Mesopotamia J. of Agric. ISSN: 2224 - 9796 (Online) مـجلــة زراعــة الـرافديـن
Vol. (49) No. (1) 2021 ISSN: 1815 - 316 X (Print) المجلد (49) العدد (1) 2021

IDENTIFICATION AND DIFFERENTIATION OF POULTRY MEAT AND PRODUCTS USING PCR-RFLP TECHNIQUE

Kamaran M. Taha[1*] Dilger M. Khdr[2] Karwan Y. Kareem[3]

[1,2,3]Department of Animal Resources, College of Agricultural Engineering Sciences, Salahaddin University-Erbil, Kurdistan Region, Iraq.
kamaran.taha@su.edu.krd

ABSTRACT

The mitochondrial cyt b gene plays a serious role in investigating untruthful meat species. This study aimed to authenticate the species of poultry products (Escallop, Nugget, Steak, and Sausage) depending on cyt b gene by using universal cyt b primer. DNA was isolated, and then a band of 359 bp of a mitochondrial cytochrome b gene was produced during the PCR amplification. The PCR products were exposed to *Hinf1 and Rsa I* restriction enzymes. The restricted fragments produced by restriction fragment length polymorphism technique (RFLP), were run by agarose gel electrophoresis. Results showed that all products had a similar band except sausage product does not follow the rule and showed mislabeling product by the REs, Two bands were yielded by HinfI RE for all products (114 and 245) bp with the differentiated sausage among other products based on the fake product (63 and 296) bp, while digestion by Rsa I produced three bands for escallop, nugget, and steak, (63, 100, 196), but only two bands for sausage was generated (148 and 211). As result, the study offered that analyzing meat products to detect the origin species via a PCR-RFLP technique by using these restriction enzymes can give reliable results. In short sausage is considered as fraud products because the results showed different bands as compared with poultry meat.

Keywords: RFLP-PCR; Poultry meat Identification; Mt cyt b gene; Restriction Enzyme.

Received: 28 / 12 / 2020, Accepted: 15 / 3 / 2021

INTRODUCTION

In most regions of the world, meat and their products intake carry on to increase and particularly in developing nations (cawthorn *et al.*, 2013 and keyvan *et al.*, 2017). Meat has always been eaten by people in different types, both after thermal processing or prepared in a way that requires long-term packing, such as dry sausages ….etc. These based products have become rare and expensive throughout the year, they are sold on the market at parentally excessive expenses, and for this purpose they are desirable goals for the meat commercial fraud. Furthermore, it is a critical part of the administrative problem of sustenance as tainted and substitution of meat has been a reliable concern for various reasons, for example, community health, religious aspects, future health and unwanted task in meat markets (rashid *et al.*, 2014; hou *et al.*, 2015 and farag *et al.*, 2015).

In order to protect clients and avoid unfair opposition, the detection and prevention of deceptive practices requires easy and rapid research methods that are appropriate for routine applications of these products. Different approaches based on the study of

species-specific components consisting of protein and DNA have been established to distinguish meat and their products originating from different species of animal and to prevent fraudulent practices. It is possible to classify organisms by analyzing proteins via various methods using immunological, chromatographic and electrophoretic methods (Kang'ethe *et al.*, 1986; Berger et al., 1988; Zerifi *et al.*, 1992; Amstrong and Leach, 1992 and Gallardo *et al.*, 1995). Nevertheless, even in the identical species, protein denaturation in meat during warmth treatment or other technological process and variation in protein composition decreases these methods. In addition, these techniques may be insufficient to discriminate between species that are closely related and not suitable for routine use, as it is difficult and time consuming to isolate species-specific protein. Moreover, these techniques can be insufficient to differentiate between species that are closely related and are not appropriate for using routinely, as the isolation of species-precise proteins is hard and time-consuming as it is tough and labor to isolate of species-specific proteins is (Hofmann, 1987; Jemmi and Schlosser, 1992 and Koh *et al*., 1998 and Kesmen *et al*., 2010).

