The Pharma Innovation Journal 2023; SP-12(9): 2040-2047

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(9): 2040-2047

www.thepharmajournal.com
Received: 14-06-2023
Accepted: 22-07-2023

Sohail Ahmed
M.V.Sc., Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, SVVU Tirupati, Andhra Pradesh, India

C Siva Swetha
Assistant Professor and Head, Department of Veterinary Public Health and Epidemiology. College of Veterinary Science, SVVU Tirupati, Andhra Pradesh, India

A Jagadeesh Babu
Professor and University Head, Department of Veterinary Public Health and Epidemiology, N.T.R College of Veterinary Science, Gannavaram, SVVU, Tirupati, Andhra Pradesh, India

S Vijaya Lakshmi
Associate Professor, Department of Veterinary Microbiology, College of Veterinary Science, SVVU Tirupati, Andhra Pradesh, India

P Ramya
Assistant Professor, Department of Veterinary Public Health and Epidemiology. College of Veterinary Science, SVVU Tirupati, Andhra Pradesh, India

M Venkata Lakshmi
M.V.Sc., Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, SVVU Tirupati, Andhra Pradesh, India

PV Siddartha
Ph.D. Scholar, Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, SVVU Tirupati, Andhra Pradesh, India

Naswar Khan Pathan
3rd Year B.V.Sc. & AH Student, College of Veterinary Science, Tirupati, Andhra Pradesh, India

Corresponding Author:
Sohail Ahmed
M.V.Sc., Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, SVVU Tirupati, Andhra Pradesh, India

Comparing methodologies of DNA extraction for analysis of raw and cooked meats

Sohail Ahmed, C Siva Swetha, A Jagadeesh Babu, S Vijaya Lakshmi, P Ramya, M Venkata Lakshmi, PV Siddartha and Naswar Khan Pathan

Abstract
The adulteration of meat stands as a pressing concern within the meat industry, exerting far-reaching impacts on the realms of health, economy, and the deeply rooted religious and ethical convictions of consumers. Addressing this issue hinges significantly upon meat speciation, with DNA-based methodologies emerging as cutting-edge and remarkably dependable, marked by their exquisite specificity and sensitivity. Diverse techniques are available for extraction of DNA from meat specimens. In this investigation, we have encompassed three distinct methodologies including, Phenol-Chloroform-Isoamyl alcohol, PCI-Isopropanol, and the commercial kit for extraction of DNA from both raw and cooked chicken, mutton, beef and pork samples. The efficiency of these three approaches was meticulously assessed through a battery of parameters encompassing gel electrophoresis, purity, concentration and PCR assay using species-specific primers targeting the mitochondrial genes of the species under study. The thermal processed samples were also tested to gauge the resilience of DNA integrity under harsh temperature conditions. Our findings underscore that the PCI and PCI-Isopropanol methods represent cost-effective alternatives, providing robust DNA extraction from both raw and cooked samples, thereby offering a viable substitute to the more expensive commercially available kit procedures.

Keywords: Meat, adulteration, DNA, extraction, PCI, PCR

1. Introduction
In recent years, there has been a notable rise in the heightened consciousness surrounding food safety and quality. In this context, the issue of meat adulteration has emerged as a profoundly significant concern, impacting health, economics, religious considerations, and regulatory aspects alike. Furthermore, these unethical practices appear to be more prevalent in nations characterized by economic challenges and large populations, exacerbated by an escalating demand for meat and meat-derived products. Consumers are increasingly mindful of the origins of their food and anticipate authenticity. Beyond mere consumer satisfaction, genuine labeling of the source of meat and meat products is imperative due to social and religious considerations, as well as the specific health risks associated with meat consumption.

Given the paramount importance of consumer health, there is a heightened emphasis on assessing food composition and ensuring its authenticity. One pivotal facet of food quality control procedures is meat speciation. Conventional methods, such as anatomical, histological, organoleptic, chemical, electrophoretic, chromatographic and immunologic techniques, are either labour-intensive or lack consistent repeatability and reproducibility. They are reported to have lower sensitivity and, therefore, are considered reliable primarily for unprocessed raw meats. With the advancement in technology and ongoing research, a multitude of techniques for discerning meat species have arisen. Two predominant methodologies stand out in detecting and identifying meat species in food which include protein-based and DNA-based methods (Nakyinsige *et al*., 2012 and Ali *et al*., 2012) [1, 2]. The efficacy of protein and lipid-based techniques has purportedly shown a diminished level of effectiveness primarily ascribed to the heightened vulnerability of the target biomarkers to modification during the intricate processing treatments which the meat undergoes (Ha *et al*., 2017; Bhat *et al*., 2016 and Aida *et al*., 2005) [3-5].

In the past two decades, DNA-based molecular techniques have kindled optimism for the creation of dependable and genuinely authentic methodologies for species identification (Saini *et al*., 2007) [6]. DNA is ubiquitous, residing within nearly all cells of an organism, and when contrasted with proteins, exhibits greater thermal stability. DNA functions as a repository for

an individual's entire genetic information, remaining conserved regardless of the specific tissues or organs in which it is found (Lockley and Bardsley, 2000) [7]. Notable among these methodologies involving DNA are, DNA hybridization and its applications (Chikuni *et al*., 1990 and Ballin *et al*., 2009), forensically informative nucleotide sequencing (FINS) of DNA (Hsieh *et al*., 2005 and Girish *et al*., 2004), polymerase chain reaction (PCR) assays and their diverse applications (Matsunga *et al*., 1999), universal primer pairs for PCR-restriction fragment length polymorphism (PCR-RFLP) analysis (Murugaiah *et al*., 2009; Uddin *et al*., 2021 and Gargouri *et al*., 2021), PCR-random amplified polymorphic DNA fingerprinting (PCR-RAPD) (Rastogi *et al*., 2007 and Calvo *et al*., 2001), amplified fragment length polymorphism (AFLP) (Sasazaki *et al*., 2004 and Zhao *et al*., 2018) and real-time PCR (RT-PCR) (Tanabe *et al*., 2007; Liu *et al*., 2021 and Li *et al*., 2021) [8-22]. The Polymerase Chain Reaction (PCR) techniques are the most precise and sensitive for species identification, offering a notable advantage in terms of speed and efficiency in contrast with the protein-based approaches (Murugaiah *et al*., 2009 and Tanabe *et al*., 2007) [13, 20].

