Arch Lebensmittelhyg 69,
99–103 (2018)
DOI 10.2376/0003-925X-69-99

ISSN 0003-925X

Korrespondenzadresse:
satish.kumar@gfsu.edu.in

Institute of Forensic Science, Gujarat Forensic Sciences University, Gandhinagar, Gujarat, India, 382 007

Investigation of meat quality using protein profiling

Untersuchung der Qualität von Fleisch durch Protein-Profiling

Pawan Shankar Singh Rathore, Nigel Fernandes, Bhargav Chamanlal Patel, Mohinder Singh Dahiya, Satish Kumar

Summary

Identification of meat from different sources and ascertaining their quality is prime concern in meat and food industry. Protein profiling or fingerprinting is an excellent method both for species identification and quality assurance of meat used for several food products. In present study protein profile of meat from cow, buffalo, chicken and goat was analysed using one dimensional sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS PAGE) in order to identify species, adulteration and age of samples. Protein bands of 170 kDa and 46 kDa were observed in chicken whereas 63 kDa protein was observed specific for bovine family. Presence of above mentioned three proteins in mixed meat sample reveal that protein profiling is alternative tool to investigate and analyse their adulteration. Time dependent degradation assay of meat showed that high molecular weight proteins were prone to degrade early while low molecular weight protein (63 kDa) was relatively most resistant to degradation.

Practical Application

- Present work provides protein profiling as a potent method for assuring the food quality and safety related to meat and its products.
- Identification and analysis of specific protein bands could further be explored for precise species identification and adulteration of meat samples which is very important for law enforcement agencies to ascertain its quality.
- Present results also hold promises for forensic investigation of food related criminal cases.

Keywords: Meat Adulteration, Species Identification, SDS-PAGE, Meat quality

Zusammenfassung

Die Identifizierung von Fleisch verschiedenen Ursprungs und die Feststellung ihrer Qualität sind in der Fleisch- und Lebensmittelindustrie von vorrangiger Bedeutung. Protein-Profiling oder Fingerprinting ist eine ausgezeichnete Methode sowohl zur Speziesidentifikation als auch zur Qualitätssicherung von Fleisch, das für verschiedene Lebensmittelprodukte verwendet wird. In der vorliegenden Studie wurde das Proteinprofil von Fleisch von Kühen, Büffeln, Hühnern und Ziegen mittels eindimensionaler Natriumdodecylsulfat-Polyacrylamid-Gelelektrophorese (SDS-PAGE) analysiert, um Spezies, Verfälschung und Alter zu identifizieren. Proteinbanden von 170 kDa und 46 kDa wurden bei Hühnern identifiziert, während 63 kDa Proteinbanden spezifisch für die Familie der Rinder (Bovinae) war. Die Anwesenheit der oben genannten drei Proteine in gemischten Fleischproben zeigte, dass Protein-Profiling ein alternatives Werkzeug zur Untersuchung von Verfälschungen ist. Zeitabhängige Degradationsuntersuchungen von Fleisch zeigten, dass Proteine mit hohem Molekulargewicht dazu neigten, sich frühzeitig abzubauen, während Protein mit niedrigem Molekulargewicht (63 kDa) widerstandsfähiger gegenüber Abbau war.

Schlüsselwörter: Fleischverfälschung, Artenbestimmung, SDS-PAGE, Fleischqualität

Journal of Food Safety and Food Quality 69, Heft 3 (2018), Seiten 77–104

Introduction

Owing to high protein, fat, vitamin and minerals content, meat is an important diet maintaining the proper metabolism of the cell. In past decades consumption of meat has increased significantly in Western, Asian and African countries. European population derive nearly 33 % of their calories from animal product whereas African people get only 6 % of their calories from animal (Bender & Smith, 1997). In a rapidly growing world population, where providing safe and healthy food for everyone is a key issue, meat is an excellent source for all necessary nutritional ingredients. Current consumption of meat implies an increase in average global consumption per capita from 32.6 kg/year to 44–54 kg/year in coming years (Keyzer et al., 2001).

