Journal of Agricultural, Food and Environmental Sciences
UDC:637.5:577.1/.2
Original scientific paper

DIFFERENT APPROACHES IN THE IDENTIFICATION OF MEAT ORIGIN BASED ON PROTEIN PROFILING AND SIMPLE PCR

Z. S. Musliji[1], M. Svetozarevic[1], T. Nestorovski[2], B. Tanaskovska[2] and Z. T. Popovski[1,2]

[1]Laboratory for Protein and DNA Technology, Faculty of Technology and Metalurgy, Skopje – Republic of Macedonia
[2]Department of Biochemistry and Genetic Engineering, Faculty of Agriculture and Food Sciences, Skopje – Republic of Macedonia

coresponding author: zoran_popovski@yahoo.com

Abstract

The meat origin, as a fundamental factor for the quality and the usability of its products, can be determined using DNA or protein analysis. In this study, different techniques are applied to determine the origin of different raw meat samples. The DNA analysis of meat is based on the Polymerase Chain Reaction whereas the techniques involving protein analysis are carried out using electrophoresis. Using the SDS-PAGE technique optimized by changing the running conditions, the amount of materials and the gel concentration, it is possible to differentiate different types of meat. We determine the differences in protein profile of poultry meat compared to the other samples in which are identified two specific fractions between 116 and 200 kD in the zone of myosin heavy chains and one bellow 45 kD in the zone of actin. In the beef samples there is a specific fraction in the zone of tropomyosin, while in pork and beef samples appeared a fraction in the zone of myosin light chain. This technique is suitable and can be only used for internal control in production and processing environments because of its low sensitivity. In practice, there are commercialized kits for the identification of meat and meat products based on DNA analysis. All those kits are dealing with specific primers for different type of meat (beef, pork, poultry, goat, horse, etc). In this case we used the primers for ryanodine receptor gene 1 (RYR1) which protein is involved in calcium pathways of the skeletal muscle cells. We amplify DNA isolated from beef, pork and poultry and DNA analysis based on partial amplification of the RYR1 gene showed the difference between mammalian and poultry meat because there is no amplification on the DNA sample isolated from chicken meat.

Keywords: meat, origin, DNA, protein, identification

Introduction

Meat origin can be determined on protein or DNA level using molecular approach. The proteins in meat can be divided into water soluble or soluble in diluted salt solutions (sarcoplasmic proteins 30-50%); soluble in concentrated salt solutions (myofibrillar proteins 50 -60 %) and insoluble in water or salt solutions (binding tissue proteins 15-20%). From myofibrils, myosin has a globular structure and the ability to retain high amounts of water; actin comes in two forms G-actin and F-actin; actomyosin that is insoluble in water and tropomyosin that is viscous and subunit of myosin. As for sarcoplasmic proteins, most important are: myogen, globulin, myoglobulin which gives the characteristic color of meat and binds the oxygen reversely. Insoluble proteins are

collagen and reticulin. (Du M., McCormick R.J.: (2009).

The identification of meat origin on a protein level could be carried out using ELISA or electrophoresis. Electrophoresis is a commonly used technique in many scientific fields, where charged molecules are separated by using an electrical field. Molecules have different migration rates depending on their total charge, size, and conformation. Most electrophoretic systems use an equal and constant voltage on all of the cross-sectional areas of different matrices employed in the electrophoretic separation. Because many biological compounds have charges and ionisable groups, electrophoresis is frequently utilized in biochemical research, as a tool for separation of complex biological molecules such as proteins, nucleic acids, peptides. The "smiling" pattern often seen on slab gel electrophoresis is result of non-uniform heating of the gel (Gersten D.M. 1996). For separation of proteins, polyacrylamide electrophoresis (PAGE) is commonly used. Sodium dodecyl sulfate (SDS) - an anionic detergent is applied to protein sample to linearize proteins and to impart a negative charge to linearized proteins, and proteins are separated only by length of their polypeptide chains. Gels are formed as acrylamide monomer polymerizes into long chains that are linked together by bis molecules (N,N'-methylenebisacrylamide). In order the polymerization to start, ammonium persulfate (APS) and N,N,N',N'-tetramethylethylenediamine (TEMED) are added to form the free radicals. TEMED accelerates the decomposition of persulfate molecules into sulfate free radicals and these in turn, initiate the polymerization. This study reports optimization of the conditions for identification of the origin of different types of raw meat using SDS-PAGE (Macdonald Wick. 2010).