The judicious selection of an optimal DNA extraction method is of great significance for achieving a successful species identification through DNA-based techniques (Auricchio *et al*., 2013) [23]. The commercial extraction kits deliver unquestionable benefits, featuring directness, safe, rapid and markedly improved efficiency compared to the traditional methods like the phenol-chloroform approach for extracting DNA from meat and meat products. But the expensive nature of the kits is also to be factored into the decision-making process when determining the appropriate DNA extraction method, especially when handling a large number of samples simultaneously (Djurkin Kušec *et al*., 2015 and Santos *et al*., 2018) [24, 25].

In this research, the efficiency of conventional methods including phenol-chloroform-isoamyl alcohol, PCI-isopropanol and commercial extraction kit in detection of four meat types *viz*., chicken, mutton, beef and pork were compared by analysis of quality, yield and purity of DNA obtained using various means. Raw, cooked and samples thermally processed at various temperatures are all included in this study.

2. Materials and Methods

2.1 Collection of samples

A total of 100 samples were studied in this research work with 25 samples each belonging to chicken, mutton, beef and pork samples. Out of each 25 samples, 4 were raw, 6 were thermal processed and 15 were collected from various restaurants, small scale hotels and roadside vendors in and around Tirupati region for the extraction of DNA. The samples weighing 75 to 100 gm were collected in sterile containers and were transported to Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Tirupati and were kept in deep freezer maintained at -20 °C until further processing.

2.2 Thermal processing of samples

To evaluate the effectiveness of the DNA extraction methods employed in this research work when analyzing commercially available meat products which typically undergo extensive cooking and processing, the collected raw meat samples were subjected to thermal processing at various temperatures. Raw meat samples were cooked in water bath at 60 °C, 70 °C, 80 °C, 90 °C and 100 °C for 30 min each and also autoclaved at 121 °C, 15 psi for 30 mins.

2.3 DNA Extraction

The DNA extracted form the collected meat samples was compared using three different methods. The first method used was based on Phenol-Chloroform-Isoamyl alcohol (PCI) described by Sambrook and Russel (2001) with slight modifications. Secondly, a PCI-Isopropanol method described by Alvardo *et al*. (2017) was employed with slight modifications [26, 27]. Lastly, a QIAGEN QIAamp® DNA FFPE power kit was also used for extraction of DNA from collected samples.

2.3.1 Phenol-Chloroform-Isoamyl alcohol (PCI) method

Meat samples (50-75 mg) were finely chopped and placed in a 1.5 ml Eppendorf tube with 450-500 µl of STE buffer. After incubating at 37 °C for 1 hour, Proteinase-K solution (10 mg/ml, 15 µl) and 10% SDS (25-30 µl) were added, followed by a 60 °C incubation for 4 hours. An additional 10 µl of Proteinase-K solution was added, and overnight incubation at 37 °C ensued with gentle swirling. The mixture was briefly mixed with an equal volume (450-500 µl) of Tris-saturated phenol for no more than 10 minutes. After centrifugation at 14,000 RPM for 20 minutes, the upper aqueous phase was collected. This material was washed with Phenol: Chloroform: Isoamyl alcohol (25:24:1), separated by another 15-minute, 14,000 RPM centrifugation, and the upper aqueous phase was collected in to a fresh tube. An equal volume of chilled 99% ethanol and 200-250 µl of 4% 3M sodium acetate were added. After gentle mixing, the tube was placed in a -20 °C freezer overnight. The following day, centrifugation at 14,000 RPM for 20 minutes yielded a DNA pellet. This pellet was washed with 70% ethanol, followed by a 10,000 RPM, 5-minute centrifugation. The DNA pellet was thoroughly dried and then dissolved in 60 µl of nuclease-free water. The extracted DNA was subjected to analysis or stored at -20 °C for further use.

2.3.2 PCI – Isopropanol method

Meat samples (50-75 mg each) were finely chopped and placed in 1.5 ml Eppendorf tubes with 750 µl of Salt Lysis buffer (containing Tris HCl, EDTA, SDS, and NaCl). After centrifugation at 3000 RPM for 2 minutes, they were incubated at 60 °C for 30 minutes. Following incubation, 600 µl of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was gently mixed in, and the tube was centrifuged at 14,000 RPM for 10 minutes. The upper aqueous phase was collected into a fresh Eppendorf tube, and 10 µl of Proteinase-K solution (10 mg/ml) was added. It was then incubated at 50 °C for 30-45 minutes. Another round of 600 µl of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added, followed by a 10-minute, 14,000 RPM centrifugation. The upper aqueous phase was collected with care to exclude any lower-phase material. Isopropanol was added in equal volume to the tube, gently mixed, and then centrifuged at 14,000 RPM for 10 minutes. The resulting DNA pellet was washed with 70% ethanol and centrifuged at 10,000 RPM for 5 minutes. After drying completely, the DNA pellet was dissolved in 60 µl of nuclease-free water. The extracted DNA was subjected to analysis or stored at -20 °C for further use.

2.3.3 QIAamp® DNA FFPE power kit method
The QIAGEN QIAamp® DNA FFPE power kit was used to extract the DNA from meat samples following the instructions given by the manufacturer.

