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SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECTROPHORESIS ANALYSIS FOR IDENTIFICATION OF RED MEAT ANIMAL SPECIES

(With One Figure)

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التحليل الكهربى للتعرف على أنواع اللحوم الحمراء للحيوانات المختلفة

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تم استخدام التحليل الكهربى بطريقة (SDS- PAGE) للتعرف على اللحوم الحمراء للحيوانات المختلفة. وكانت الصورة البروتينية لعينات عضلات أخذت من الحيوانات المختلفة والتي تم تفريدها على جيل ١٢% وصبغها بالكوماسى الأزرق قد أظهرت اختلافات فى الأنواع المختلفة (الأبقار ، الجمال ، الخيول ، الأغنام ، الخنازير والكلاب). وكان الوزن الجزيئى للشرائط البروتينية فى كل الأنواع يتراوح ما بين ١٤,٣٠٠ حتى ٦٦,٠٠٠ كيلو دالتون فيما عدا الجمال فكان يوجد شريط بروتينى واضح أعلى من ٦٦,٠٠٠ كيلو دالتون. وفى الخيول كان يوجد شريط واضح فى الوسط عند ٣٤,٧٠٠ كيلو دالتون. أما فى الأغنام والخنازير والكلاب فلوحظ اختلافات طفيفة فى الصورة البروتينية ولكنها كانت مميزة لكل نوع. والخلاصة أنه يمكن تحديد نوع اللحوم بإستخدام الفصل الكهربى حيث أنها طريقة دقيقة وسهلة وسريعة مقارنة بالطرق الأخرى للكشف على الغش فى اللحوم ومنتجاتها.

SUMMARY

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS- PAGE) analysis was used to identify red meat of various animal species. The protein profiles of whole muscle samples of different animal species after Coomassie- stained 12% gel showed different bands in different species. In all species (cattle, camel, horse, sheep, pig and dog), the molecular weight of the protein bands was ranged from 14.300 to 66.000 kDa, except camel, one prominent distinct band appeared

above 66.000 kDa. In horse, the band pattern showing control distinct band at 34.7000. In sheep, pig and dog there were slight differences in their bands pattern which characteristic for each species. In conclusion, species speciation of meat can be done by SDS- PAGE and it is accurate, easy and comparatively rapid technique than many other methods which used for detection of adulteration of meat and meat product.

Key words: *Reel meat, animal species, electrophoresis*

INTRODUCTION

In recent years, species identification of meat have been gaining in practical importance. This has been caused, on the one hand, by a lack of confidence of consumers regarding the origin of marketed products. On the other hand, by the concern of producers to ensure the safety of the consumers products. The need for meat species identification derives from the lack of confidence regarding the statement of the manufacturer concerning the meat species as well as some religious consideration (Hindus do not eat beef, followers of Islam and Judaism do not eat pork) as well as the prohibition of trading meat derived from animal species under protection or threatened with extinction.

It is necessary to control meat products which were subjected during their manufacture to various technological processes causing significant property changes of the applied raw materials which may, additionally, contribute to difficulties in the process of their identification. It may also be necessary to determine the percentages composition of an article manufactured using different meat species.

Electrophoretic methods can be used to identify meat of various animal species (Montowska and Pospiech, 2007).

PAGE electrophoresis can be used for the protein identification of meats derived from: pigs, cattle, horses, sheep, fish, reindeer, mouse, goats and bears.

The SDS- PAGE (sodium dodecyl sulphate- polyacrylamide gel electrophoresis) method for identification of meats derived from different animals species are based on the examination of muscle extracts.

The present study was focused on the species identification of meat with the assistance of electrophoretic method.

MATERIALS and METHODS

Samples:

10 gram fresh meat samples of cattle, sheep, pig, horses, dogs and camels were obtained following slaughter procedures. All samples were used immediately upon removal for preparation of purified whole muscle homogenates.

Whole muscles preparation:

The whole muscles were prepared by using a modification of the method of Bechtel and Parrish (1983). A 4 gm of muscle was knife-minced, added to 10 ml of a solution (25°C) containing 2% (Wt/ Vol) SDS, 10 mM sodium phosphate buffer 7.0 and homogenized with a motor homogenizer. The samples were centrifuged at 1.500 x g for 15 min at 25°C to remove traces of insoluble components.