Various molecular approaches have been developed to recognize origin meat species. These methods can reduce the insufficiencies of common techniques (Girish *et al.*, 2005). PCR, AFLP, RAPD, DNA hybridization and RFLP are included in molecular markers (Rodriguez *et al.*, 1991; Arslan *et al.*, 2005 and Alves *et al.*, 2002). Polymerase chain response-based methods are outstandingly fast and reliable, and presently they have proved to be a standard for meat distinguishable confirmation in the industry (Kesmen *et al.*, 2010). Particularly mtDNA has been the foremost studied eukaryotic genomes district which has expected an essential work being created of population and developmental hereditary qualities (Abou-Hadeed *et al.*, 2011and De Masi *et al.*, 2015). The gene cytochrome b (cyt b) expresses one of the best-known proteins that make up the mitochondrial phosphorylation matrix comlpex III and is the only one expressed by the mitochondrial genome. The cyt b gene is used in valid drug and molecular evolution research as a critical utility tool (Prusak *et al.*, 2004; Al-Sanjary, 2009; Abou-Hadeed *et al.*, 2011and Farag et al., 2015).

The aim of the study was to authenticate and detect commercial fraude in certain meat products such as escallop, nugget, steak and sausage obtained from different markets in Erbil-Iraq and the results will be used to compare labels written on products resulting from chicken meat, whether true or not, using the PCR-RFLP molecular technique for mitochondrial cytochrome b gene analysis, using 2 restriction enzymes (HinfI and Rsa I). These restriction enzymes have not been used for identification before in Kurdistan region- Iraq.

MATERIALS AND METHODS

Sample Preparation and DNA Isolation

This study was done on meat of four types of poultry products (Escallop, Nugget, Steak and Sausage), in laboratory of molecular genetics in Salahaddin University- Erbil, college of agricultural engineering sciences and a laboratory in genome company in Erbil. Ten samples in each type were collected from different markets in Erbil governorate (mixed together to make 4 main samples as a polled sample from same

origin), and chicken meat in different parts (breast, wing, leg) was taken as used as a positive control for comparisons.

Meat samples of the concerned products were stored in aluminum foil at -20 °C (until the all samples were collected for about 2 weeks) for DNA extraction. DNA was extracted by using Blood-Animal-Plant DNA Preparation Kit (Spin column based genomic DNA purification, Jena Bioscience GmbH, Germany) according to manufacturer's instructions. The isolated DNA was labeled and stored at -20 °C for the next stage. The purity of DNA was checked by Nanodrop spectrophotometer (Thermo scientific UK) and gel electrophoresis.

PCR Primers

Polymerase chain Reaction (PCR) occurred utilizing a modification of the forced restriction fragment length polymorphism (RFLP) strategy. The primer sequences used for this research were showed in Table 1.

Table (1): Sequence of cyt b primers

Gene name	Nucleotide Sequences	amplified size	Reference
Cyt b (NP_904340.1, gene ID: Gene ID: 17711)	F: 5'CCATCCAACATCTCAGCATGATGAAA-3'	359 bp	Meyer *et al* (1995)
	R: 5'-GCCCCTCAGAATGATATTTGTCCTCA-3'		

PCR Amplification

The target DNA (mtcyt b gene, forward and reverse primers) for each species was amplified by PCR (Applied Biosystems® Veriti® 96-Well Fast Thermal Cycler, USA). The final reaction volume for each one was of 25µl. The PCR component for amplification of cyt b gene is shown in Table 2. The cycling conditions consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles consisting of denaturation at 95°C for 0.30 min, annealing 50°C 1 min, and extension at 72°C for 45 sec, with final extension 72°C for 7 min then holded at 4 °C for infinity. The PCR products were screened in a 2% agarose gel containing ethidium bromide (LOT:110802BB197, Bio Basic Inc.) in Tris-borate EDTA buffer and visualized under UV transillumination (Biostep-UST-20M-8K).

