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Meat species identification of raw muscles by isoelectric focusing of the myoglobins

K. HOFMANN
Federal Centre for Meat Research; Institute of Chemistry and Physics, D-8650 Kulmbach
Federal Republic of Germany

The determination of meat species is an important task in the food inspection because the amounts of imported game meats which have to be controlled are increasing. Usually the terms "identification" and "differentiation" are used as synonyms. However, it seems to be justified to distinguish those cases, in which the species of meat in question has to be clarified (identification) from those in which an undesirable meat species should be excluded (differentiation).

Isoelectric focusing of the total sarcoplasmic proteins which has been reported to be successful for species identification of meat from slaughter animals, results in complicated protein patterns. Their judgement is difficult because the patterns depend on the type of muscle and on several other influences (HOFMANN 1985a, 1986). The methods and problems of meat species identification were recently discussed on a Scientific Workshop (see HOFMANN 1985b; PATTERSON 1985).

If the gels used for electrophoresis are thick enough the myoglobin bands can be recognized by their own red-brownish colour. Therefore a time-consuming staining procedure is not needed. We found that polyacrylamide gels 0.5 - 1 mm thick used for isoelectric focusing are adaquate for this purpose. Most of the species investigated show two myoglobin bands with characteristic positions (isoelectric points) which allow a definite identification of the meat species.

HÖYEM and THORSON (1970), POZO-LORA and LOPEZ-GIMENEZ (1973) and SPELL (1974) were able to distinguish meat from different species by their myoglobins using polyacrylamide or discelectrophoresis, whereas the isoelectric focusing was applied at first by SLATTERY and SINCLAIR (1983). The latter were able to distinguish beef and buffalo as well as red and grey kangoroo, which could not be differentiated by serological methods. The differentiation between donkey and horse meat, resp. sheep and goat meat was not possible by this technique.

Material and methods

Principle of isoelectric focusing

Isoelectric focusing (IEF) is a special electrophoretic technique. Under the influence of an electric field a pH gradient is formed in a gel prepared with the addition of amphoteric substances (ampholytes). The separation of proteins by IEF uses the fact that the net charge of a protein varies with the pH value of its environment. Consequently the proteins stop moving when they have reached that pH at which their net charge is getting zero (isoelectric point). As the proteins have different isoelectric points the IEF results in different bands distributed in the gel over the pH gradient used.

Apparatus

For isoelectric focusing the LKB Multiphor System was applied (Multiphor 2117, Macrodrive 5 Power Supply, Multitemp, mould for preparing gels). Polyacrylamidegels with different pH gradients (3-10, 5-9, 6-9) were prepared using Servalyt® carrier ampholytes. Furthermore LKB Ampholine PAGplates (ready-prepared thin-layer polyacrylamide gels) pH 5.5 - 8.5 were used. This range proved to be the most suitable. In this case 0.1 M NaOH was used as electrolyte solution for the cathode and 0.4 M HEPES [N-(2-hydroxyethyl)piperazine-N'-2-ethansulfonic acid] for the anode.

Procedure

The principle of the procedure used was described by GÖRG et al. (1979) and in the instructions of LKB (from Dr. H. Schickle and F. Horneff). In general samples of 20 µl of juice or drip from fresh or frozen meat were dropped on EF-Sample Application Pieces (LKB) by means of a micropipette. These were placed on the gel slabs (0.5 mm thick) in a distance of about 3 cm from the anode. In several cases freeze dried meat extracts were available (freeze drying has no influence on the quality of the patterns of the myoglobins in the gel). They were dissolved with 4 parts of water before use for IEF.

Meat extracts are not useful in this procedure because their myoglobin concentration is usually to low. Samples of meat juice from pale meats (e.g. special pork or poultry muscles) which may also be low in their myoglobin content have to be concentrated prior to application.

During IEF the temperature was kept at +4°C and the "Power Supply" was adjusted to 1500 V, 50 mA and 25 W. Under this conditions the focusing was finished after 2 hours.

Measurement of the isoelectric points (pI)

The pI values of single myoglobin bands were measured by means of a special surface pH electrode (Multiphor Elektrode LKB 2217-111) on PAGplates pH 5.5 - 8.5 at 24°C. The electrode was put on the surface of the gel by its own weight, so the diaphragm and the glass membran formed one line with the myoglobin band in the gel.