The analysis and identification of meat origin on DNA level can be done using PCR based methods (Mullis K., Faloona F., Scharf S., Saiki R., Horn G., and Erlich H.: 1986 and Поповски З., Танасковска Б., Мискоска – Милеска Е.: 2013) The basis of molecular identification of meat origin on DNA level is an amplification of specific sequence for appropriate animal genome.

Today, modern kits for identification are available (Ilhak I., Arslan A. 2007), but keeping in mind their price this study shows simple techniques that can be used in teaching purposes and in production management. In that sense we applied SDS – PAGE for identification of meat origin on a protein level and simple PCR for amplification of part of RYR1 gene which is commonly present in mammals (Popovski Z. *et al.* 2002)

Materials and methods

As a starting material for this study were used raw beef, pork and chicken meat samples.

The protein analysis was done by SDS-PAGE, where as a referent point is used mixture of 5 proteins with molecular weight of 200 kD, 116 kD, 97 kD, 66 kD, and 45 kD.

The preparation of the reagents for SDS-PAGE was done according to the procedure described by Macdonald Wick (2010).

The gel used for electrophoresis was divided into an upper stacking gel with lower concentration and lower resolving gel with smaller pores. The stacking gel has a role to deposit the proteins at the top of the resolving gel as a narrow band.

The electrophoresis was performed under the voltage of 185 V for 45 min. The analysis of the gels was done with Gene Tools software using G-box device.

Sample preparation. The samples were prepared using 100 mg meat sample mixed with 100 ml Reducing Buffer (2x). This mixture was denatured on 100°C for 3 minutes.

DNA analysis. Isolation of DNA was made using Promega kit for isolation of DNA from animal tissues. The analysis of DNA integrity was made by 0.8% agarose gel electrophoresis (table 1).

Table 1. AGE-reagents

Reagents	Volume
Agarose	0.24 g
dH_2O	30 mL
Etidium Bromide	1 μL (10g/mL)

Table 2. Reagents for master mix

Reagents	Volume
dH_2O	10.65 μL x number of PCR reactions
10x PCR buffer with Mg	2.5 μL x no. of PCR reactions
25 mM $MgCl_2$	2.0 μL x no. of PCR reactions
1 mM Dntp	2.5 μL x no. of PCR reactions
Forward primer (10 pmol)	1 μL x no. of PCR reactions
Reverse primer (10 pmol)	1 μL x no. of PCR reactions
Taq Gold (5 u/mL)	(2u) 0.4 μL x no. of PCR reactions
Total	**20 μL x no. of PCR reactions**

PCR reaction for amplification of part of RYR1 gene was prepared following the protocol in table 2 (Popovski et al. 2002). In 20 μL of master mix were added 5 μL of DNA sample. The PCR was carried out by the following conditions: 95°C for 3 min (initial denaturation); 95°C for 15 sec (denaturation), 65°C for 15 sec (annealing), 72 °C for 45 sec (polymerization)in 40 cycles and terminal elongation at 72°C for 10 min.

Results and discussion

The protein content is the essence of the analysis that can be used for identification of its origin. The most convenient way for determination of the meat protein profile is electrophoresis. Although there are many factors that can affect these data, such as time of slaughter, way of meat storage, the idea is to find a simpler method that excludes these parameters, but is still reliable.

In order to analyze the protein profile, meat samples that contain about 2 μg proteins are shown in figure 1.

Except for the band in the zone under 46 kD at chicken sample, there are two more protein fractions at the same sample between 116 and 200 kD. These bands are also visible in the mixed samples that contain chicken meat (#5,6,8). Taking in consideration the molecular mass of the fractions, it is possible that they stand for isoforms of myosine that vary in these samples.

In order to make distinction between beef and pork, the concentration of the gel was increased from 10% to 15%, so that a better resolution is obtained (figure 2).