2.4 Gel Electrophoretic analysis of DNA
Horizontal sub-marine gel electrophoresis was used to determine the quality of isolated DNA as described by Sambrook and Russel (2006) with slight modifications [28]. Agarose 1% w/v suspension in 1.0 X SBB (Sodium Borate Buffer) with ethidium bromide (10 mg/ml) was used for preparation of gel. About 5 – 7 µl of DNA was mixed with 2 µl of 6X gel loading dye (Xylene Cyanol and Bromophenol Blue) and loaded in to the wells. Electrophoresis was performed at 70 - 80 volts until the dye running on gel covered 60 per cent of the gel. After the electrophoresis, the gel was visualized under UV-transilluminator (Genei, Bengaluru) and documented through gel documentation system (BIO-RAD, USA).

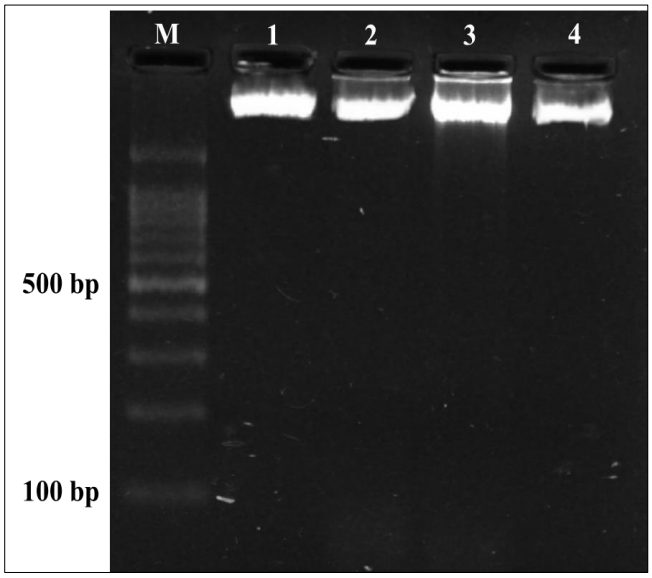
2.5 Nanodrop Analysis of DNA
The concentration and purity of the extracted DNA was checked using 1µl of DNA in Nanodrop (Thermo Scientific, USA) at an absorbance of OD_{260}: OD_{280}.

2.6 Suitability of extracted DNA for PCR assay
The extracted DNA was subjected to PCR assay using species-specific primers targeting the specific mitochondrial genes. The details of the oligonucleotides used were given in the Table 1.

Table 1: Oligonucleotide primers used in this study.

Species	(Target gene)	Primer sequence 5'-3'		Amplicon size (bp)	Reference
Chicken	12SrRNA	F	TGAGAACTACGAGCACAAAC	183	Dalmasso *et al*. (2004) [29]
		R	GGGCTATTGAGCTCACTGTT		
Mutton	ND5	F	TTCCTCCCTCACACTAGTCACC	263	Uddin *et al*. (2021) [14]
		R	CTGGAACGAATATTATTGAGAAGAAGTC		
Beef	ND5	F	GGTTTCATTTTAGCAATAGCATGG	106	Hossain *et al*. (2017) [30]
		R	GTCCAATCAAGGGTATGTTTGAG		
Pork	ND5	F	GATTCCTAACCCACTCAAACG	73	
		R	GGTATGTTTGGGCATTCATTG		

3. Results
3.1 Gel Electrophoretic analysis of DNA
The DNA extracted using all three methodologies were subjected to horizontal gel electrophoresis to assess the quality. DNA is deemed to be of superior quality when it displays no signs of shearing during electrophoresis. DNA of chicken and pork samples extracted using PCI method did not show any shearing and hence considered to be of good quality but the DNA extracted from mutton and beef samples exhibited slight shearing during electrophoresis. The DNA extracted from all four species employing PCI-Isopropanol (Fig. 1) and kit method did not show any shearing and were hence deemed to be of good quality.



Lane M-100bp molecular marker; Lane 1-Chicken DNA;
Lane 2-Mutton DNA; Lane 3-Beef DNA; Lane 4-Pork DNA.

Fig 1: Gel electrophoresis of DNA extracted using PCI-Isopropanol Method

3.2 Nanodrop Analysis of DNA
3.2.1 Phenol-Chloroform-Isoamyl alcohol method
The PCI method employed for DNA extraction proved successful for chicken and pork samples, with consistent results observed across raw, thermally processed, and commercially collected meat samples. However, it exhibited a different outcome when applied to mutton and beef samples, as indicated by an error in Nanodrop readings which stated excessively high absorbance at the measurement wavelength. This method yielded DNA concentrations ranging from 100 to 140 ng/µl, with purity levels falling within the range of 1.5 to 2.0 for chicken samples. In case of pork samples, the DNA concentrations ranged between 80 to 110 ng/µl, with purity levels between 1.4 and 2.1.

3.2.2 PCI – Isopropanol method
The PCI-Isopropanol method proved to be highly effective in extracting DNA from all four species under investigation. The DNA extracted from all raw, thermally processed, and commercially collected samples demonstrated substantial concentration and purity levels suitable for subsequent analysis. Specifically, the DNA obtained from chicken samples exhibited concentrations ranging from 100 to 120 ng/µl, with purity levels falling within the range of 1.4 to 2.1. Pork samples yielded DNA concentrations ranging from 80 to 100 ng/µl, with purity levels spanning from 1.3 to 2.3. In the case of mutton samples, DNA concentrations ranged from 60 to 110 ng/µl, with purity levels between 1.5 and 2.3. Finally, the DNA extracted from beef samples displayed purity levels ranging from 1.4 to 2.0, with concentrations ranging from 50 to 100 ng/µl.

