Myoglobin Electrophoretic Patterns in Identification of Meat from Different Animal Species

The identification of meat from different animal species used as human food, employing disc electrophoresis on polyacrylamide gels, is described. The method is based upon the migration of the myoglobin bands, which, in the electrophoretic system employed, differs sufficiently to give a clear-cut identification of low-cost meats, like whale and horse meat, in mixtures with valuable meat such as beef.

Determination of the origin of meat component(s) in meat products is an important and challenging task in food hygiene and food control. Addition of low-cost meat like horse or whale meat to, for instance, beef in the preparation of meat products is not uncommon. Identification of meat may also be of great importance in veterinary forensic medicine.

Several methods for the identification of meat based upon chemical-chromatographic, chemical-physiological, and electrophoretic principles have been developed. These methods have not, so far, come into practical use to any considerable extent. The routine methods are dominated by serological methods, fundamentally based upon the work of Uhlenhuth (1901).

Serological methods are based on the fact that proteins foreign to animal organisms, by parenteral application, cause the production of specific antibodies in the blood, which may be employed in serological reactions. Precipitation tests are the ones employed in the majority of laboratories, and may also, according to Rosenberg (1926, 1928), be used for heated meat products.

Several authors (Ouchterlony, 1949; Scheibner, 1960) have adopted the precipitin test to the agar-plate method, by which the precipitation is clearly visible, and a specific precipitation is easily distinguishable from a nonspecific one.

Herrmann and Kotter (1968) found the agar-plate diffusion method more sensitive than the precipitin test, and this method can also be used for turbid antigen-antiserum solutions. The method is practical and can easily be brought into use, even in small laboratories. However, the cross-reaction problem asserts itself even in this method.

Cook and Sturgeon (1966) identified horse, pork, and beef meat by the use of gas chromatography on the unsaponifiable matter, first fractionated by column chromatography.

Species identification using electrophoretic techniques has been employed by several authors. Hill *et al.* (1966) applied electrophoresis using agar gel as the supporting medium in the identification of fish species. Thompson (1960, 1961) employed starch gel zone electrophoresis for identification of protein extracts, for fish species identification, and later, for the identification of animal species. Mackie (1968), Chu (1968), and Manusco (1964) used disc electrophoresis for the identification of fish species, while Cowie (1968) employed thin-slab polyacrylamide gel electrophoresis for the same purpose.

The need for a fairly rapid, inexpensive, and reliable method to detect, in the first instance, whale meat and horse meat in mixture with other more valuable meats prompted us to develop a method for the identification of meat species. This paper describes the identification of the species mentioned by evaluating the myoglobin band patterns produced in polyacrylamide electrophoresis.

MATERIALS AND METHODS

Materials. The meat samples used for the experiments were fresh or frozen material. Whale (*Balaenoptera borealis*) and bear (*Ursus arctos*) meat samples were available only in the frozen state. *Balaenoptera borealis* is the most important whale species caught for human consumption off the coast of Norway.

Representative samples of fresh meat from cattle, horse, sheep, goat, pig, moose (*Alces alces*) and reindeer (*Rangifer tarandus*) were collected at slaughterhouses.

Reagents. The chemicals employed for making the polyacrylamide gels, acrylamide, *N,N*-methylene-bis-acrylamide, *N,N,N',N'*-tetramethyl ethylenediamine, were purchased from Eastman Organic Chemicals.

The other reagents used were *pro analysi* reagents from various manufacturers.

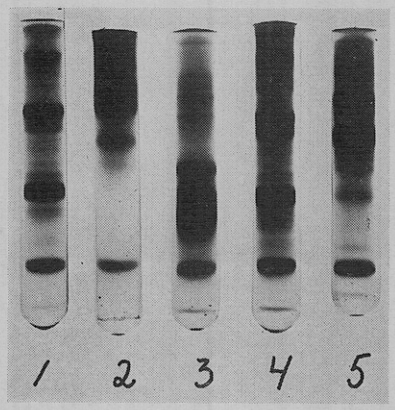

Figure 1. Polyacrylamide gel protein patterns, stained with Amido Black, of beef (1), whale (2), horse (3), reindeer (4), and bear (5)

Experimental conditions as described in the text

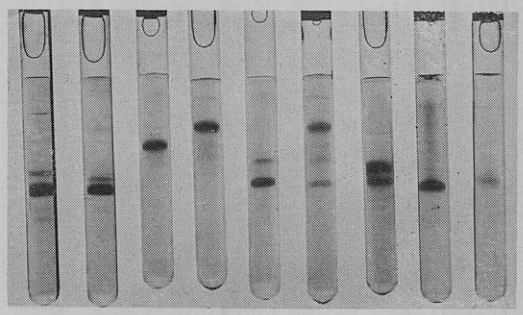

Figure 2. Polyacrylamide gel patterns of myoglobin from various meat species

No staining. From left to right: moose, reindeer, bear, whale, beef, mixture of whale and beef, horse, beef minced meat, and beef/pork minced meat

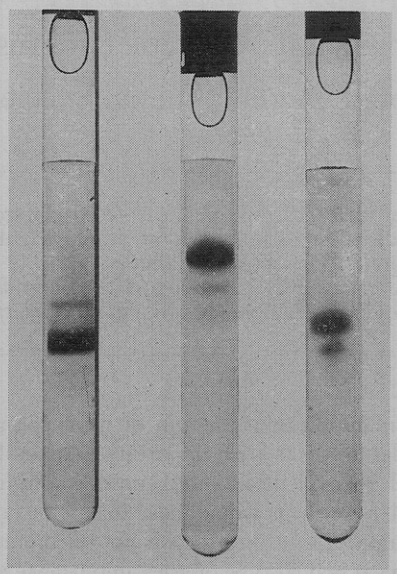

Figure 3. From left to right: Myoglobin bands of beef, whale and horse

Note the position of the secondary myoglobin band for horse meat

Table I. R_B Values (see Text) of Myoglobin Bands for Various Animal Species

Species	R_B values	
	Major band	Secondary band
Whale	0.28	...
Cow	0.51	0.40
Horse	0.43	0.50
Sheep	0.50	0.41
Moose	0.49	0.43
Reindeer	0.50	0.44
Goat	0.51	0.41
Pig	0.52	...
Bear	0.35	...