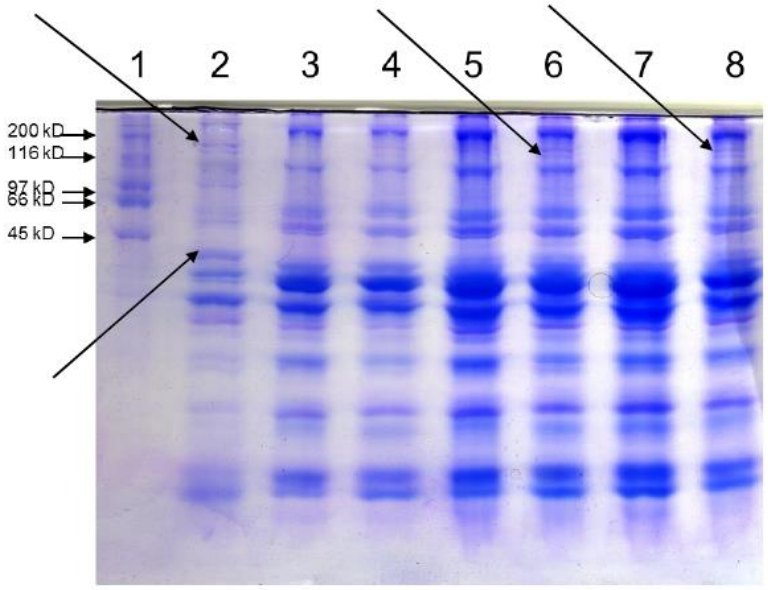

Figure 1. 10% SDS PAGE of meat proteins. #1. 2 μL **standards #2. 2** μL **chicken #3. 2** μL **pork #4. 2** μL **beef #5. 2** μL **chicken + 2** μL **pork #6. 2** μL **chicken + 2** μL **beef #7. 2** μL **beef + 2** μL **pork #8. 0.7** μL **chicken + 0.7** μL **beef + 0.7** μL **pork.**

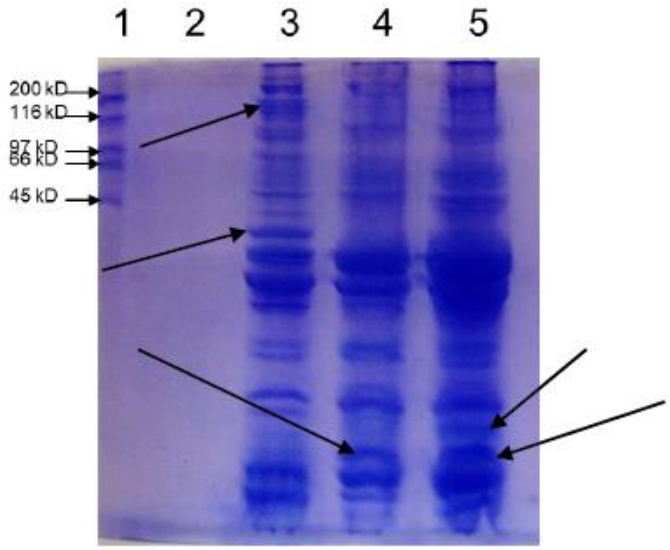

Figure 2. 15% SDS PAGE of meat protein. #1. 4 μL **standards #2. empty #3. 5** μL **chicken #4. 5** μL **pork #5. 5** μL **beef**

Because of the increased gel concentration, more bands can be distinguished in every sample. Besides the presence of two bands in the zone of 116-120 kD and one under 45 kD in the chicken sample compared to the beef sample, another variations are visible that can be used for distinction between pork and beef meat. In the zone of low molecular mass (> 20 kD) at pork and beef samples a protein fractions was showed up that doesn't appear in the chicken sample, whereas at the pork sample, above these fraction, a specific band is visible characteristic only for the pork sample.

There are still issues for the sensibility of this technique. The presence of extremly low amount of meat from different origin is

practicaly impossible to identify by using this method. That's why it is necessary to introduse techniques such as DNA analysis based on PCR.

Apart of the protein analysis that implies identification of protein fractions present or absent in different types of meat, DNA analysis is based on determination of DNA fragment characteristic for appropriate animal genome. The greatest part of the commercial kits are based on target sequences for specific animals for which are designed appropriate primers that are used for detection of the presence certain meat type.

In this study, analysis of the origin of different meat types is done by amplification of RYR1 gene fragment. It is used as marker gene in pig selection because it is part of the intercellular metabolism of calcium, and mutation of its functional segment brings cellular calcium deficiency and presence of pale, watery meat.