3.2.3 QIAamp® DNA FFPE power kit method
The QIAGEN QIAamp® DNA FFPE power kit successfully yielded DNA with high purity ranging from 1.7 -1.9 for all four species *viz*., chicken, sheep, cattle and pig. While, the DNA Concentrations obtained were low when compared to

other methods, ranging between 15- 20 ng/µl, 8 - 15 ng/µl, 8 – 14 ng/µl and 12 – 20 ng/µl were for chicken, mutton, beef and pork samples respectively. An overview of comparison of three methods employed in this study by Nanodrop was given in Table 2.

Table 2: Comparison of DNA extraction method by Nanodrop analysis.

S. No	Species	PCI method		PCI-Isopropanol method		QIAGEN QIAamp® DNA FFPE power kit	
		Concentration (ng/µl)	$OD_{260:280}$	Concentration (ng/µl)	$OD_{260:280}$	Concentration (ng/µl)	$OD_{260:280}$
1.	Chicken	100-140	1.5-2.0	100-120	1.4-2.1	15-20	1.7-1.9
2.	Mutton	-	-	60 – 110	1.5-2.3	8 – 15	1.7-1.9
3.	Beef	-	-	50 – 100	1.4-2.0	8 – 14	1.7-1.9
4.	Pork	80-110	1.4-2.1	80-100	1.3-2.3	12 - 20	1.7-1.9

3.3 Thermal processed samples

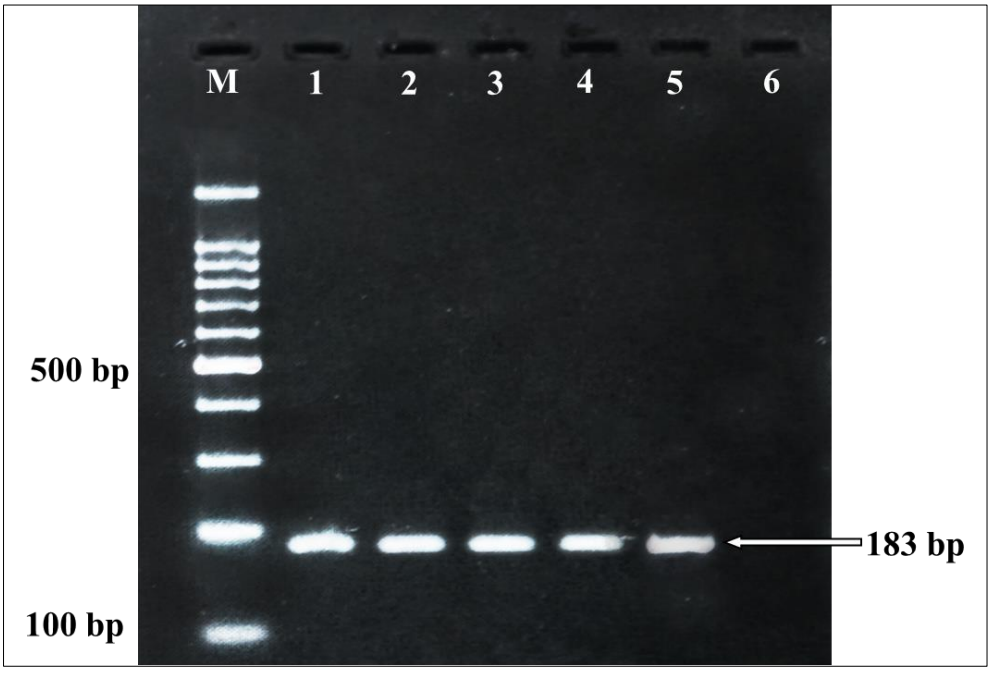
The raw samples of chicken, mutton, beef and pork were thermally processed at 60 °C, 70 °C, 80 °C, 90 °C, 100 °C and autoclaved (at 121 °C, 15 psi) for 30 min each to know the stability of extracted DNA. PCI-Isopropanol method was used to extract the DNA from these processed samples. The concentration and purity of DNA extracted from these processed samples were estimated by Nano drop and the details are given in Table 3. Despite the varying range of processing temperatures, the DNA obtained exhibited both good concentration and purity, indicating that thermal processing had little to no discernible impact on the quality of the extracted DNA.

Table 3: Concentrations (ng/µl) and purity (OD260: OD280) of DNA extracted from thermal processed samples at various temperatures.

Species	60 °C	70 °C	80 °C	90 °C	100 °C	121 °C
Chicken	139.97 (1.93)	112.45 (1.91)	116.14 (1.92)	110.45 (1.81)	105.67 (1.85)	128.47 (1.84)
Mutton	89.63 (1.77)	97.01 (1.80)	88.63 (1.71)	90.92 (1.68)	101.39 (1.96)	94.66 (1.67)
Beef	81.08 (1.78)	84.26 (1.66)	84.22 (1.75)	89.80 (1.86)	89.42 (1.72)	87.60 (1.92)
Pork	94.32 (1.82)	85.61 (1.72)	89.38 (1.59)	83.01 (1.75)	110.14 (1.92)	92.71 (1.66)

