

Gel preparation and electrophesis condition for zone electrophoresis
Polyacrylamide gels (5, 7.5 and 10% T and 3%C) containing 0.375 M Tris/HCl buffer pH 8.9 are prepared on polyester films by cassette technique (LKB information sheet SD/RE-058) for horizontal electrophoresis and following the producer's instructions for the vertical technique. The stacking gels for the discontinous zone electrophoresis contain 5% total acrylamide, 3% crosslinker and 0.125 M Tris/HCl buffer pH 6.8. Porous gradient gels are made within a range of 5 to 10% total acrylamide.
Zone electrophoresis is carried out at a constant currency of 50 mA (horizontal technique, gel dimension 125x260x0.5 mm) and 20 mA (vertical technique, gel dimension 70x80x0.75 mm) using a 0.05 M Tris/ 0.38 M Glycine electrode buffer pH 8.3. It is finished after the bromphenolblue marker has reached the cathodic end of the gel.

Protein staining with Coomassie Brilliant Blue G-250 (NEUHOFF et al., 1985)
After fixation in 10% trichloracetic acid (TCA) (w/v) for 1 hour the gels are incubated in a staining solution containing 0.1% Coomassie Brilliant Blue G-250, 2% phosphoric acid (w/v) and 6% ammonium sulfate (w/v). The staining solution is prepared by mixing an aqueous solution of Coomassie (1g/20ml) with the phosphoric acid/ammonium sulfate solution. After staining for at least 12 hours the gels are washed in 0.1 M buffer pH 6.5 for 3 minutes and in 25% methanol (v/v) for 1 minute.

Protein staining with Serva Violet 17 (PATESTOS et al., 1988)
After fixation in 10% TCA and washing with 3% phosphoric acid (w/v) the gels are put into the staining solution which consists of 1 part stock solution (1 g Serva Violet 17 dissolved in 100 ml water) plus 9 parts 11% phosphoric acid (w/v) for at least 10 minutes and then are destained with 3% phosphoric acid (w/v).

Esterase staining (LKB information sheet SD/RE - 066)
200 ml 0.1 M phosphate buffer pH 7.0 is poured over the gel, then a solution of 60 mg α-naphthyl-acetate in 3 ml acetone and a solution of 100 mg Fast Blue B (resp. Fast Blue RR, Fast Blue BB, Fast Red TR, Fast Black K, Fast Garnet GBC in 3-5 ml water (only Fast Garnet GBC should be dissolved in methanol) is added. As soon as the bands have developed sufficiently the staining procedure is stopped by a solution of 20% methanol (v/v) and 10% acetic acid (v/v).

Myoglobin staining (BAUER & HOFMANN 1987a-c)
0.1 g o-dianisidine is dissolved in 30 ml ethanol followed by the addition of 70 ml 0.1 M citrate/0.2 M phosphate buffer pH 5 and directly before use 2 ml hydrogen peroxide. Within 15 minutes the formation of an intense brown-red colour is finished.

Conservation
Gels on polyester support are incubated in a solution of 0.5% acetic acid (v/v) and 2% glycerol (v/v) and dried at room temperature. Other gels are sealed in vacuum bags after incubation in the acetic acid/glycerol solution.

RESULTS

Electrophoretic techniques
By means of IEF the investigated species cattle, pig, sheep, horse, rabbit, chicken and turkey can be identified, if the separated proteins are made visible by Coomassie Brilliant Blue G-250 (CI. 42655) or Serva Violet 17 (CI. 42650) (Fig.1a). Applying the specific myoglobin staining technique these meat species with exception of very closely related animals like chicken and turkey can be proved quickly and reliably (Fig.1b).

esterase staining different patterns can be made out, but species identification can be difficult or even impossible because of the very pale bands of some species e.g. beef and pork Fig.1c). IEF is carried out in rehydratable polyacrylamide gels. The advantages of this technique in general (FREY et al., 1986) and especially for meat species identification have already been described (BAUER & HOFMANN 1987 a-c).

By continous zone electrophoresis and protein staining beef and mutton can be distinguished only by slightly developed side bands, which however could not be clearly visualized in all our experiments (Fig.2a). Using only the main bands for identification, as described by HEINERT et al. (1988) these two species could not be distinguished. All the other investigated species could be identified because of their main bands. Comparing the myoglobin bands beef and mutton as well as chicken and turkey cannot be distinguished (Fig.2b). Furthermore, difficulties may arise because meat with low myoglobin content (chicken, rabbit, pale pork) cannot or only very weakly made visible by pseudoperoxidase staining. By means of esterase staining the investigated meat species could be identified minding the same conditions as in IEF (Fig.2c).

