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Effect of novel polymerase spiral reaction assay (PSR) for species identification of Chevon

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Abstract
The present study aims at development of a novel Polymerase Spiral Reaction (PSR) assay to detect the presence of goat meat (chevon) in adulterated meat. Many PSR assays have been developed to identify bacteria, fungi and even viruses. But, PSR based assays have not yet been reported for confirming meat species, to the best of our knowledge. The DNA from meat samples were extracted employing the alkaline lysis (AL) technique. The extracted DNA were evaluated for its purity by spectrophotometer analysis. The samples had an Optical Density (OD) ratio of 1.6-1.8 between a wavelength of 260 and 280 nm. In this study, a pair of PSR chevon-specific primers were sequenced targeting goat specific mitochondrial DNA (mtDNA). The possibility of cross-amplification of the primers was ruled out by considering related species. The results were analysed by (1) Observation of visual colour change by the addition of SYBR Green I stain to the amplified product, (2) Measuring the intensity of colour developed and (3) Visualization of bands on agarose gel electrophoresis (2%). The specificity of the chevon-specific PSR was determined using DNA extracted from meat samples obtained from different meat species *viz.*, buffalo, cattle, chicken, pig, sheep and ostrich. There was no cross amplification observed when the PSR assay was carried out with DNA of other species. The experiment was repeated six times and consistent results were obtained. Results obtained in the present study are reliable and can be used for field purposes which take about 90 min when combined with the alkaline lysis method of DNA extraction.

Keywords: Polymerase spiral reaction, mitochondrial DNA, single nucleotide polymorphisms

Introduction
Meat is a nutritious food and rich source of proteins, minerals and vitamins [1]. Although expensive, goat meat (chevon) is one of the most popular meats especially in the south Asia. Due to the higher prices, meat traders often illegally mislabel other species cheaper meats as chevon for economic gains including slaughter banned species [2]. Small ruminants (sheep and goat) significantly contribute to the national economy of some low- and middle-income countries; further, their meats could be misrepresented interchangeably due to disparities in their market prices. In order to authenticate origin of the meat, sensitive speciation tools are needed so as to prevent fraudulent acts [3] and protect consumer's sentiments [4]. Several meat speciation tests *viz.* anatomical or histological, protein based, spectrometric and DNA based methods are in vogue for determining authenticity of the meat. However, each method has its own merit and demerits; of which, DNA based molecular techniques have proven to be superior over the other methods owing to versatile and discriminatory nature of the DNA, especially the mitochondrial targets [5]. Various PCR based DNA amplification techniques have been developed for the authentication of mutton or sheep such as restriction fragment length polymorphism (RFLP) [6], random amplified polymorphic DNA (RAPD) fingerprinting [7], arbitrarily primed PCR, DNA sequence analysis [8], duplex/multiplex PCR [9], species specific PCR [2, 10], touchdown-duplex PCR [11] and real time PCR [12]. The PCR based DNA amplification tools even though are conclusive for species identification, require costly thermal cycler and post-PCR signal visualization instruments such as electrophoresis assembly and gel documentation system [13] making cumbersome, time-taking process and inability in resource deficient establishments. However, isothermal amplification techniques such as Loop mediated isothermal amplification (LAMP), rolling circle replication (RCR) and strand displacement amplification (SDA) have been proven as less complex and quicker in detections. Isothermal techniques have some limitations; for instance, LAMP assay requires multiple primers, stringent optimizations and leftover contamination could even lead to false-positives [14].