Increasing demand and limited supply of meat leads to adulteration and supply of old product for consumption. Generally meat can be adulterated either by blending with inferior quality product or by substitution of meat of one species by another species. Substandard quality of meat compromise economic loss and food safety of consumers (Cota-Rivas & Vallejo-Cordoba, 1997). There are several reports where interspecies contamination of meat were observed (Walker et al., 2013). In one of the case it was observed that 5 dried beef brands were contaminated by pork (Zilhadia & Ummi, 2014). High nutritional components of meat also make them very susceptible to spoilage. Due to higher cost and susceptibility to spoilage, restaurants owners and meat producers tend to spike fresh meat with old meat. Considering the global impact of meat and its products, assuring the composition and quality of meat is inevitable for food safety and security. Methods for determining age and adulteration of meat or their products have been comprehensively reviewed earlier (Kartheek et al., 2011). Techniques to detect adulteration and age of meats ranges from simple physical tests to sophisticated molecular biological techniques (Kremar & Rencova, 2001). Enzyme-linked immunosorbent assay (ELISA) was preferred technique for species identification but suffers lack of sensitivity and rapid saturation of antibody in presence of high amount of antigen was another limitation for detecting adulteration (Kang'ethe et al., 1982;Walker et al., 2013). Polymerase chain reaction (PCR) methods such as the random amplified polymorphic DNA fingerprints and multiplex PCR (Koh et al., 1998; Bhat et al., 2016) have been reported for the detection of species adulteration.

In present study we explored protein profiling method using sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) to distinguish meat from different sources and to estimate their age. With the fact that nearly 25–30 % of meat sold in India and Asian subcontinent is adulterated (Singh & Neelam, 2011) it is imperative to establish an easy method to detect interspecies or intraspecies mixing of meat. In present study we also investigated if present approach can be explored to ensure the purity of meat. As the proteome is defined as a set of protein of a given cell at a particular time, analysis of protein profile, which are the end product of gene expression are better alternative for the quality assurance of meat and meat products. Additionally, stability of peptide bonds in protein compared to hydrogen bonds of DNA make protein based techniques more suitable for older or degraded samples. Our results show that some proteins are specific for bovine family while some are for aves, which can be effectively explored to for detecting interspecies or intraspecies adulteration of meat. In our study different proteins were observed to exhibit different degradation pattern over the time, thus indicating how old the meat sample could be. Taken together, present approach appeared to be accurate and relatively easy for accessing the quality of meat.

Materials and Methods

Source and storage of meat

Fresh meat (approximately 30 min after death) of buffalo *(Bubalus bubalis)* chicken *(Gallus gallus domesticus)* and goat *(Capra hircus)* was obtained from local butcher shop maintaining hygienic condition. Three samples of fresh meat from each species were taken in present study. Since slaughtering and treading of cow meat is banned in Gujarat state of India, cow *(Bostaurus indicus)* meat was kindly provided by Directorate of Forensic Science, Gandhinagar, Gujarat, India. All samples were transported and stored at 4 ºC unless stated elsewhere.

Sample Preparation

Whole muscle protein from different meat samples were extracted as described previously (Sinha et al., 2012). Briefly, nearly 5.5 g of tissue was trimmed of fat and thoroughly washed with ice-cold homogenate buffer (50 mm Tris-HCl, pH 7.4, 150 mm NaCl, 2 mm $MgCl_2$). Tissue was cut into small pieces in 30 ml of same buffer and homogenised with mortar and pestle containing the homogeniser on ice. Homogenate was centrifuged at 9000 rpm using the nuve NF 800R centrifuge for 30 minutes at 4 ºC. Pellet containing cellular debris and particulate matter was discarded and supernatant containing protein was collected and kept at 4 ºC until further analysis. Protein concentration in each sample was quantified by Folin Lowry's assay using bovine serum albumin as standards. To monitor the adulteration of meat, ~2.5 g fresh meat of cow and chicken was mixed, homogenised and protein was extracted as described above before subjecting to SDS-PAGE. Time dependant degradation analysis of meat protein was analysed by keeping the cow tissues at 28 ºC for 0, 8, and 17 days followed by extracting the protein and running the SDS-PAGE (0 day represent freshly obtained meat). Further, time dependent protein degradation was also monitored by UV-Visible spectroscopy. Protein extracted from cow meat at different time interval was diluted (1000 fold) before measuring the absorbance at 210 nm and 222 nm using double beam UV-Visible Spectrophotometer. All samples were measured against buffer blank.