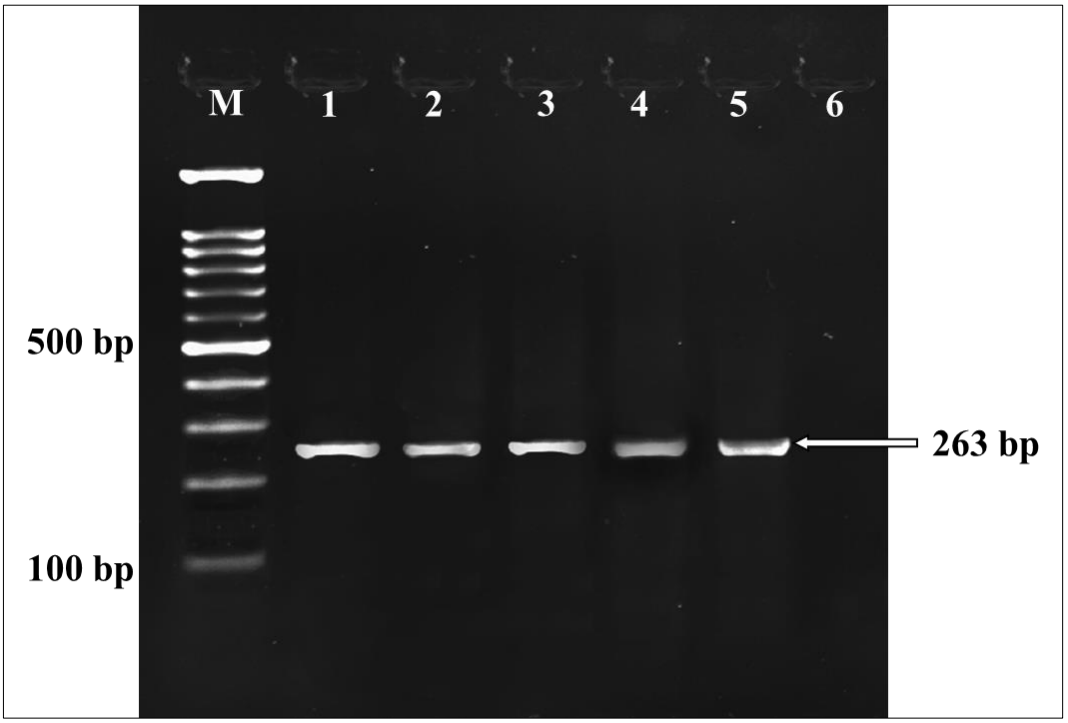
3.4 PCR assay

The DNA extracted from the raw, thermal processed and commercially sourced samples was subjected to PCR assay utilizing established primers designed to target the specific mitochondrial genes within the four species of interest. The PCR assay yielded the desired amplicon size of 183 bp for chicken (Fig. 2), 263 bp for mutton (Fig. 3), 106 bp for beef (Fig. 4) and 73bp for pork (Fig. 5) successfully.



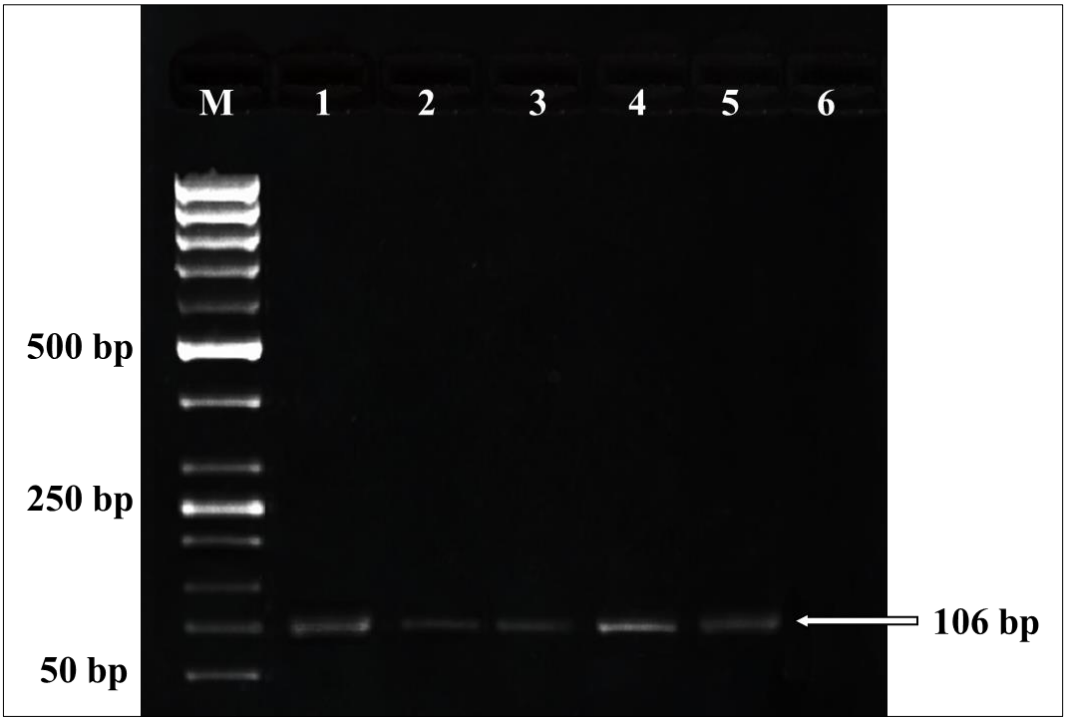
Lane 1: Raw chicken sample;
Lane 2: Thermal processed sample @ 60 °C;
Lane 3: Thermal processed sample @ 100 °C;
Lane 4: Autoclaved sample;
Lane 5: Commercially procured chicken sample; Lane 6: Negative control.