Protein concentrations of the supernatants were determined by using the bicinchoninic acid (BCA) method (Smith *et al.,* 1985). Samples were diluted with water for 6.4 mg/ml and then prepared for SDS- PAGE.

Preparation of whole muscle homogenate for SDS- PAGE:

The whole muscle homogenates suspended in 5 mM tris-HCl (pH 8.0) were diluted to 3.2 mg/ ml and then 1 vol of each sample was immediately combined with 0.5 Vol. of 25°C sample buffer/ traching dye solution (3 mM EDTA, 3% [wt/ vol] SDS, 30% [vol/ vol] glycerol, 0.003% [wt/ vol] pyronin Y, and 30 mM tris- HCl, pH 8.0) (Wang, 1982) and 0.1 vol of 2-mercaptoethanol for a final protein concentration of 2 mg/ ml. the samples were heated at 50°C for 20 min before loading onto polyacrylamide gels.

Gel system:

The SDS- PAGE analysis was done according to the procedure of Laemmli (1970), with modification according to Huff- Lonergan *et al.* (1995) to accommodate separation of proteins with widely different molecular weights. A 12% polyacrylamide separating gel was made from a 30% stock solution of acrylamide (acrylamide: N, N'- bis-methylene acrylamide = 37: 1) and .375 M tris- HCl (pH 8.0), 2 mM EDTA, 0.1% (wt/ vol) SDS, 0.67% (vol/ vol) N,N,N'N'-tetramethylethyl- enediamine (TEMED), and 0.1% (wt/ vol) ammonium persulfate (APS). The protein was separated on SDS- polyacrylamide slab gels using Heofer mini- gel system (SE 250, Mighty small II) with PS 500 XT power supply (Hoefer Scientific Instruments, San Francisco,

CA). The gel dimensions = 100 mm high X 80 mm wide X 1.5 mm thick. The running buffer used in both the upper and lower buffer chambers consisted of 25 mM tris, 192 mM glycine, 2 mM EDTA and 0.1% SDS. Fifty micrograms of protein was loaded per lane onto the slab gel. Electrophoresis was performed at room temperature at a constant voltage of 100 v until the bromophenol blue dye reached 1 cm from the bottom.

A mixture of molecular weight standards (SIGMA- ALDRICH) consists of 6 proteins was applied to the gel. The standards were SDS-PAGE marker, (14.300 kDa - 66.000 kDa), Bovine serum albumin, 66.000, Ovalbumin, 45.000; Pepsin, 34.700; Trypsinogen, 24.000; β-lactoglobulin, 18.400 & Lyzozyme, 14.300. For visualization of all protein bands, gels were stained a minimum of 12 h in an excess of 0.1% (wt/ vol) Coomassie bailliant blue R-250, 40% (vol/ vol) ethanol, and 7% (vol/ vol) glacial acetic acid. Gels were destained in an excess of the same solution without the Coomassie brilliant blue R-250.

RESULTS and DISCUSSION

Band pattern:

The protein profiles of whole muscle samples of different animal species after Coomassie- stained 12% SDS- PAGE showed different bands in different species. In all species the molecular weight of the protein bands was ranged from 14.300 to 66.000 KDA. In cattle, the bands were ranged from 7- 9 in no, from which there are 3 distinct bands, one prominent thick band at 66.000 kDa, one between 45.000 and 34.000 kDa and one band at 18.400 kDa. In camel, the protein bands were similar as in cattle, but there is one prominent distinct band above 66.000 KDA. The horse showed very characteristic banding pattern in which bands were only 3- 4 in No. with central distinct band at 34.700 kDa. In sheep, pig and dog, the band pattern are similar in no and evenly distributed. In sheep, there were 3 distinct bands at 37.700 KDA, 24.000 KDA and 14.300. On the other hand, pig showed 4 distinct bands appear at 34.700 kDa, 24.000 kDa, 18.400 kDa and 14.300 kDa. In dog, distinct difference was observed in the intensity of the stained bands which were very faint than sheep or pig.