Table (2): PCR reaction mixture for amplification of cyt b gene

PCR mixture	**Required concentration**	**Amount µl**
DNA template	50ng	5 µl
AMPLICON red Master Mix (2 X)	1X	12.5 µl
Pair Primers (40pmol/µl F, R)	10 pmol for each primer	2 µl
DNAs free water	-	5.5 µl
Final reaction volume		25 µl

RFLP Analysis

The obtained PCR products were digested by exposing to the selected HinfI 5'-G/ANTC-3' / 3' – CTNA/G-5' and Rsa I 5' GT/AC-3' / 3'- CA/TG-5' (ADR6201 00001211493, Promega- USA) restriction enzymes for each PCR products separately.

The digestion mixture consists of (2 µl of 10X Reaction buffer, 0.5 µl (5 U) of Reaction Enzyme, 10 µl PCR product and filled with 7.5 µl of free deionized water to complete the final volume 20 µl), This mixtures were incubated from 2 to 4 h, (37 °C) according to the restriction enzymes manufacture instructions. 10µl of the digested samples were loaded, in 2.5% agarose gel. The length of the fragments produced in digestion was matched with the 100 bp DNA Ladder RTU (Cat NO. DM012-R500, Promega- USA).

RESULTS AND DISCUSSION

Mitochondrial DNA was isolated successfully and Purity of DNA ratio ranged from 1.7 to 1.9. The universal cytochrome b gene was clearly produced 359bp as shown in figure 1, with no differences for each sample when run in 2% agarose gel. Then amplicons were exposed to restriction enzymes HinfI and Rsa I, different positions of amplified DNA were cut by these enzymes. Formerly, digested fragments were separated by 3% agarose gel. The samples displayed different fragment sizes, as shown in Table 3 and Figure 2, 3, when they were imaged via UV Transilluminator and linked with the standard size ladder for comparisons.

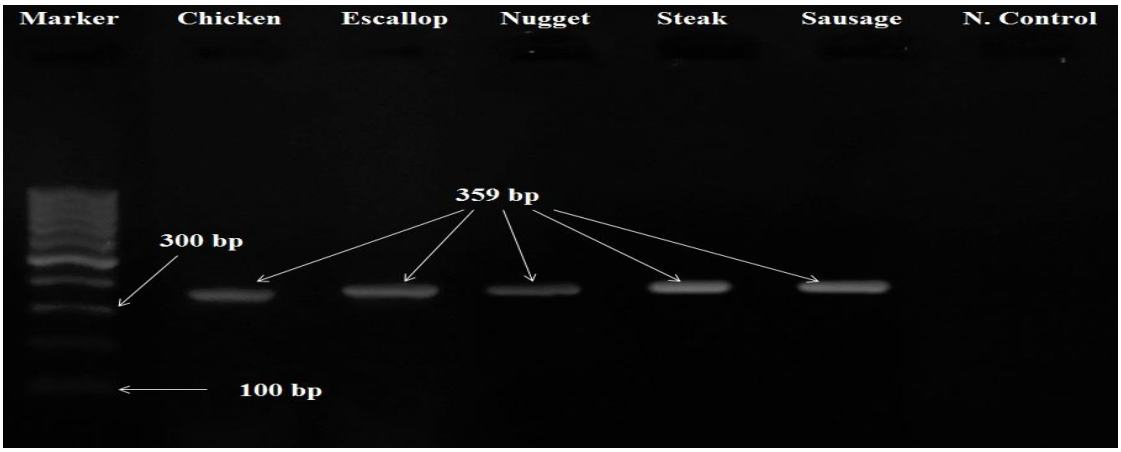

Figure (1): PCR product of poultry products**,** Escallop, Nugget, Steak and Sausage and chicken meat (control).

Table (3): Number of bands and their sizes of poultry products produced by (*HinfI*, *RsaI*) REs.