Documentation

The myoglobin patterns were photographed immediately after finishing the IEF. Furthermore the bands were drawn on a mylar foil by means of an overhead pen. The foil was put on a glass plate covering the gel avoiding direct contact with the gel.

The fixing of the myoglobin bands was achieved by heating the gel covered with a second mylar foil in a boiling water bath for 2 min. Trichloric acetic acid was not useful for fixing because the bands were discoloured.

Results

Some results of isoelectric focusing in polyacrylamide gels with different pH-gradients are shown in figs. 1-3.

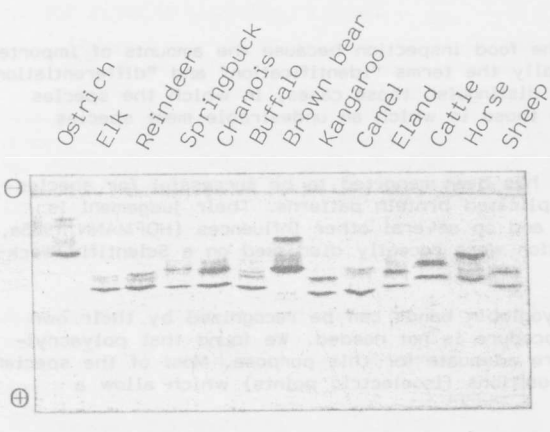

Fig. 1: Isoelectric focusing of the myoglobins of different species in pH gradient 5-9

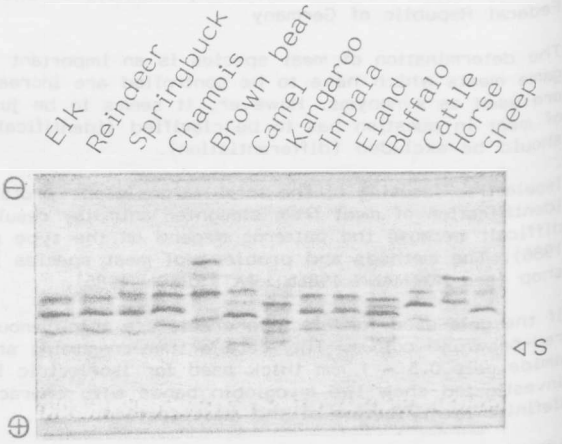

Fig. 2: Isoelectric focusing of the myoglobins of different species in pH gradient 6-9. The application points of the samples are marked with "S"

The best results were achieved when the pH gradient 5.5 - 8.5 was used (fig. 3). Therefore these gels were used to measure the pI values of the myoglobin bands the results of which are presented in table 1 and fig. 4 (atlas of myoglobins).

In the literature there are only few data about pI values of myoglobins of different species. Therefore our results could be only compared with those from horse and beef (tab. 2). The results presented by different authors differ considerably (tab. 2); these which do agree with those be found in our experiments are marked by bold type.

Table 1. pI values of the myoglobins of muscles from different species measured on polyacrylamid gels with pH gradient 5.5-8.5 (at 24°C)

Meat species	main bands		minor bands			
Cattle	7,1	6,8	6,4	6,2		
Buffalo	6,85	6,6	7,3	7,1	7,0	6,35
Horse	7,25	6,85	7,0			
Pig	6,5	6,0				
Sheep	7,05	6,75	7,4	7,2		
Kangaroo, grey	6,9	6,5	6,75			
Camel	7,15	6,7	6,85	6,25		
Brown bear	7,6	7,05	7,2	6,55		
Rabbit	7,4	7,3	6,3			
Hare	7,0	6,6				
Deer	7,05	6,75	6,85	6,4		
Fallow deer	7,05	6,75	6,4			
Elk	7,05	6,75	6,4			
Reindeer	7,05	6,75	6,85	6,4		
Chamois	7,05	6,75	7,4	7,25	6,85	6,4
Impala	7,05	6,75	6,4			
Eland	7,05	6,75	6,4			
Black buck	7,05	6,75	7,4	7,25	6,4	
Blesbock	7,05	6,75	6,4			
Springbuck	7,05	6,75	7,4	7,25	6,85	6,4
Saiga	7,05	6,75	7,4	7,25		
Duck	8,1	-				
Wild duck	8,1	7,5				
Ostrich	8,05	7,45				

The existing deviations show clearly that the pI values determined cannot be taken as absolut and constant under all circumstances. Their reproducibility depends on the conditions of focusing which are difficult to keep constant as the practical experiences show.