Fig 2: Chicken DNA yielding 183 bp from various samples



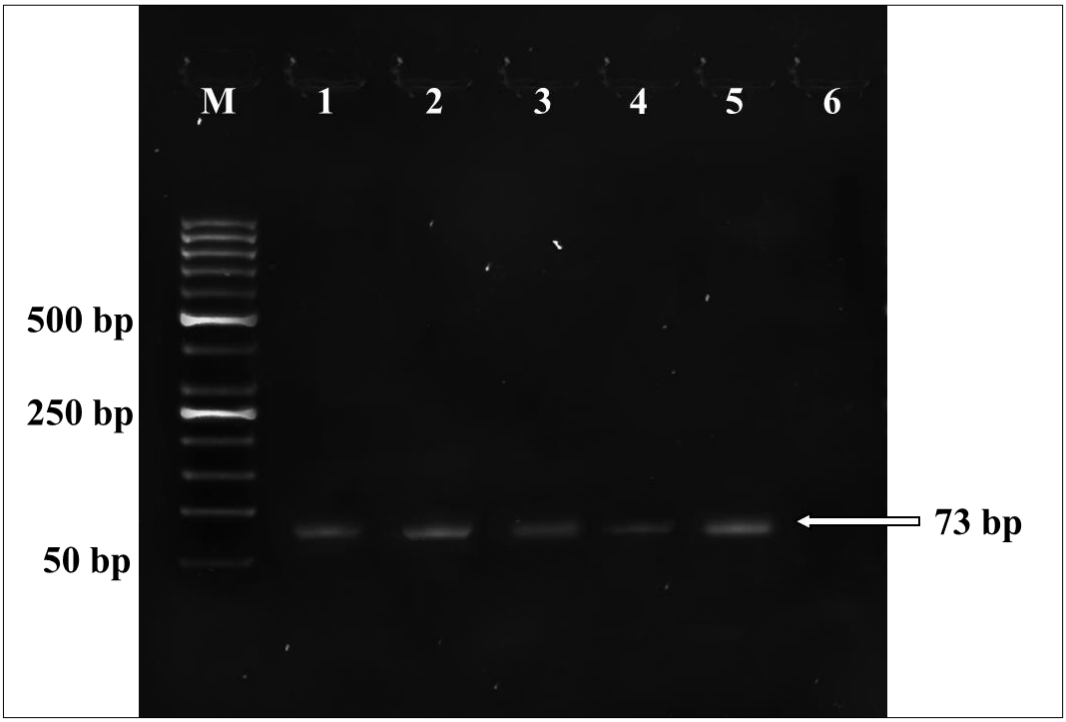
Lane 1: Raw mutton sample;
Lane 2: Thermal processed sample @ 60 °C;
Lane 3: Thermal processed sample @ 100 °C;
Lane 4: Autoclaved sample;
Lane 5: Commercially procured mutton sample;
Lane 6: Negative control.

Fig 3: Mutton DNA yielding 263 bp from various samples



Lane 1: Raw beef sample;
Lane 2: Autoclaved sample;
Lane 3: Thermal processed sample @ 100 °C;
Lane 4: Thermal processed sample @ 60 °C;
Lane 5: Commercially procured beef sample;
Lane 6: Negative control.

Fig 4: Beef DNA yielding 106 bp from various samples



Lane 1: Raw mutton sample;
Lane 2: Thermal processed sample@ 60 °C;
Lane 3: Thermal processed sample @ 100 °C;
Lane 4: Autoclaved sample;
Lane 5: Commercially procured mutton sample;
Lane 6: Negative control.

Fig 5: Pork DNA yielding 73 bp from various samples

4. Discussion

A total of 100 samples were analyzed in this research work with 25 samples each belonging to chicken, mutton, beef and pork samples. In this investigation, three methodologies were employed to extract DNA from meat samples. Firstly, PCI technique given by Sambrook and Russel, 2001 was used with slight modifications. Secondly another method based on PCI-Isopropanol documented by Alvardo *et al*. (2017) was employed with slight modifications [26, 27]. Lastly, commercially available QIAGEN QIAamp® DNA FFPE power kit was used to extract the DNA. The quality and quantity of the extracted DNA was assessed by gel electrophoresis, nanodrop and PCR assay which involved the use of species-specific primer pairs which target the mitochondrial genes of the species under study.

The gel electrophoresis method revealed that the DNA extracted from chicken and pork using PCI method exhibited no sign of shearing. However, slight shearing was noticed when DNA extracted from mutton and beef samples were subjected to electrophoresis. The DNA samples extracted using PCI-Isopropanol method and Qiagen kit also showed no signs of shearing under electrophoresis. When analyzed using the Nanodrop, the chicken and pork DNA extracted using PCI method showed high concentration and a good range of purity. The DNA samples of mutton and beef showed error in nanodrop readings revealing that the absorbance at the measurement wavelength is too high. In contrast, the mutton and beef DNA obtained by PCI-Isopropanol method showed good measurement in Nanodrop in terms of purity and concentration. The nanodrop results for chicken and pork DNA from PCI-Isopropanol were similar to that of PCI method. The DNA extracted using kit method from all four species showed a high uniform purity ranging from 1.7 to 1.9. However, when the DNA was quantified, the resultant concentration was found to be in much lesser quantities when compared to PCI and PCI-Isopropanol methods. The extracted DNA from all three methodologies when subjected to PCR assay using primers targeting species-specific mitochondrial genes revealed the desirable amplicons of 183 bp, 263 bp, 106 bp and 73 bp from chicken, mutton, beef and pork samples respectively. The mutton and beef DNA extracted using PCI method were also successfully amplified by PCR assay despite the shearing and error in the nanodrop readings, proving that the DNA extracted was of sufficient grade for amplification.

The DNA was extracted from thermally processed samples to know the extent of any DNA degradation in the processed samples during heat treatment. The raw samples of all four species under the study were subjected to various cooking temperatures in a water bath *viz*., 60 °C, 70 °C, 80 °C, 90 °C and 100 °C for 30 min each and autoclaved at 121 °C, 15 psi for 30 min. The DNA extracted from these samples was tested in Nanodrop for its concentration and purity. The PCI and PCI-Isopropanol methods were able to successfully extract the DNA from these thermal processed meat samples. The samples cooked at various temperatures up to 100 °C and autoclaved at 121° C, 15 psi for 30 min also gave healthy concentrations of DNA with decent purity. Some researchers have also studied the DNA integrity of thermally processed meat samples. Karabasanavar *et al*. (2017) was able to identify beef DNA in raw, cooked (60 °C, 80 °C and 100 °C for 30 min), autoclaved (121 °C for 30 min) and micro-oven processed meat samples using beef-specific PCR [31]. In contrast, Haunshi *et al*. (2009) found that pig specific marker could not be amplified from the DNA extracted from autoclaved pork samples [32].