Polymerase spiral reaction (PSR) is known to overcome such limitations [15]. The PSR meets ASSURED (affordable, sensitive, specific, user friendly, robust and rapid, equipment free and deliverable) guidelines proposed by the World Health Organization for developing diagnostic techniques [16]. The PSR is a recent isothermal DNA amplification test known to possess higher specificity, sensitivity, reliability and suitability for DNA based detections. Like the PCR, the PSR uses only one pair of primers and unlike PCR, amplification is carried at isothermal as in RCR or self-sustained sequence replication reaction (3SR). Additional steps as involved in other isothermal amplification tests could be precluded in the PCR; for instance, isothermal amplification methods *viz*. LAMP and SDA (strand displacement amplification) require an initial incubation (95 °C); DNA helicase is used in reaction mixture in HDA (helicase-dependent amplification) assay for the denaturation of Watson-Crick double strands [15]. The PSR does not require initial denaturation step, reaction proceeds as soon as temperature reaches optimal amplification temperature. One enzyme and one set of primers are all the reagents required to initiate PSR; hence, PSR has been the revolutionary isothermal nucleic acid amplification technique. The PSR does not require special equipment as the reaction takes place at constant single temperature, this is an added advantage of this technique; PSR possesses higher sensitivity, specificity and rapidity; hence it is suitable for the on-site and point-of-care (PoC) applications. Additionally, observance of visual change in colour to read to test results makes it a user-friendly test for the low resource laboratory systems. All the PSR techniques developed so far have been tested for detection of bacteria [15, 17], fungi such as *Candida albicans* [18] and viruses [19]. Till date no PSR has been developed and validated for the purpose of meat species identification. Hence, keeping in view the versatility of the PSR as a DNA based tool, present work was undertaken with the objective of development and validation of a novel PSR assay for the goat meat and evaluate its suitability in raw, cooked and adulterated meat samples.

Materials and Methods

A study on development of novel Polymerase Spiral Reaction (PSR) assay for detection of chevon was carried out at Indian Council of Agricultural Research – National Research Centre on Meat (ICAR - NRCM), Chengicherla, Uppal, Hyderabad with the aim to provide a fast and quick assay for detection of chevon.

Collection of Samples

Samples of chevon, mutton and chicken were collected from experimental abattoir, ICAR – National Research Centre on Meat, Hyderabad, Telangana State, India. Samples of beef and carabeef were obtained from the Municipal Slaughterhouse, Chengicherla, Hyderabad, Telangana State, India. Pork was sourced from verified convenience stores at Secunderabad, Telangana State, India. Samples of ostrich meat was obtained from the Ostrich farm, Hassan District, Karnataka State, India. These samples were collected in a sterile manner and labelled appropriately, with the date of collection and name of the sample collected, for better traceability. The samples were placed in a sterile container and transported to ICAR – National Research Centre on Meat, India under refrigerated temperature and were stored at - 20 °C till further use.

Nucleic Acid Extraction

The chemicals used for the nucleic acid extraction from meat samples are mentioned. The isolation of DNA was done by Alkaline lysis (AL) method as per Girish *et al*. (2013) [10] as described below.

1. About 500 mg of fresh meat sample was taken and triturated with eight volumes of 0.2 N sodium hydroxide (4 mL) in an autoclaved pestle and motor.
2. 5 µL of this extract was taken in a 1 mL centrifuge tube and mixed with another eight volumes of 0.2 N sodium hydroxide solution (40 µL) and incubated at 75 °C in water bath for 20 min.
3. After thermal lysis, the reaction mix was neutralised using eight volumes of 0.04 M, pH 7 Tris-Hcl (360 µL).
4. 1 µL of the final mix was used to for detection of DNA concentration and purity.
5. The DNA samples were stored at - 20 °C for further use.

Checking Quality, Purity and Concentration of DNA

Quality of DNA

A horizontal submarine agarose gel electrophoresis was employed to examine the quality of genomic DNA. First, the gel was cast in a gel caster with a comb in such a way that there is still a gap of 0.5 mm between the comb teeth and tray floor, in order to thoroughly seal the wells with agarose. Afterwards, 0.8% agarose w/v suspension in 1 X Tris-Acetate-Ethylenediamine tetra acetic acid (TAE) buffer was prepared, heated in the microwave until the agarose was thoroughly melted and dissolved, and a clear transparent solution was obtained. Ethidium bromide (10 mg/mL) was added to the mixture after cooling. A levelled casting tray was filled with the agarose solution. The gel formed had a thickness of around 4 mm. After allowing the agarose to set, the comb was carefully removed, and the gel casting platform was submerged into the electrophoresis tank filled with 1 X TAE buffer. After combining 5 µL of diluted DNA with 2 µL of the 6 X gel loading dye (Xylene Cyanol and Bromophenol blue), the diluted DNA samples mixed with dye were loaded into the wells. One of the wells was loaded with a 1 kb DNA marker. For 1 hr, electrophoresis was carried out at 90 V. After the electrophoresis, the gel was viewed under a UV transilluminator and photographed for documentation. For further examination, only DNA samples with an intact band and minimal smearing were used.