SDS-PAGE (Laemmli Buffer System)

Approximately 4 µg protein samples along with protein ladder were loaded onto 1.0 mm thick mini polyacrylamide gels (8 % stacking and 12.5 % resolving) and separated by electrophoresis in a running buffer for 1 h at a constant voltage of 190 V. After electrophoresis, gels were stained with 0.025 % (w/v) Coomassie brilliant blue followed by multiple destaining before visualizing the protein bands.

Results and Discussion

Nutrient composition of cow, buffalo, mutton and chicken

Table 1 presents the protein concentration of raw meat from buffalo, cow, chicken and mutton. Estimated by Folin Lowry's assay protein content was observed highest in mutton followed by cow, buffalo and chicken. Nutritional component of protein from 5.5 g of mutton was around 348 mg/ml while cow, buffalo and chicken contain approxi-

TABLE 1: *Table 1 shows the estimation of protein from 5.5 gm of freshly obtained meat samples of mutton, cow, buffalo and chicken. 5.5 gm of meat sample was homogenated in 30 ml buffer and after centrifugation, supernatant was quantified by Folin Lowry's method.*

Sample	Concentration (mg/ml)
Mutton	347.9
Cow	230.8
Buffalo	228.5
Chicken	187.9

mately 230, 229, and 187 mg/ml of protein respectively. It is apparent from our observation that red meat contains more protein than chicken, which is frequently consumed as white meat. Owing to their high nutritional value and recommendation of International Congress of Nutrition, 2013 where at least 25 to 30 g of high quality protein was prescribed for optimal health and growth (Binnie et al., 2014), red meat is a preferred dietary source. As meat holds high nutritional value which varies significantly from species to species, it is very common practice to spike lower grade meat to higher one in order to save money. It is thus urgent to device or upgrade techniques which could easily and effectively used to determine the quality of meat and its products.

Protein profile of various meat samples and investigating their adulteration

First of all, various meat samples were evaluated on the basis of their protein profile. Figure 1A shows the protein bands from fresh meat of buffalo, cow, chicken and goat (Lane 2, 3, 4 and 5 respectively). At first glance protein profile of all meat samples were appeared identical; however there were differences when observing minutely. Protein of approximately 90 kDa was observed in all meat samples namely buffalo, cow, goat and chicken. However electophoretic mobility of this protein was slightly higher in chicken (Lane 4). From earlier work it was assumed that 90 kDa protein may represent either α-actinin or SERCA1 (Pittner et al., 2015). The specific protein bands of molecular weight 170 kDa and 46k Da were observed only in chicken (Lane 4) but not in other meat samples. These proteins could be cardiac muscle isoform (128 kDa) and beta-enolase (47 kDa) respectively of *Gallus gallus* (Montowska & Pospiech, 2013). Presence of these proteins could be used to distinguish chicken meat from the group of above mentioned meat samples. Figure 1A also revealed presence of 63 kDa protein in buffalo, cow and goat (Lane 2, 3 and 5) but this was not prevalent in chicken (Lane 4). This finding indicates that 63 kDa protein which is a type of myofibrils is present in bovine family but not in aves family, however detail proteomic analysis is inevitable to establish the link between these groups of animals.

From figure 1A, it is apparent that 170 kDa and 46 kDa protein is specific to chicken while 63 kDa protein is specific to bovine family. Profound variation in protein profile of meat samples from chicken, cow and buffalo reveals functional genetic variability in species. Specific protein bands could be explored for species determination for both the food industry and forensic investigation particularly in wild life forensic where determination of animals from residual carcasses is a challenge.

As fraudulent adulteration of superior quality meat with inferior quality meat is a practice that has been observed all over the world (Yosef et al., 2014), we aimed to explore SDS PAGE as a simple and effective technique to investigate adulteration of meat. After proper mixing of freshly obtained cow and chicken tissue, cellular extract containing protein was subjected to denaturing PAGE along with fresh tissue extract from both cow and chicken individually as a positive control (Figure 1B). Lane 1 showing profile of cow protein indicates the presence of 63 kDa protein which is not present in chicken, while 170 kDa and 46 kDa protein band was observed only in chicken but it was absent in cow (Lane 2). Lane 3 of figure 1B showing the protein profile of mixed cow and chicken meat indicate the presence of protein band mainly 170 kDa and 46 kDa protein which were present in chicken and 63 kDa which was present only in cow. This finding clearly indicates that presence of different protein band in a particular meat sample could be the indicator of adulteration. With the earlier finding where adulteration in uncooked Kashmiri mutton was probed by multiplex PCR (Bhat et al., 2016) our finding suggests that protein profile from SDS-PAGE can easily been explored to detect the adulteration of meat samples. However, the requirement of protein database exclusively from commonly used animal is inevitable for accurate identification of source of meat.