Poultry Products	*HinfI* fragment size pb	*RsaI* fragment size pb
Escallop	2 bands (245,114)	3 bands (63, 100, 196)
Nugget	2 bands (245,114)	3 bands (63, 100, 196)
Steak	2 bands (245,114)	3 bands (63, 100, 196)
Sausage	2 bands (63,296)	2 bands (148,211)

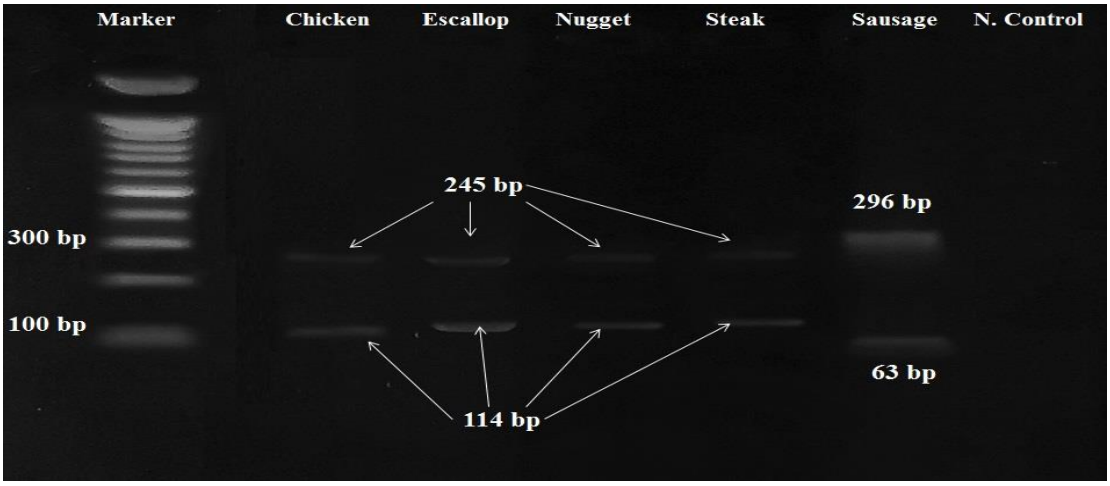

Figure (2): Digestion of PCR product of poultry products**,** Escallop, Nugget, Steak and Sausage and chicken meat (control) with *HinfI* restriction enzyme of Cyt b gene fragments.

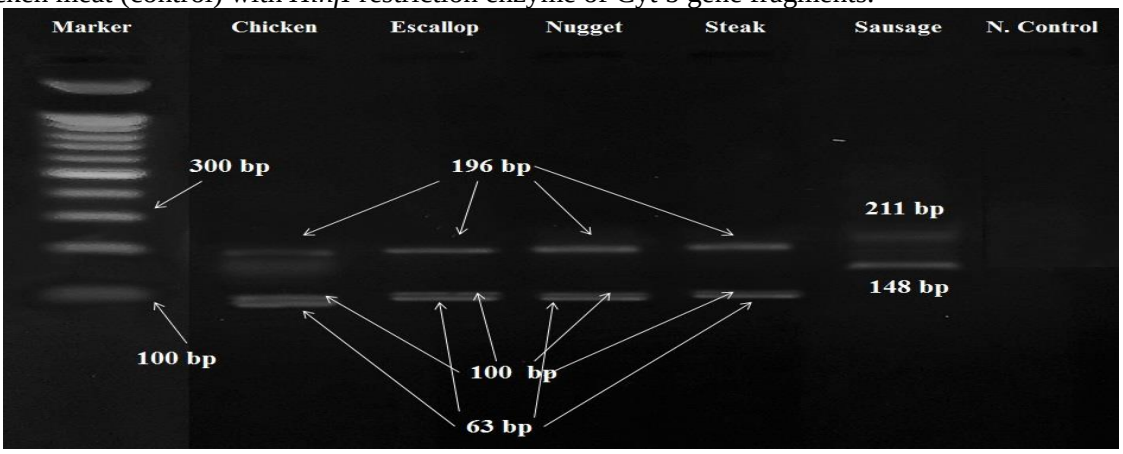

Figure (3): Digestion of PCR product of poultry products, Escallop, Nugget, Steak and Sausage and chicken meat (control) with RsaI restriction enzyme of Cyt b gene fragments.