Purity of DNA

The purity of the DNA isolated by the AL method was assessed with the help of Nano Spectrophotometer (DeNovix, Model: Ds-11 FX + Spectrophotometer/ Fluorometer) for purity at OD260/280. 1 µL of DNA was utilized for taking the readings against 1 µL of 1 X Tris-Ethylenediamine tetra acetic acid (TE) buffer as a blank. The DNA sample of OD260/280 between 1.7 to 1.9 were considered good and were used for further study (PSR amplification).

Concentration of DNA

The concentration of the DNA was assessed with the help of Nano spectrophotometer (DeNovix, Model: Ds-11 FX + Spectrophotometer/ Fluorometer) for quantity at 260 nm. For estimating the concentration of DNA, following formula was employed.

A total of 12.5 µL of standardized reaction mix consisted of 10X Bsm buffer (1.25 µL), Bsm DNA polymerase large

fragment 1600 Units, 8 Units/ µL (0.5 µL), 100 mM MgSO4 (1 µL), 10 mM dNTP mix (1.75 µL), 10 µM PSR forward primer (1 µL), 10 µM PSR backward primer (1 µL), 5 M betaine (2 µL), template DNA (1.5 µL) and volume was made up to 12.5 µL using nuclease free water. All the chemicals, reagents and buffers were obtained from Thermo Fisher Scientific, Telangana state, India. The reaction mix was incubated at 62 °C for 60 min in a dry bath. After the PSR reaction was performed, 0.5 µL (1:10 of 10,000 X concentrate in Dimethyl sulfoxide) of SYBR™ Green I nucleic acid gel stain (Invitrogen, Thermo Fisher Scientific, India) was added for the visualization of presence of amplification.

Visual colour change

Validation of the PSR amplified products was done by visualization of the fluorescence developed. After the PSR reaction was performed, 0.5 µL (1:10 of 10,000 X concentrate in Dimethyl sulfoxide) of SYBR™ Green I nucleic acid gel stain (Invitrogen, Thermo Fisher Scientific, India) was added for the visualization of amplification. A positive reaction developed green fluorescence and a negative reaction remained orange in colour.

Fluorometric assay

The intensity of fluorescence developed is measured with the help of a fluorometer (DeNovix, Model: Ds-11 FX + Spectrophotometer/ Fluorometer). After visualisation of the colour developed in the negative and positive reactions, the amplified products were then employed to measure the intensity of colour developed. The fluorometer reading of each of the amplified products stained with SYBR Green I stain was performed using fluorometer (DeNovix, Model: Ds-11 FX + Spectrophotometer/ Fluorometer). The amplified products were diluted with 187.5 µL of nuclease free water to make the volume to 200 µL. The reading is taken for the diluted amplified products at Relative Fluorescence Units (RFU) 565-650 nm. The SYBR Green I stain was excited most at 497 nm wavelength and emits fluorescence with a peak at 520 nm wavelength.

Agarose Gel Electrophoresis

Finally, the PSR results were confirmed by agarose gel electrophoresis. The chemicals used for the agarose gel electrophoresis to check the quality of DNA.

Specificity of Psr Assay

By conducting PSR reactions with DNA from different animals *viz*., buffalo, cattle, chicken, pig, sheep and ostrich, the specificity of the chevon-specificity of PSR was evaluated. This test was repeated 3 times.