Time dependant degradation of raw meat

Quality of meat and meat products depends on the age of animal and time after butchering the carcass. As time progresses, myofibrils and associated proteins start degrading under the influence of proteolytic enzyme thus leading to spoilage and deteriorating the quality of meat (Contreras, et al., 2016). It is a very common practice to use old meats by restaurants and meat industries, thus compromising the health and hygiene of the consumers. To address, if age of meat sample could be determined by the protein profiling, we explored SDS-PAGE to monitor the protein degradation of cow meat at different time points. Figure 2 shows the protein profile of cow meat at different time of post-mortem storage at 28 ºC. Extract from fresh meat sample showed whole range of protein (Lane 1) which were observed to be degraded slowly over the period of 8 and 17 days (Lane 2 and 3 respectively). After 8 days of post-mortem storage, protein bands of 63 kDa, 40 kDa and 30 kDa were

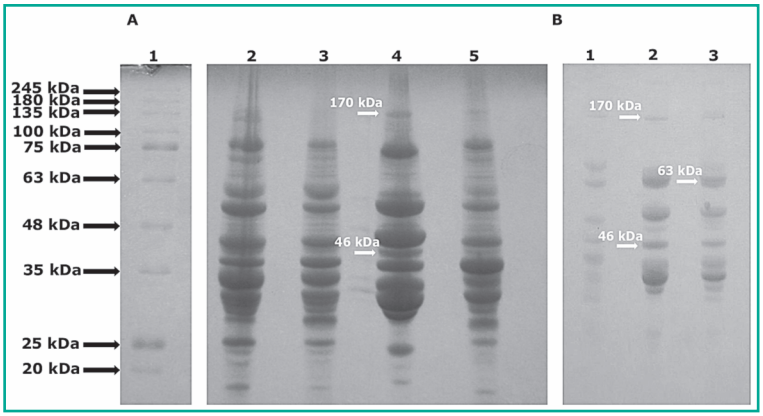

FIGURE 1: *(A) Image of a representative gel for the study of protein fingerprint identification of four meat samples is shown here. Lane 1, 2, 3, 4 and 5 shows the standard protein ladder and protein profile of buffalo, cow, chicken and goat respectively. (B) Adulteration of meat using SDS-PAGE is shown here. Protein profile of different lane represents as Lane 1, 2 and 3 shows protein from fresh cow, fresh chicken meat and adulterated fresh cow and chicken meat respectively.*

Journal of Food Safety and Food Quality 69, Heft 3 (2018), Seiten 77–104

intact while other proteins were either disappeared or their band intensity became weak (Lane 2). Figure 2 indicates that 90 kDa, 48 kDa and most of the proteins between 25 to 35 kDa are more prone to enzymatic degradation within the time window of 8 days. Our observation was in agreement with earlier work where higher molecular weight protein like SERCA1 was observed to be degraded within the first 10 days post-mortem (Pittner et al., 2015). As discussed earlier in figure 1A that 90 kDa protein could either be α-actinin or SERCA1. With the available literature that α-actinin is never degraded within initial 10 days (Pittner et al., 2015), degradation of 90 kDa band indicate the presence of SERCA1.

After 8 days we did not observed either decrease in band intensity or degradation of 63 kDa protein (Lane 2). For the same time window proteolytic products of both 40 kDa and 28 kDa proteins were observed. From earlier works it is observed that 40 kDa cardiac troponin (cTnT) of pig muscle showed degradation product of 37 kDa and 35 kDa after 120 ± 8.5 and 132.0 ± 6.9 post-mortem hour respectively (Pittner et al., 2015). Our data also reveals that 40 kDa protein band which could be of cardiac troponin was also degraded in cow meat yielding small fragments.