In comparison, identification the source of meat and the products by molecular techniques offer satisfied and optimistic result over traditional morphological or protein identification and so on, for that reason DNA based methods are the best method for identifying species in both commercial foods and animal products (Ali *et al*., 2015; Andrea *et al*., 2015). Currently, meat adulteration has become a common in many poor countries, therefore it is vital to identify and confirm the commercial fraud meat and products for the public, which cannot be observed with touching or sensing organism, and discover the origin species for those products. Because it is critical for food safety, consumer demands, and law. For these using PCR based method is very trustworthy for the quality of food generally and the meat products specifically (Al-Sanjary, 2009; Farag *et al.*, 2015, Khan *et al*., 2018 and Dilger *et al.*, 2020). As a result of both restriction enzymes that were done all samples separately at the same time, we can notice that the sausage was displayed mislabeling and marked as fraudulent because was not parallel to the chicken meat (positive control) and chicken meat was not used during the process of sausages. Although it was labeled as a chicken meat and there was possibly other meat species that may be replaced with the original meat.

Studying the mitochondria cytochrome b gene via RFLP- PCR based method shown brilliant power and actual tool for separating all products samples such as (Escallop, Nugget, Steak and Sausage) because there were no unclear amplified fragments when cleaved with restriction endonucleases among them. Hence, restriction enzymes offered

exact bands for all samples and there was no need to use statistical analysis to identify significant or nonsignificant among the samples. It is more probable that meat of two or more species is mixed for fraudulent purpose during meat processing such as grinders, cutters, knives, choppers. In order to detect traces of meat mixtures samples have to be kept for PCR-RFLP method due to is a very powerful method (Khan *et al*., 2018). The results were achieved in utilizing the HinfI, RsaI restriction enzymes absolutely recommended that these results were suitable to authenticity and demonstrate unreal meat source and proposed to utilize other types to detect the real sausages species.

In general, meat identifications previous Study was done based on tissues and mixed processing (Ong *et al*., 2007, Ghovvati *et al*., 2009 and Ciupa *et al*., 2012), while in this paper focused on products in general which were commonly used for human daily nutrients, that labeled as a chicken meat, so that the obtained results were a vital and established a very effective determination in all used products by cyt b mt DNA. Moreover, this research approved that there was a satisfactory level of the aimed DNA to amplify in a PCR. Then this is a confirmatory point to settle the benefits of utilizing the mitochondrial DNA more than nuDNA (Pakendorf and Stoneking, 2005). Ayaz *et al*. (2006) stated that 11 of 28 (39.2%) sausage testers that were affirmed as beef were established mixing beef and poultry meat together. Also, the results were agreement with the research done by (Keyvan and coworker, 2017) that detected the adulteration with sausage products. Kesmen and collegues (2007) used species-specific primers for identification of horse, donkey, pig, beef and sheep DNA in sausage for amplification of various parts of mt DNA, and then they observed one in a hundred ng DNA in every sample.

CONCLUSION

The current study revealed that the RFLP-PCR undeniably is a reliable and prevailing method for detection species meat from Fraud Products beside that using the mitochondrial cytochrome b gene, with the help of two restriction endonucleases (HinfI and RsaI) can be very effective and powerful. Moreover, it is a rapid technique and does not need hardworking and inexpensive method. This research indicated that, the meat source that used to make sausage is not produced with chicken meat, and does not obey the public health, low as well, which offered to a public as a chicken meat product. A fraudulent product in any country is a risk and protection are a must. To keep public health and avoid fake products meat products must be frequently analyzed by quality control in governmental organizations.