Investigations were carried out by vertical as well as horizontal techniques. In the latter case the polyacrylamide gels were poured on polyester films. What concerns our experience the horizontal technique seems to be particularly suitable because of the better handling of gels in the staining procedures and the easy way of documenting them. Besides in horizontal zone electrophoresis no U-shaped bands appear whereas they do in vertical technique especially at high protein concentrations. The use of polyacrylamide gels with a total acrylamide concentration of 7.5% seemed to be most suitable for the optimal separation of the most important proteins for species identification.

The application of a porous gradient gel and also of the discontinous zone electrophoresis show no advantages compared to the continous zone electrophoresis what concerns the distinguishing of meat species but their preparation takes more time.

Staining methods

The protein staining with Coomassie Brilliant Blue in colloidal state means an improvement of the traditional Coomassie staining in water/methanol/acetic acid concerning sensitivity and background staining. By the low concentrations of acrylamide a completly clear background cannot be obtained. However the well coloured protein bands contrast to the pale blue background. This procedure takes the same time as the Coomassie staining in water/methanol/acetic acid destaining included. Another staining method which uses a dye in colloidal state is the protein staining with Serva Violet. Its advantage is the fact that its procedure takes only one to two hours with 0.5 mm gels which enables a very quick species identification.

For the proof of esterases α-naphthyl-acetate was used as substrate and various diazonium salts recommended in the literature (BLAICH, 1978; ÖHLENSCHLÄGER et al., 1980; GAAl et al, 1980) were checked for their suitability for species identification in what concerns their sensitivity and background staining. According to our experience Fast Blue B and Fast Red TR seemed to be most suitable chromogens although they are not the most sensitive ones. The bands are clearly developed and there are no smears in bands of meat with a high esterase content. Fast Blue RR and BB are more sensitive and often show these smears but they are better for investigestions of meat with low esterase content. Fast Black K and Fast Garnet GBC have been neglected because of their dark background. The specific myoglobin staining method by o-dianisidine was described in detail and discussed in former publications (BAUER & HOFMANN, 1987 a-c).