The PCI method has yielded the highest DNA concentration among all methods. This is in accordance with results obtained by Suadi *et al*. (2020) who compared the efficiency of PCI method and commercially available kit in extraction of porcine, beef and chicken DNA [33]. Piskata *et al*. (2019) also acquired similar results with PCI method extracting the highest DNA concentration compared to eight commercially available kits from raw, heat processed products of chicken, beef and pork [34]. Hence recommending the conventional PCI method as an excellent alternative to more expensive extraction kits. However, the concentration obtained using PCI method was still significantly low when compared to Suadi *et al*. (2020) who documented the highest DNA concentrations by PCI method *viz*., 4,957.5 ng/µl, 5,285 ng/µl and 2,550 ng/µl from chicken, beef and pork samples respectively [33]. The concentration and purity results yielded by the Qiagen tissue kit were in concordance to results of Piskata *et al*. (2019) [34]. Similarly, Ali *et al*. (2015) also reported high quality of DNA ranging between 1.7 and 2 using Yeastern Genomic DNA Mini Kit [35]. Although the purity of 1.3 to 2.3 obtained from PCI and PCI - Isopropanol methods varies far from the perfect purity value of 1.8 reported by Somma, 2004, the minimum level of purity required for amplification of DNA is 1.0 as reported by Bourke *et al*. (1999) [36, 37]. The results of PCR assay were in accordance with the results of Dalmasso *et al*. (2004) yielding 183 bp amplicon from chicken samples. The 263 bp amplicon obtained from mutton samples was parallel to results of Uddin *et al*. (2021) [29, 14]. The 106 bp and 73 bp amplicons acquired from beef and pork respectively were in accordance with the records of Hossain *et al*. (2017) [30].

5. Conclusion

In this investigation involving three methodologies of DNA extraction, the PCI method was found to be producing the best results. The PCI-Isopropanol method falls slightly behind the PCI method in an average but sufficient enough for further processing. The kit method used produced lesser concentrations compared to other two methods but of higher purity. The analysis of DNA extracted from thermal processed samples revealed no effect of heat treatment on integrity of DNA. Though the PCI and PCI-Isopropanol methods are time consuming, they serve as cost-effective techniques yielding DNA from raw and cooked samples of sufficient grade in terms of concentration and purity required for further procedures. Hence can be used as an alternative to more expensive commercially available kit procedures.

6. Acknowledgement

My sincere thanks to Department of Veterinary Public Health and Epidemiology, Department of Veterinary Microbiology and Department of Animal Genetics and Breeding of C.V.Sc, SVVU, Tirupati for provision of facilities to carry out my research work.

7. References

1. Nakyinsige K, Man YBC, Sazili AQ. Halal authenticity issues in meat and meat products. Meat science. 2012;91(3):207-214.
2. Ali ME, Kashif M, Uddin K, Hashim U, Mustafa S, Che Man YB. Species authentication methods in foods and feeds: the present, past, and future of halal forensics. Food Analytical Methods. 2012;*5*:935-955.
3. Ha J, Kim S, Lee J, Lee S, Lee H, Choi Y, *et al*. Identification of pork adulteration in processed meat products using the developed mitochondrial DNA-based primers. Korean Journal for Food Science of Animal Resources. 2017;37(3):464.
4. Bhat MM, Salahuddin M, Mantoo IA, Adil S, Jalal H, Pal, MA. Species-specific identification of adulteration in cooked mutton Rista (a Kashmiri Wazwan cuisine product) with beef and buffalo meat through multiplex polymerase chain reaction. Veterinary world. 2016;9(3):226.
5. Aida AA, Man YC, Wong CMVL, Raha AR, Son R. Analysis of raw meats and fats of pigs using polymerase chain reaction for Halal authentication. Meat science. 2005;69(1):47-52.
6. Saini M, Das DK, Dhara A, Swarup D, Yadav MP, Gupta PK. Characterization of peacock (*Pavo cristatus*) mitochondrial 12S rRNA sequence and its use in differentiation from closely related poultry species. British poultry science. 2007;48(2):162-166.
7. Lockley AK, Bardsley RG. DNA-based methods for food authentication. Trends in Food Science & Technology. 2000;11(2):67-77.
8. Chikuni K, Ozutsumi K, Koishikawa T, Kato S. Species identification of cooked meats by DNA hybridization assay. Meat science. 1990;27(2):119-128.
9. Ballin NZ, Vogensen FK, Karlsson AH. Species determination–Can we detect and quantify meat adulteration? Meat science. 2009;83(2):165-174.
10. Hsieh HM, Tsai CC, Tsai LC, Chiang HL, Huang NE, Shih RTP, *et al*. Species identification of meat products using the cytochrome b gene. Forensic Sci. J. 2005;4:29-36.
11. Girish PS, Anjaneyulu ASR, Viswas KN, Anand M, Rajkumar N, Shivakumar, BM, *et al*. Sequence analysis of mitochondrial 12S rRNA gene can identify meat species. Meat Science. 2004;66(3):551-556.
12. Matsunaga T, Chikuni K, Tanabe R, Muroya S, Shibata K, Yamada J, *et al*. A quick and simple method for the identification of meat species and meat products by PCR assay. Meat science. 1999;51(2):143-148.
13. Murugaiah C, Noor ZM, Mastakim M, Bilung LM, Selamat J, Radu S. Meat species identification and Halal authentication analysis using mitochondrial DNA. Meat science. 2009;83(1):57-61.
14. Uddin SMK, Hossain MM, Chowdhury ZZ, Johan MRB. Short targeting multiplex PCR assay to detect and discriminate beef, buffalo, chicken, duck, goat, sheep and pork DNA in food products. Food Additives & Contaminants: Part A. 2021;38(8):1273-1288.
15. Gargouri H, Moalla N, Kacem HH. PCR–RFLP and species-specific PCR efficiency for the identification of adulteries in meat and meat products. European Food Research and Technology. 2021;247(9):2183-2192.
16. Rastogi G, Dharne MS, Walujkar S, Kumar A, Patole MS, Shouche YS. Species identification and authentication of tissues of animal origin using mitochondrial and nuclear markers. Meat science. 2007;76(4):666-674.
17. Calvo JH, Zaragoza P, Osta R. A quick and more sensitive method to identify pork in processed and unprocessed food by PCR amplification of a new specific DNA fragment. Journal of animal science. 2001;79(8):2108-2112.
18. Sasazaki S, Itoh K, Arimitsu S, Imada T, Takasuga A,