ACKNOWLEGEMENTS

The authors are very grateful to the Salahaddin University–Erbil for supporting this research.

DISCLOSURE STATEMENT

The authors declare that they have no conflicts of interest.

Mesopotamia J. of Agric. ISSN: 2224 - 9796 (Online) مـجلــة زراعــة الـرافديـن
Vol. (49) No. (1) 2021 ISSN: 1815 - 316 X (Print) المجلد (49) العدد (1) 2021

تحديد وتمييز لحوم الدواجن ومنتوجاتها باستخدام تقنية تباين أطوال قطع التقييد– تفاعل البلمرة المتسلسل

[1*]كامران مصطفى طه [2]دلكير مغديد خدر [3]كاروان ياسين كريم

[1،2،3] قسم الثروة الحيوانية, كلية علوم الهندسة الزراعية. جامعة صلاح الدين–اربيل–اقليم الكردستان–العراق.

Kamaran.taha@su.edu.krd

الخلاصة

يلعب جين cyt b المايتوكوندريا دورا مهما في التحديد انواع لحوم الغير الصحية. هدفت الدراسة الحالية الى التحقيق من انواع منتوجات لحوم الدواجن (Escallop, Nugget, Steak, Sausage) باستخدام البادئ المعروف عالميا cyt b. تم عزل الحمض النووي DNA ومن ثم تضخيم قطعة DNA 359 قاعدة زوجيه في تقنية التفاعل البلمرة المتسلسل PCR.ثم تم هضم القطع المضخمة مع الأنزيمات القاطعة Hinf1 و RsaI. ثم الترحيل القطع المضخمة الذي تم انتاجها عن طريق تباين أطوال قطع التقييد (RFLP) بواسطة الترحيل الكهربائي بالهلام (agarose).أظهرت النتائج أن جميع المنتجات لها نفس الشروط الانتاجية باستثناء منتج Sausage الذي لم يتبع الشروط وكانت تسميته المنتوج خاطئة بالاعتماد على الانزيمات القاطعة, حيث تم انتاج قطعتين bp 114 و bp 245 لكل من Escallop و Nugget و Steak بينما كانت القطعتين bp 63 و bp 296 قاعدة زوجية مختلفة لمنتوج Sausage بوسطة انزيم القطع Hinf1، في حين انتج الهضم الانزيمى بانزيم القطع RSaI ثلاث حزم (63 bp, 100 و 196) قاعدة زوجية لجميع منتجات عدا منتوج Sausage الذي اظهر بحزمتين (148 و 211) قاعدة زوجية. استنادا على النتائج هذه الدراسة، فان استخدام تقنية PCR-RFLP باستخدام إنزيمات القاطعة يمكن أن تعطي نتائج موثوقة لتحليل وكشف عن أصل منتجات اللحوم. بالقصير Sausage يعتبر من المنتجات المغشوش, لان النتائج تظهر الحزمة المختلفة اذا قارن مع الحزمة اللحوم الدجاج.

الكلمات المفتاحية: RFLP-PCR، تحديد لحوم الدواجن، الجين مايتوكوندريا سايتوكروم بي، الانزيمات القاطعة

تاريخ تسلم البحث: 28 / 12 / 2020، وقبوله: 15 / 3 / 2021

REFERENCES

Abou-Hadeed, A. H., Mervat ,H. G., & Mayada, R. F. (2011). Species Identification of Some Non-Domestic Animals Using PCR-RFLP Analysis of Cytochrome b Gene. *Zagazig Veternary Journal*, 39(4), 37-46.

Ali, E., Abdur, R., Sharifah, B. A., Mahfujur, R., Amin, A.l., Abd Rashid, A.J. & Asing, N.R. (2015). Multiplex PCR assay for the detection of five meat species forbidden in Islamic foods. *Food Chemistry*, 177(15), 214-224.