The isolated DNA from chicken, pork and beef was amplifyed and analyzed on 3% agarose gel. (figure 3).

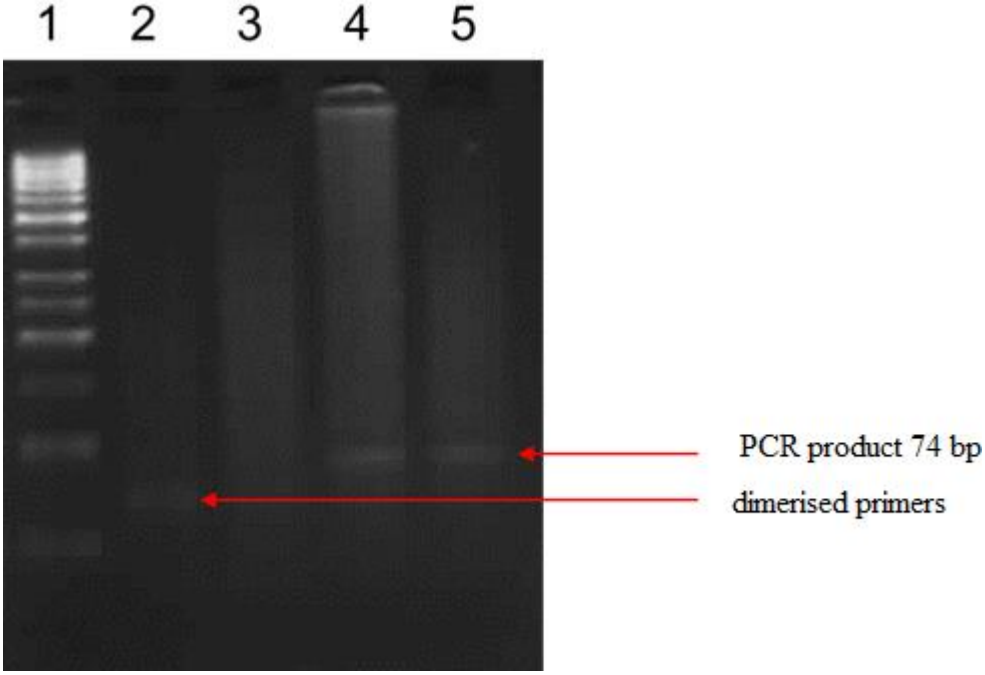

Figure 3. Agarose gel electrophoresis on PCR fragments from RYR1 gene from different meat samples. #1. DNK Ladder 50 -1000 bp # 2. blank. #3. chicken. #4. pork #5. beef.

Based on this electrophoregram can be concluded that there is no amplification of DNA sample from chicken, whereas the specific fragment with 74 bp is present after the amplification of DNA isolated from beef and pork meat.

Conclusion

SDS PAGE of meat protein profile can be used only for screening the meat origin during the production proceses. The sensibility of SDS PAGE could not recommend this method for identifying small amounts of undeclared or forged meat.

DNA analysis based on detection of RYR1 gene fragment is not a good choice for distinguishing different types of meat, because this technique allows only to make difference between poultry and mammal meat.

References

1. Du M., McCormick R.J.: (2009) Applied Muscle Biology and Meat Science. Taylor an Francis Group. LLC
2. Gersten D.M. (1996), Gel Electrophoresis (Essential Techniques Series). New York: Wiley

3. Macdonald W. (2010) Laboratory Manual. Ohio State University. Department for animal Science.
4. Mullis K., Faloona F., Scharf S., Saiki R., Horn G., and Erlich H.: (1986) Specific enzymatic amplification of DNA in vitro. Polymerase chain reaction. Cold Spring Harbor Symp. Quant. Biology 51: 263,.
5. Поповски З., Танасковска Б., Мискоска – Милеска Е.: (2013) Прирачник за методи базирани на PCR. ФЗНХ – УКИМ
6. Irfan I., Arslan A.: (2007) Identification of Meat Species by Polymerase Chain Reaction Technique. Turkish Journal of Veterinary and Animal Science. 31 (3). p. 159-163
7. Popovski Z., Tanaskovska B., Porchu K., Vukovic V., Andonov S., Palasevski B, (2002).: New Approach in Detection of Porcine Stress Syndrome. Biotechnology in Animal Husbandry, 18 (5-6), p. 73-80