Apparatus and Electrophoresis Procedure. Gel electrophoresis of the meat extracts was carried out on 7.5% polyacrylamide gels, essentially using the procedure of Ornstein and Davis (1964). However, the sample and spacer gels were omitted and replaced by a Sephadex-sucrose-buffer mixture as described by Broome (1963). The gels were 10 cm long and had a diameter of 0.5 cm. Running time was approximately 40 min with a constant current of 4 mA per tube. The electrophoresis was performed at room temperature and with bromophenol blue as tracking dye.

After completion of electrophoresis, the gels were removed from the glass tubing and stained for protein with 1% Amido Black in 7% acetic acid, or fixation of the naturally-colored myoglobin bands was carried out by submerging the gels in 3% hydrochloric acid, containing 0.5% potassium rhodanide to brighten the color of the myoglobin.

Preparation of the Meat Extracts. Five grams of meat (if possible freed from fatty tissue) was homogenized in a mortar with 5 ml of distilled water. The homogenate was centrifuged at 1.400 × g for 15 min.

Aliquots (150 μl) of the supernatant were used for electrophoresis. The aliquot was mixed thoroughly with the Sephadex–sucrose–buffer mixture (0.2 ml) used on top of the polyacrylamide column.

RESULTS AND DISCUSSION

The total protein patterns of each animal species examined, as revealed by staining with Amido Black, turned out to be rather specific, as shown in Figure 1. Sex, age, or storage in the frozen state for several weeks did not alter the patterns significantly (Skaare and Høyem, 1968).

It was realized, however, that the primary aim for this study, to identify whale and horse meat in admixtures with other meat proteins, could be approached simply by comparing the brownish myoglobin bands which can be readily observed during and after electrophoresis without staining with Amido Black.

Distinct myoglobin bands were obtained with all species examined (Figure 2). For some species two bands were obtained, one major band and one secondary band. The migration rates of the various myoglobin bands compared to the migration rate of the bromophenol blue are listed in Table I.

As seen from Table I, whale meat has one myoglobin band with a R_B value of 0.28 (R_B value = migration distance for myoglobin band/migration distance for bromophenol blue). This is considerably less than the R_B values of the myoglobin bands of any of the other meat species examined. Horse myoglobin has a major band at R_B 0.43, which is among the lower R_B values, and in addition a second, minor band migrating at R_B 0.50. This is the only observed case where the

main myoglobin band has a lower R_B value than the secondary one. The features mentioned will easily distinguish horse and whale meat from other meat species.

As far as meat from ruminants and pig is concerned, the myoglobin electrophoretic patterns are rather similar and do not distinguish sufficiently between these species. For the identification of these species the total protein patterns, as revealed by staining with Amido Black, should be evaluated.

The method described has been used for identification of the meat species used in commercially available meat products (Figure 3). Falsifications with whale or horse meat are easily detected.

Efforts to make the method work for heated products have failed, in spite of numerous modifications in extraction techniques and in the composition of the polyacrylamide gels. For such products one presumably has to find other methods for meat identification than electrophoretic and serological ones.

ACKNOWLEDGMENT

This work was supported by a grant from The Agricultural Research Council of Norway, Oslo, Norway.

LITERATURE CITED

Broome, J., *Nature* (London) **199,** 179 (1963).
Chu, R., *J. Ass. Offic. Agr. Chem.* **51,** 754 (1968).
Cook, H. R., Sturgeon, J. D., *J. Ass. Offic. Agr. Chem.* **49,** 877 (1966).
Cowie, W. P., *J. Sci. Food Agr.*, **19,** 226 (1968).
Herrmann, C., Kotter, L., *Arch. Lebensmittelhyg.* **12,** 266 (1968).
Hill, W. S., Learson, R. J., Lane, J. P., *J. Ass. Offic. Agr. Chem.* **49,** 1245 (1966).
Mackie, I. M., *Analyst* **93,** 458 (1968).
Manusco, V. M., *J. Ass. Offic. Agr. Chem.* **47,** 5 (1964).
Ornstein, L., Davis, B. J., *Ann. N. Y. Acad. Sci.* **121,** (1964).
Ouchterlony, Ö., *Med. Diss.* (Stockholm) (1949).
Rosenberg, R., *Zentralbl. Bakteriol. Parasitenk. Infektionskr., Abt. 1: Orig.* **98,** 259 (1926).
Rosenberg, R., *Zentralbl. Bakteriol. Parasitenk. Infektionskr., Abt. 1: Orig.* **107,** 448 (1928).
Scheibner, G., *Monatsh. Veterinaermed.* **15,** 341 (1960).
Skaare, K., Hoyem, T., unpublished, 1968.
Thompson, R. R., *J. Ass. Offic. Agr. Chem.* **43,** 763 (1960).
Thompson, R. R., *J. Ass. Offic. Agr. Chem.* **44,** 787 (1961).
Uhlenhuth, P., *Dent. Med. Woch. Schr.* **27,** 780 (1901).

Tore Höyem
Björn Thorson

Department of Food Hygiene
Veterinary College of Norway
Oslo, Norway

Received for review October 1, 1969. Accepted April 17, 1970.