Alves, E, Castellanos, C., Ovilo, Silio, C. L., & Rodriguez, C. (2002). Differentiation of the Raw Material of the Iberian Pig Meat Industry Based on the Use of Amplified Fragment Length Polymorphism. *Meat Science*, 61: 157–162.

Al-Sanjary R.A. (2009). Identification of beef using restriction fragment length polymorphism - polymerase chain reaction. *Iraqi Journal of Veterinary Sciences*, 23 (1), 43-46.

Amstrong, S. G., & Leach, D. N. (1992). The use of HPLC protein profiles in fish species identification. *Food Chemistry*, 44, 147-155.

Andrea, A., Lisa, G., Lorenzo, C., Priscilla, D., Antonino, M., Renato, M., Daniela, G., & Alessandra, G. (2015). DNA and Mini-DNA barcoding for the identification of Porgies species (family Sparidae) of commercial interest on the international market. *Food Control*, 50, 589-596.

Arslan, A., Ilhak, I., Calicioglu M., & Karahan, M., (2005). Identification of Meats Using Random Amplified Polymorphic DNA (RAPD) Technique. *Journal of Muscular Foods*, 16: 37–45.

Ayaz, Y., Ayaz, N.D., & Erol, I. (2006). Detection of species in meat and meat products using enzyme-linked immunosorbent assay. *Journal of Muscular Foods*, 17, 214-220.

Berger, R. G., Mageau, R. P., Schwap, B., & Johnston, R. W. (1988). Detection of poultry and pork in cooked and canned meat foods by enzyme linked immunosorbent assays. *Journal-Association of official Analytical Chemistry*, 71(2), 406-409.

Cawthorn, D., Steinman, H. A., & Hoffman, L. C. (2013). A high incidence of species substitution and mislabeling detected in meat products sold in South Africa. *Food Control*, 32, 440-449.

Ciupa, A., Mihaiu, M., Dan, S. D., Lapuşan, A., Jecan, C., & Cordiş, I. (2012). Using PCR Techniques for Rapid Detection of Animal Species in Meat Products. *Veterinary Medicine*, 69,1-2.

DeMasi, L., Adelfi, M. G., Pignone, D., & Laratta, B. (2015). Identification ofDoris Verrucosamollusc Via Mitochondrial 16S rDNA. *Biochemistry System Ecology*, 58: 21-29.

Dilger, M. Kh., Kamaran, M. T. Azad B. S. (2020) PCR-RFLP Technique for Species Origin Identification of Imported Buffalo Meat. Mesopotamia Journal of Agriculture, 48(4), 104-113.

Farag, M. R, Imam, T. S. & Dhama, K. (2015). Identification of some domestic animal species (camel, buffalo and sheep) by PCR-RFLP analysis of the mitochondrial cytochrome b gene. *Advances in Animal and Veterinary Science*, 3(2), 136-142.

Gallardo, J. M., Sotelo, C. G., Pineiro, C., & Perez-Martin, R. I. (1995). Use of capillary zone electrophoresis for fish species identification. Differentiation of flatfish species. *Journal of Agriculture Food Chemistry*, 43, 1238-1244.

Ghovvati, S., Nassiri, M. R., Mirhoseini, S.Z., HeraviMoussavi, A. & Javadmanesh, A. (2009). Fraud identification in industrial meat products by multiplex PCR assay. *Food Control*, 20, 696–699.

Girish, P. S., Anjaneyulu, A. S. R., Viswas, K. N., Shivakumar, B. M., Anand, M. & Patel, M. (2005). Meat Species Identification by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) of Mitochondrial 12S rRNA Gene. *Meat Science*, 70: 107–112.

Hofmann, K. (1987). Fundemental problems in identifying the animal species of muscle meat using electrophoretic methods. *Fleischwirtshaft*. 67, 820-826.

Hou, B., Xianrong, M., Liyuan, Z., Jinyue, G., Shaowen, L., & Hui, J. (2015). Development of a Sensitive and Specific Multiplex PCR Method for the

Simultaneous Detection of Chicken, Duck and Goose DNAin Meat Products. *Meat Science*, 101: 90-94.