Lane 3 of figure 2 shows the electrophoretic profiles of cow meat 17 days post-mortem. Except 63 kDa, most of protein bands disappeared when meat was stored at 28 ºC. Our observation indicates that 63 kDa protein is most resistant to degradation. Earlier work by Sazili and co-workers also reported that 63 kDa protein is thermally most stable under the various freezing-thawing conditions (Adeyemi et al., 2014). Similar results were also found in previous study where degradation of 63 kDa stable proteins were observed only under specific or controlled conditions, such as very high temperature and specific enzymatic degradation (Dutson, T.R. 1982). One striking observation after 17 days post-mortem of cow meat was the presence of a new protein band of 32 kDa (Lane 3) which was not observed in fresh (0 days) and 8 days old meat sample (Lane 1 and 2). From the previous work it is reported that cardiac troponin protein, cTnT (40 kDa) undergo proteolytic degradation yielding 32 kDa product (Labugger et al., 2000). Therefore new protein band of 32 kDa (Lane 3) could be degradation product of cTnT. It is worth mentioning that cTnT is not restricted to the heart muscle but is also present in skeletal muscle (Bodor et al., 1997), therefore degradation pattern of cTnT could be used to estimate the age of meat sample.

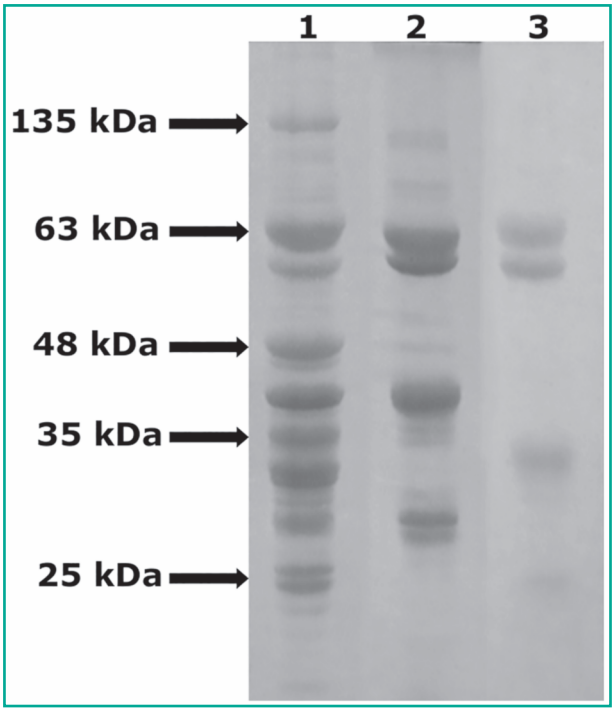

FIGURE 2: *SDS-PAGE showing time dependent degradation pattern of cow meat. Lane 1, 2 and 3 showing the protein profile of cow meat at 0, 8 and 17 days of incubation at room temperature.*

After monitoring the protein degradation by SDS-PAGE, we inclined to study the same phenomenon by spectroscopy. Peptide bond display characteristic absorption at 210 nm and 222 nm thus decrease in absorbance at these wavelengths are indicators of peptide bond hydrolysis (Varma et al., 2017). Degradation of protein eventually results into hydrolysis of peptide bonds, indicates the loss of structure. Figure 3A and 3B showing gradual decline in absorbance at 210 nm and 222 nm respectively mirrors the degradation of cow proteins over the period of 16 days. Compared to freshly extracted cow protein, absorbance after 6 days at 210 nm was observed to decrease by ~53 % (Fig 3A). After 10 days of storage at 28 ºC, absorbance of protein was decreased to ~16% of originally observed value. With further saturation till 16 days, we observed that ~84% proteins are degraded under present condition. While monitoring the same phenomenon at 222 nm we observed that after 10 days of storage, absorption of proteins was decreased to ~58% which further decreased by 25 % after 14 days (Fig 3B). Though rate of protein degradation observed from 210 nm and 222 nm was little different, figure 3A and 3B collectively supports protein degradation over the time window of 14 days which was evident from figure 2.