Most of the 24 meat species investigated exhibited two main myoglobin bands and a various number of minor bands. The positions and, consequently, the pH values of the main bands are different and characteristic in the case of cattle, buffalo, horse, pig, kangaroo, camel, bear, rabbit, hare, duck and ostrich. This means that these species can be identified by their myoglobin patterns.

It should be emphasized that even the myoglobin bands of beef and buffalo meat are so different from each other that they can be differentiated easily. In addition this fact shows that both species may not be as near related as it was assumed in the past.

Different from the myoglobin patterns discussed so far are those from a series of partly near related species as deer, fallow deer, red deer, reindeer, elk, chamois, eland, black buck, blesbock, springbuck and saiga (fig. 4). With regard to the main bands these species do not differ from each other. Therefore they have to be distinguished by additional criteria. In some cases the minor bands will be useful for this purpose (see fig. 3 and 4). In addition when the gel is looked over with a black background by light shining from the side different bands of the sarcoplasmic proteins can be recognized by their white opalescence which may be

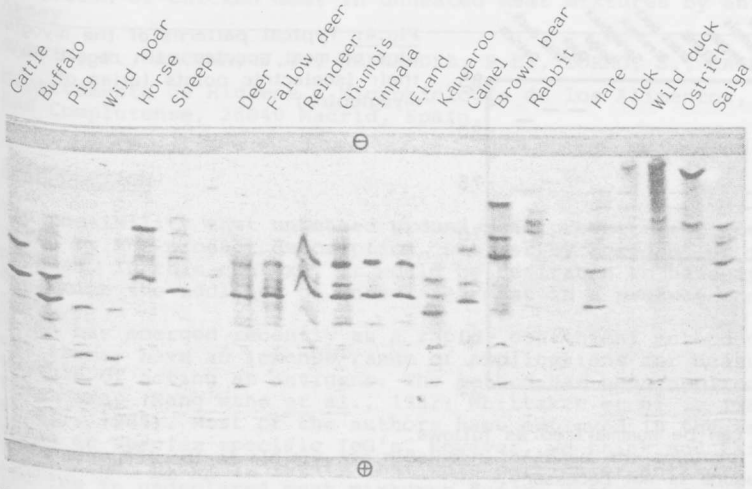

Fig.3: Isoelectric focusing of the myoglobins of different species in pH gradient 5.5-8.5 (PAG plates)

also useful for further identification. The myoglobin patterns did not depend on the type of muscle or on the time of storage of the meat. However, the intensity of the single bands varied from muscle to muscle, from animal to animal and also in some cases when the experiment was repeated. But according to our practical experiences this does not influence the usefulness of the method.

It is not be expected that the hemoglobin of residual blood in the muscle will interfere with the identification of meat species by the myoglobins because the possible concentration of hemoglobin is much lower than those of myoglobin. As known from literature even the patterns of the sarcoplasmic proteins of extremely uncomplete bleeded slaughter animals did not contain any additional bands which were due to a higher amount of hemoglobin (STOLLE et al., 1983). Additionally we found that the hemoglobin bands, e.g. from cattle, pig, sheep and horse, were different in their positions from those of the corresponding myoglobin bands.

It should be mentioned that this method for meat species identification can be applied to unheated materials only because heating splits obviously the hem component from the globin in the myoglobin molecule. Therefore, it is also impossible to use 8 M urea which dissolves proteins denatured by heat.

Discussion

The content of myoglobin in the muscle of meat animals is varying within a wide range and depends on several influences like breed, sex, feeding, age and the kind of live stock keeping. The myoglobin concentration in muscles of meat animals can vary from 20 (rabbit) to 910 (whale) or, in the case of slaughter animals, from 30 (pig) to 880 (horse) (see GINGER et al., 1954; GRAU, 1969; LAWRIE, 1968; SHENK et al., 1934). The relation of the myoglobin content in beef, mutton, pork and veal was estimated to be 7:4:2:1(BROUMAND et al.1958).