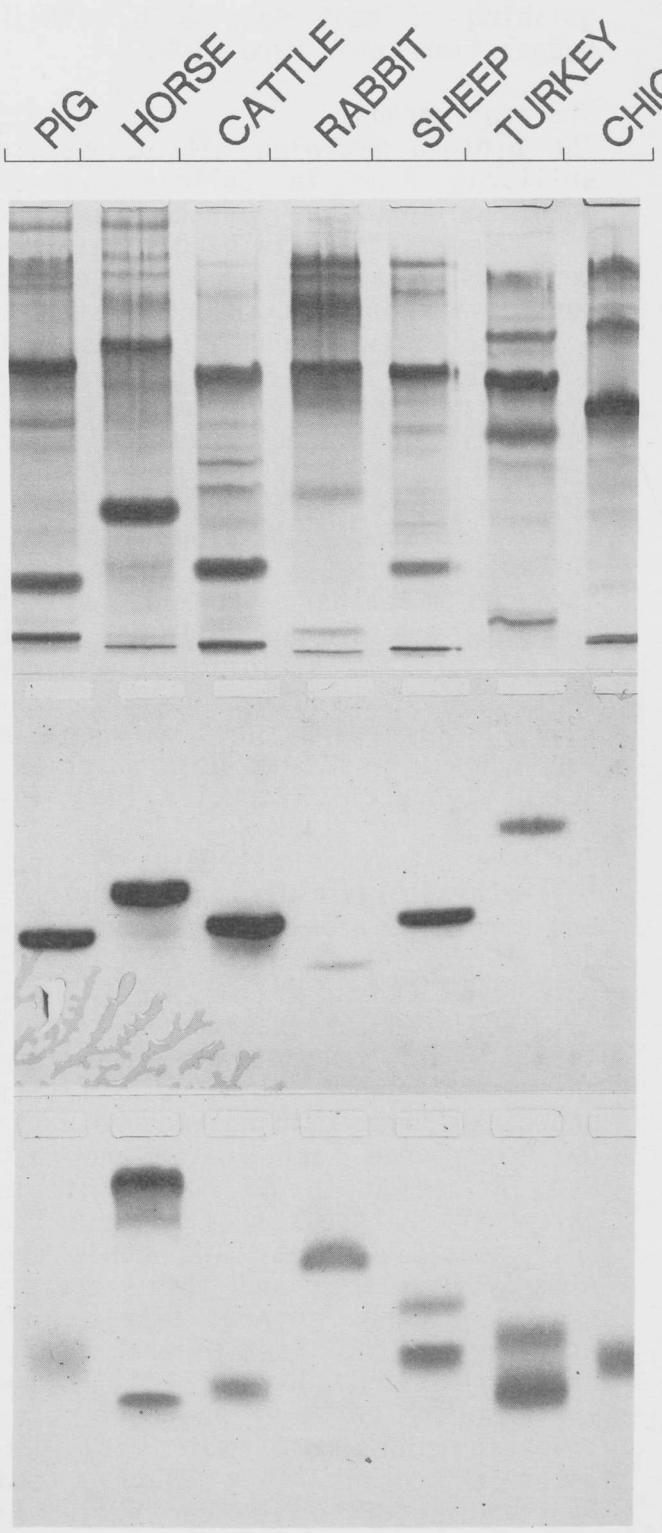

Fig.2a: Zone electrophoresis, 7.5%T, staining with Coomassie Brilliant Blue G-250, cathode on the top.

Fig.2b: Porous gradient gel electrophoresis, 5-10%T, pseudoperoxidase staining with o-dianisidine, cathode on the top.

Fig.2c: Zone electrophoresis, 7.5%T, esterase staining with α-naphthyl-acetate and Fast Blue B, cathode on the top.

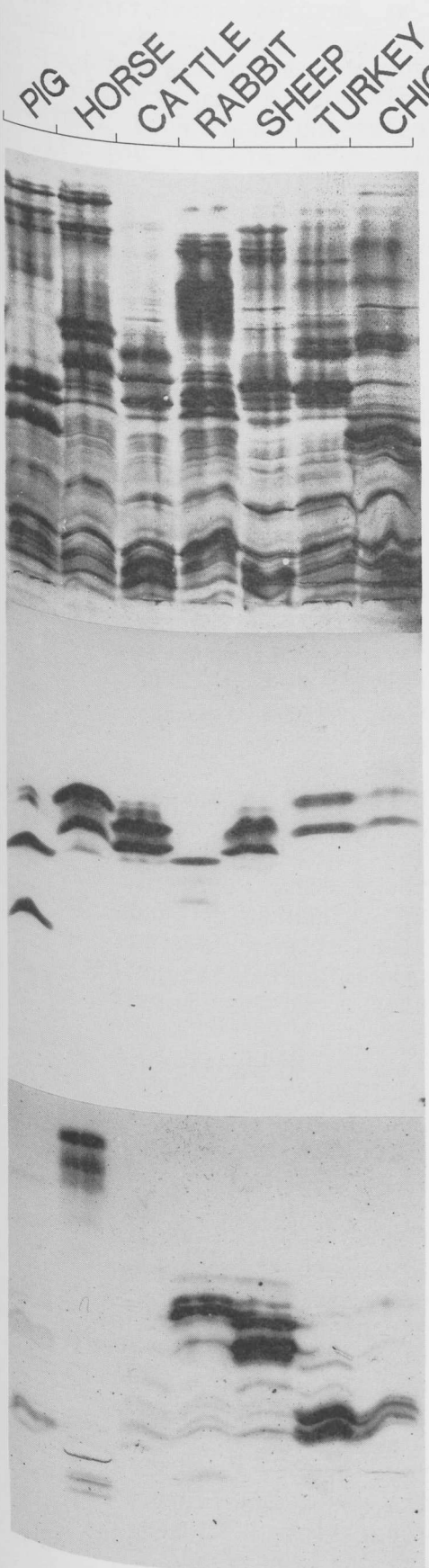

Fig.1a: Isoelectric focusing, pH gradient 3.5 - 9.5, staining with Serva Violet 17, cathode on the top.

Fig.1b: Isoelectric focusing, pH gradient 3.5 - 9.5, pseudo-peroxidase staining with o-dianisidine, cathode on the top.

Fig.1c: Isoelectric focusing, pH gradient 3.5 - 9.5, esterase staining with α-naphthyl-acetate and Fast Blue B, cathode on the top.

CONCLUSIONS

The comparsion between various methods of electrophoresis and staining showed, that none of them can be preferred unrestrictedly. The method should be chosen according to the specific aims of the investigation. For a quick and informative species identification the proof of the myoglobin bands is the method of choice. If a distinction between closely related species is desired the patterns of the whole sarcoplasmic proteins will be necessary. Attention must be paid to the influence of various circumstances which can change the patterns in IEF (HOFMANN, 1985) as well as in zone electrophoresis (HEINERT et al., 1988). Also the esterase patterns depend on different influences as demonstrated by the pherogramms in the publication of HEINERT et al. (1988). The content of esterases varies from one species to another and even different parts of the same animal show some differences concerning intensity and position of the bands. Therefore in our opinion meat species identification is only possible to a certain extent using only some of the bands and neglecting the inconstant other ones. The safest method is still the direct comparsion between the bands of the samples in question and an authentic one, applied side by side. Species identification can only be taken for sure, if all bands correspond with the reference sample.

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