Protein based techniques like electrophoretic, enzymic assay and chromatographic methods complemented with mass spectrometry (MS) technique are preferred choice for analysis of meat product than polymerase chain reaction based methods which is primarily used for species identification in meat industry. Use of two-dimensional electrophoresis (2-DE) which can separate about 10000 proteins simultaneously and can quantify less than 1 ng protein (López, J.L. 2007), is an excellent tool for authenticating meat and meat product. Further, for large scale samples, combination of 2-DE with matrix assisted laser desorption ionization-time of flight (MALDI-TOF) – MS can be used to discriminate meats of very closely related species.

Conclusion

Our results show that protein profiling could be used to distinguish between different types of meat. Specific protein bands of 170 kDa and 46 kDa were only observed in chicken meat whereas 63 kDa band was specific in bovine meat sample. Present work reveals that presence of species specific bands can efficiently be explored to ascertain the quality and freshness of meat. Though complete proteome analysis is inevitable to pin point the adulteration at species level. Time dependent proteolytic degradation profile of proteins was found to determine the age of meat sample. In present study, higher molecular weight protein was observed to be degraded early while 63 kDa protein was most resistant to degradation. In summary, present work can easily and routinely be used by law enforcement agencies and food industries to ascertain meat quality.

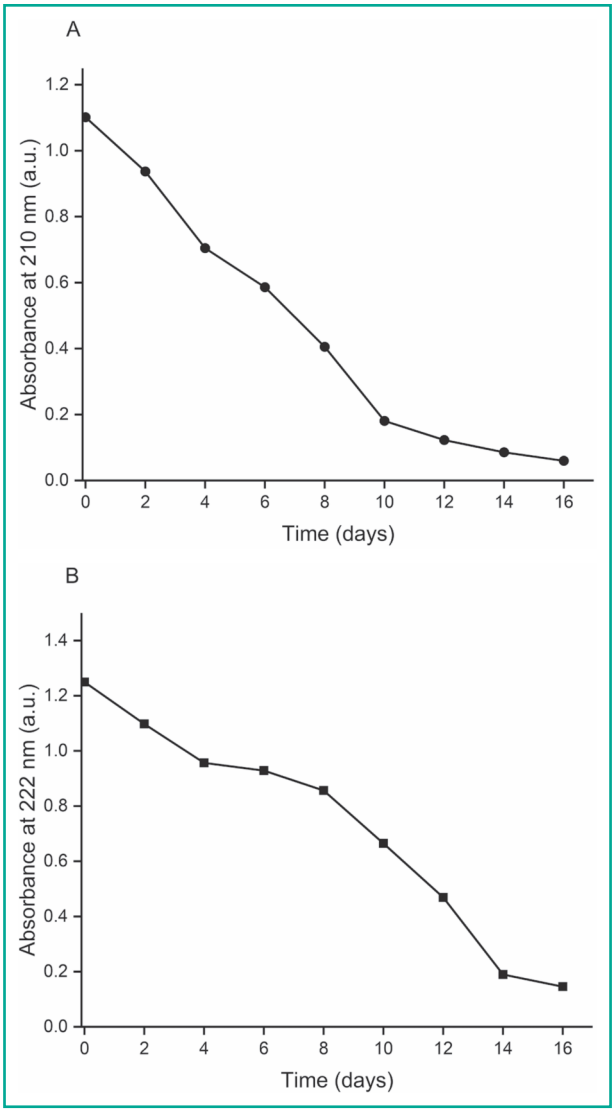

FIGURE 3: *Time dependent protein degradation of cow meat monitored by the absorbance at 210 nm (A) and 222 nm (B).*

Acknowledgements

Authers acknowledge the support rendered by Mr. Neelakant Varma of Institute of Forensic Science, Gujarat Forensic Sciences University (GFSU) for enhancing the quality and content of the manuscript. The experimental facilities and infrastructure provided by GFSU is highly acknowledged that made this work possible.

Conflict of interest

Authors have no conflict of interest.

References

Adeyemi KD, Mislan N, Aghwan ZA, Sarah SA. Sazili AQ (2014): Myofibrillar protein profile of Pectoralis major muscle in broiler chickens subjected to different freezing and thawing methods. International Food Research Journal 21: 1125–1129.

Bender W, Smith M (1997): Population, food and nutrition. Population Bulletin 51: 2–47.