Ivona, D. K., Danijela, S., Vladimir, M., Žarko, R., Dragutin, V., & Goran, K. (2016). Efficiency of PCR-RFLP and Species-specific PCR for the Identification of Meat Origin in Dry Sausages. *Czech Journal Food Science,* doi: 10.17221/243.

Jemmi, T., & Schlosser, H. (1992). Tierartenbestimmung bei erhitztem fleisch von haus-und wildwiederkäuern mittels isoelektrische fokussierung. *Fleischwirtshaft,* 72, 1191-1195.

Kang'ethe, E. K., Gathuma, J. M., & Landqvist, K. J. (1986). Identification of the species of origin of fresh, cooked and canned meat on meat products using antisera to thermostable muscle antigens by Ouctherlony's double diffusion test. *Journal of Science Food Agriculture*, 37, 157-162.

Kesmen, Z., Yetim, H., & Şahin, F. (2010). Identification of Different Meat Species Used in Sucuk Production by PCR Assay. *GIDA*, 35: 81–87.

Keyvan, E., İplikçioğlu, C. G., Çinar, K. B., Bilgen, N., & Şireli, U. T. (2017). Identification of meat species in different types of meat products by PCR. *Ankara University Veterinary Fakulty Dergerse*, 64, 261-266.

Khan, W. A., Hamid, M., Umara Amir-u-Din, M. Y., Adeela, A., Khalid, M., & Imran, M. (2018). Identification of species-specific molecular markers in different farm animals by PCR-RFLP analysis. *Pure and Applied Biology*, 7 (1), 338-342.

Koh, M. C., Lim, C. H., Chua, S. B., Chew, S. T. & Phang, S. T. W. (1998).Random amplified polymorhic DNA (RAPD) fingerprints for identification of red meat animal species. *Meat Science*, 48, (3/4) 275-2859.

Meyer, R., Hofelein, C., Luthy, J., & Candrian, U. (1995). Polymerase chain reactionrestriction fragment length polymorphism analysis: a simple method for species identification in food. *Journal of AOAC International*, 78, 1542-1551.

Ong, S. B.., Zuraini, M. I., Chai, L. C., Jurin, W.G., Haryani Y., Cheah, Y. K., Ghazali, F. M., Tunung, R., & Son, R. (2007). Meat Molecular Detection: Sensitivity of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism in Species Differentiation of Meat from Animal Origin. *ASEAN Food Journal*, 14, 51-59.

Pakendorf, B., & Stoneking, M. (2005). Mitochondrial DNA and human evolution. *Annual Review of Genomics and Human Genetics,* 6, 165-183.

Prusak, B., Grzybowski, G., & Zieba, G. (2004). Taxonomic Position of Bison Bison (Linnaeus, 1758) and Bison Bonasus (Linnaeus, 1758) Based on Analysis of Cytbgene. *Animal Science Paper Reproductive*, 22(1), 27-35.

Rashid, P. M., Babashekh, M. O., Marouf, A. S., & Amin, K. M. (2014). Identification of Animal Species in Meat Broth by Simplex and Multiplex PCR. *Journal of Zankoy Sulaimani- Part A (JZS-A),* 16(1), 97-102.

Rodriguez, M. A., Gracia, T., Gonalez, I., Asensio, L., Fernandiz, A. & Lobo, E. (1991). Identification of Goose (Anser) and Mule Duck (Anas Platyrhynchos X Carina Moschata) Foegras by Multiplex Polymerase Chain Reaction Amplification of the 5S rDNA Gene. *Journal of Agriculture Food Chemistry*, 49, 2717–2721.

Zerifi, A., Labie, C., & Benard, G. (1992). SDS-PAGE Technique for the species identification of cooked meat. *Fleishwirtschaft*, 1, 54-59.