Nagaishi H, *et al*. Development of breed identification markers derived from AFLP in beef cattle. Meat Science. 2004;67(2):275-280.
19. Zhao J, Li T, Xu Z, Wang Z, Yang S, Chen A. AFLP markers for meat traceability of cattle in the Chinese market. Food Control. 2018;91:421-426.
20. Tanabe S, Hase M, Yano T, Sato M, Fujimura T, Akiyama H. A real-time quantitative PCR detection method for pork, chicken, beef, mutton, and horseflesh in foods. Bioscience, biotechnology, and biochemistry. 2007;71(12):3131-3135.
21. Liu GQ, Luo JX, Xu WL, Li CD, Guo YS, Guo L. Improved triplex real-time PCR with endogenous control for synchronous identification of DNA from chicken, duck, and goose meat. Food Science & Nutrition. 2021;9(6):3130-3141.
22. Li T, Wang J, Wang Z, Qiao L, Liu R, Li S, *et al*. Quantitative determination of mutton adulteration with single-copy nuclear genes by real-time PCR. Food Chemistry. 2021;344:128622.
23. Auricchio B, Anniballi F, Fiore A, Skiby JE, De Medici D. Evaluation of DNA extraction methods suitable for PCR-based detection and genotyping of Clostridium botulinum. Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science. 2013;11(S1):S200-S206.
24. Djurkin Kušec I, Radišić Ž, Komlenić M, Kušec G. Comparison of commercial DNA kits and traditional DNA extraction procedure in PCR detection of pork in dry/fermented sausages. Poljoprivreda. 2015;21(1 Supplement):199-202.
25. Santos ALF, Oliveira CQ, Arruda GNP, Martins JK. Comparison of DNA extraction using proteinase K and extraction kit: analysis of the quality of the genetic material. Journal Brasileiro de Patologia e Medicina Laboratorial. 2018;54:70-75.
26. Sambrook J, Russell DW. Purification of nucleic acids by extraction with phenol: chloroform. Commonly used techniques in molecular cloning. Molecular cloning; c2001. Appendix, 8, 7.
27. Alvarado PE, Barrios RMM, Xóchihua JAM, Hernández JFC. Fast and reliable DNA extraction protocol for identification of species in raw and processed meat products sold on the commercial market. Open Agriculture. 2017;2(1):469-472.
28. Sambrook J, Russell DW. Purification of nucleic acids by extraction with phenol: chloroform. Cold Spring Harbor Protocols, 2006(1):pdb-prot4455.
29. Dalmasso A, Fontanella E, Piatti P, Civera T, Rosati S, Bottero MT. A multiplex PCR assay for the identification of animal species in feedstuffs. Molecular and cellular probes. 2004;18(2):81-87.
30. Hossain MM, Ali ME, Abd Hamid SB, Mustafa S, Desa MNM, Zaidul ISM. Targeting double genes in multiplex PCR for discriminating bovine, buffalo and porcine materials in food chain. Food Control. 2017;73:175-184.
31. Karabasanavar N, Girish PS, Kumar D, Singh SP. Detection of beef adulteration by mitochondrial D-loop based species-specific polymerase chain reaction. International Journal of Food Properties. 2017;20(sup2):2264-2271.
32. Haunshi S, Basumatary R, Girish PS, Doley S, Bardoloi RK, Kumar A. Identification of chicken, duck, pigeon and pig meat by species-specific markers of mitochondrial origin. Meat science. 2009;83(3):454-459.
33. Suadi Z, Bilung LM, Apun K, Azmi AA. Efficiency of traditional DNA extraction method in PCR detection of porcine DNA in meat mixtures. Journal Teknologi (Science & Engineering). 2020;82(5):85-90.
34. Piskata Z, Servusova E, Babak V, Nesvadbova M, Borilova G. The quality of DNA isolated from processed food and feed via different extraction procedures. Molecules. 2019;24(6):1188.
35. Ali ME, Razzak MA, Abd Hamid SB, Rahman MM, Al Amin, M, Abd Rashid NR. Multiplex PCR assay for the detection of five meat species forbidden in Islamic foods. Food chemistry. 2015;77:214-224.
36. Somma M, Querci M. The Analysis of Food Samples for the Presence of Genetically Modified Organisms. Session 4. Extraction and Purification of DNA. European Commission Joint Research Centre; c2004.
37. Bourke MT, Scherczinger CA, Ladd C, Lee HC. NaOH treatment to neutralize inhibitors of Taq polymerase. Journal of Forensic sciences. 1999;44(5):1046-1050.