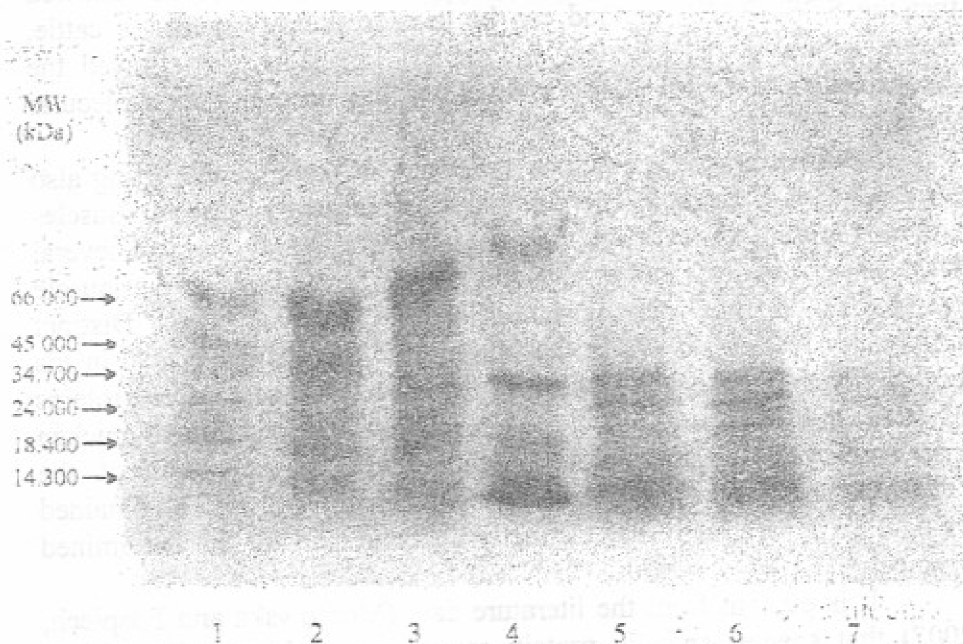

Fig. 1: Protein profiles of whole muscle samples of different animal species after coomassie- stained 12% gel.

Lane 1: Low molecular weight marker.
Lane 2: Cattle samples.
Lane 3: Camel sample.
Lane 4: Horse sample.
Lane 5: Sheep sample.
Lane 6: Pig sample.
Lane 7: Dog sample.

Among the electrophoretic methods, the most suitable for the comparison of proteins from different animal species is SDS- PAGE (Parisi and Aguiari, 1985). With the assistance of two- dimensional electrophoresis (2- DE), it is possible to identify meat of various related species of fish, birds and mammals. The obtained protein separation is visible with naked eye (in the case of proteins containing colour component) or following appropriate staining. In the latter case, the most

common dyes include: Coomasie blue, silver salts or enzymatic staining (Hofmann, 1997).

Investigation carried out by Parisi and Aguiari (1985) showed that the SDS- PAGE method can be used to identify meats of cattle, sheep, lambs, goats, red deer and rabbit. The technique allowed the identification of myofibrillar protein taking into account their molecular weights.

Hofmann (1985) obtained somewhat different results using also the SDS- PAGE method. The aim of his experiments, in which muscles and their water extracts were used, was to identify and compare several muscles derived from cattle, pigs and horses. Separations of the muscle proteins of the three animal species showed identical bands. Distinct differences were observed in the intensity of the stained bands. In his further investigations, the isoelectric focusing (IEF) of soluble proteins of cattle, pig, wild boar, horse, roe deer, fallow deer, rabbit and chicken was employed for the species identification on the basis of the determination and comparison of the bands of myoglobin. The obtained myoglobin separations were distinctly characteristic for the determined species.

It is evident from the literature data (Montowska and Pospiech, 2007) that species specific protein separations yield proteins of low molecular weight made up of three light chains of myosin (14- 25 kDa), tropanin (19- 30 kDa) and parvalbumin (about 12 kDa).

In conclusion, species speciation of meat can be done by SDS-PAGE and it is accurate, easy and comparatively rapid technique than many other methods which used for detection of adulteration of meat and meat product.

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