Bhat M. M, Ganai TAS, Rehman M U, Salahuddin M, Sofi AH, Malik A H (2016): Detection of Adulteration in Uncooked Kashmiri Mutton Product (Rista) with Cattle and Buffalo Meat. Curr.Tren.in Biotechnol. and Pharmacy. 10: 355–361.

Binnie MA, Barlow K, Johnson V, Harrison C (2014): Red meats: Time for a paradigm shift in dietary advice. Meat Science 98: 445–451.

Bodor GS, Survant L, Voss EM, Smith S, Porterfield D Apple FS (1997): Cardiac troponin T composition in normal and regenerating human skeletal muscle Clin Chem 43: 476–484.

Contreras-castillo CJ, Lomiwes D, Wu G, Frost D, Farouk MM (2016): The effect of electrical stimulation on post mortem myofibrillar protein degradation and small heat shock protein kinetics in bull beef. Meat Sci. 113: 65–72.

Cota-Rivas M, Vallejo-Cordoba B (1997): Capillary Electrophoresis for Meat Species Differentiation. J. Capil. Electro 4: 195–199.

Dutson, TR (1982): Meat proteolysis. Food proteins. (London: Science Publication) pp. 333–336.

Kang'ethe EK, Jones SJ, Patterson RLS (1982): Identification of the species origin of fresh meat using enzyme-linked immunosorbent assay procedure. Meat Sci 7: 229–240.

Kartheek M, Smith AA, Muthu AK, Manavalan R (2011): Determination of Adulterants in Food : A Review. J. Chem. Pharm. Res. 3: 629–636.

Keyzer MA, Merbis MD, Pavel IFPW (2001): Can we feed the animals? Origin and implications of rising meat demand. Centre for World Food Studies: Amsterdam.

Koh MC, Lim CH. Chua SB, Chew ST, Phang STW (1998): Random Amplified Polymorphic DNA (RAPD) Fingerprints for Identification of Red Meat Animal Species. Meat Sci., 48: 215–285.

Kremar P, Rencova E (2001): Identification of bovine specific DNA in feed stuffs. J. Food Prot. 64: 117–119.

Labugger R, Organ L, Collier C, Atar D, Van Eyk JE (2000): Extensive troponin I and T modification detected in serum from patients with acute myocardial infarction. Circulation; 102: 1221–6.

López JL (2007): Two-dimensional electrophoresis in proteome expression analysis. J. Chrom. B 849: 190–202.

Montowska M, Pospiech T (2007): Species identification by electrophoretic methods. Acta Sci. Pol., Technol. Aliment. 6: 5–16.

Pittner S, Monticelli FC, Pfisterer A, Zissler A, Sänger AM, Stoiber W, Steinbacher P (2015): Postmortem degradation of skeletal muscle proteins : a novel approach to determine the time since death. Int J LegalMed (online) DOI 10.1007/s00414-015-1210-6

Singh VP, Neelam S (2011): Meat Species Specifications to Ensure the Quality of Meat-A Review. International Journal of Meat Sci. 1: 15–26.

Sinha M, Lalwani S, Mir R, Sharma S, Dogra TD, Singh TP (2012): A preliminary molecular study on protein profile of vital organs: A new direction for post mortem interval determination. J Indian Acad Forensic Med 34: 292–293.

Varma N, Singh I, Dahiya MS, Ravi VK, Kumar S (2017): Structural perturbation by arsenic triggers the aggregation of hen eggwhite lysozyme by promoting oligomers formation. Int. J. Biol. Macromol. DOI: 10.1016/j.ijbiomac.2017.11.096

Walker MJ, Burns M, Burns DT (2013): Horse Meat in Beef Products- Species Substitution 2013. Journal of the Association of Public Analysts (Online) 4167–106.

Yosef TA, Al-Julaifi MZ, AL-Rizqi AM (2014): Food Forensics: Using DNA-Based Technology for the Detection of Animal Species in Meat Products. Nature and Science 12: 82–90.

Zilhadia Ofa SB, Ummi C (2014): Protein profiles of beef, pork, and sausages by using SDS-PAGE method. Journal of Food and Pharmaceutical Sciences 2: 46–51.

Address of corresponding author:
Dr. Satish Kumar
Institute of Forensic Science
Gujarat Forensic Sciences University
Gandhinagar, Gujarat
India, 382 007
satish.kumar@gfsu.edu.in