Table 2: pI values of the myoglobins of horse and cattle mentioned in literature

Meat Species	pI		Temperature	Literature
Horse	6,47 6,60 6,83 7,17	6,53 6,79 6,92	20°C	van den OORD et al., 1969
	6,76	7,16	4°C	BEELEY et al., 1972
	6,8 7,27	6,86 7,76	4°C	VESTERBERG and SVENSSON, 19,66
	6,8 7,1	7,0 7,5	(?)	SALAMAN, 1971
	7,31	6,83	25°C	RADOLA, 1973
	7,3		4°C	reported by SERVA (Heidelberg)
Cattle	6,56 7,01	6,74	20°C	van den OORD et al., 1969

The variability of the myoglobin content explains the different intensities of the myoglobin bands which were also observed in the SDS electrophoresis (HOFMANN,1985c,1986).

The question is, whether the existence of diverse myoglobin bands is due to different isomeres or to the presence of different forms or steps of oxidation. QUINN and PEARSON (1964) and QUINN et al. (1964) found that beef muscles contained at least three kinds of myoglobin with different electrophoretic behaviour. They postulated that the different myoglobins were characterized by differences in the binding between the protein (globin) and the prostetic group (hem). Also in investigations with myoglobins of horse, cattle and whale (RADOLA, 1969) and the myoglobins of human being and rat (HERBERTZ, 1972) resulted in the appearance of diverse bands. In the latter experiment the myoglobin was stabilized by the formation of an Fe (III) cyano complex so the myoglobin did not exist in different steps of oxidation. This result promotes the assumption that native muscles may contain several isomeres of myoglobin.

Conclusions

Isoelectric focusing of the proteins of undiluted meat juices in polyacrylamide gels (0.5 mm thick, pH gradient 5.5-8.5) exhibits red-brownish bands of myoglobin which are characteristic for most of the species investigated. The position of two main bands each enables to identify the meat from cattle, buffalo, horse, pig, kangaroo, camel, bear, rabbit, hare, duck and ostrich. Nearly related species like different kinds of deer and antilope being identical in their main myoglobin bands, may be differentiated by further characteristics. The isoelectric points of the myoglobins were determined. They can be used for identification of meat species in question without having a pool of standards.

The myoglobin patterns in the gels do not depend upon the type of muscle and other factors as the patterns of the total sarcoplasmic proteins do (HOFMANN, 1985c, 1986).

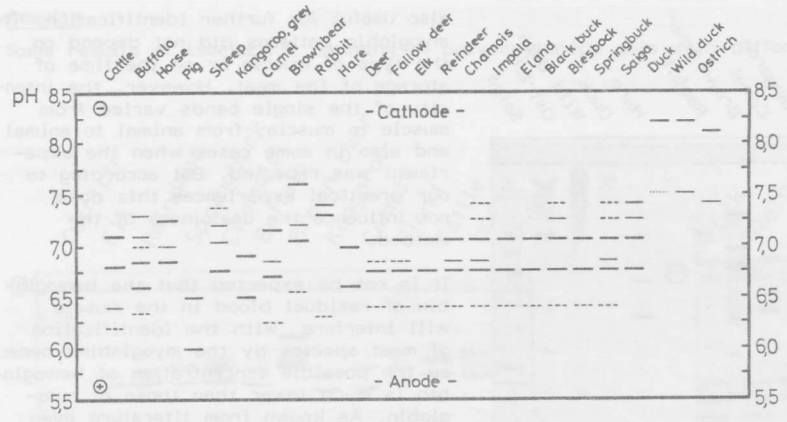

Fig.4: Typical patterns of the myoglobins of meat species with regard to their isoelectric points (atlas of myoglobins)

The advantages of the "myoglobin method" can be summarized as follows.

1. In contrast to most of the sarcoplasmic proteins the bands of myoglobin are visible in the IEF gels used so that a time-consuming staining procedure is not necessary.
2. The myoglobin patterns are simple and can be judged easily in contrast to the patterns of the whole sarcoplasmic proteins usually being complicated.
3. The positions resp. pI values of the myoglobin bands were found to be constant and did not depend on several factors as e.g. the kind of muscle or the time of storage post mortem.
4. As the identification of meat species can be managed by measurement of the pI values with the aid of a surface pH electrode no pool of standards is needed.
5. Drip from stored or thawed frozen meat often being available in practice may be used directly as a sample for isoelectric focusing without additional preparation.

Acknowledgements

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