Sensitivity of PSR Assay

The DNA of goat meat was two-fold serially diluted with 1 X TE buffer to get various concentrations *viz*., 200, 100, 50, 25, 10, 5, 2.5 and 0.5 ng/µL, in order to determine the sensitivity of chevon-specific PSR. The PSR reaction was performed thrice for each of these DNA concentrations.

Validation of PSR Assay

Assessing heat-treated samples. Samples were prepared by first cooking the samples at 60 °C, 80 °C, 100 °C and 121 °C for 30 min in water bath (Model number: STXUWB14, Stericox company, India) followed by extraction of DNA. Later, PSR reaction was performed for each of these samples.

Detection of chevon in admixture meat samples

Admixture meat of different percentages (w/w) of chevon mixed with mutton was prepared as follows: 50%, 40%, 30%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.6%, 0.4%, 0.2% and 0.1% and DNA was extracted from these admixture meat samples. Then, PSR reaction was performed for each of the admixed samples to determine the level up to which the goat DNA can be detected in the admixed samples.

Statistical Analysis

The performance of chevon-specific PSR assay was assessed by determining sensitivity, specificity and accuracy. Each experiment was repeated thrice. The mean and standard error values were calculated using MS-Excel program (Microsoft Corporation). A bar graph was prepared in MS-Excel program (Microsoft Corporation) for the mean fluorometer values with error bars for standard error and R squared value.

Preperation of Binary Meat Mixtures

Binary meat mixtures of chevon mixed with mutton were prepared. Different proportions of chevon in mutton were made in various percentages as shown each mixture was made up to 500 mg and the samples were labelled from BM 1 to BM 13 accordingly. All the samples were stored at - 20 °C until further processing. Prior to processing the samples were thawed for 10-15 min and thoroughly mixed. Meat mixtures were subjected for DNA extraction using AL method. The DNA extracted from these samples BM 1 to BM 13 were used for detection of chevon in admixture meat. The lower limit was set up to 0.1% for this test.

Results and Discussion

The DNA was extracted by Alkaline lysis (AL) method as described by Girish *et al*. (2013) [10]. The quality of DNA samples was checked by performing agarose gel electrophoresis (0.8%) for 1 hr and the results were visualized in a gel documentation system. The DNA was extracted from different species *viz*., buffalo, cattle, chicken, pig, sheep, goat and ostrich. The extracted DNA quality was checked by agarose gel electrophoresis (0.8%). DNA extracted from binary meat mixtures as chevon is frequently combined with mutton, mutton was employed for binary meat mixes. Different proportions of meat mixtures of chevon and mutton were prepared with each mixture sample weighing 500 mg and labelled the binary meat mixture samples from BM 1 to BM 13. The binary meat mixture samples with different proportions of goat and sheep meat were then subjected to AL method of DNA extraction. The purity of the goatsheep binary meat mixtures was in the range of 1.6 to 2.0 at OD260/280. The quantity analysis was done for DNA samples that were having clear bands with minimal shearing and were considered for performing the PSR reaction. The concentrations that were in the range of 70 - 120 ng/µL were considered for performing the PSR test. The PSR procedure was effectively optimised for betaine and magnesium sulphate concentrations in the PSR reaction mix. Temperature and duration of the PSR assay was also optimised. The concentrations of betaine used for standardisation are from 4.2 M to 5 M. While, magnesium sulphate concentrations utilised for standardisation are in the range of 96 mm to 102 mM. Temperatures from 60 °C to 65 °C were considered for

optimisation. The standardisation was done based on colour change corroborated with fluorometric measurement and ladder pattern visualised on agarose gel electrophoresis. Each procedure was repeated thrice in order to avoid any errors. The change of colour was observed after addition of SYBR Green I nucleic acid gel stain (Invitrogen, Thermo Fisher Scientific, India) to the PSR amplified products after completion of the reaction. The fluorometer reading was measured in a fluorometer (DeNovix, Model: Ds-11 FX+ Spectrophotometer/fluorometer). The samples after observation of the visual colour change were diluted up to 200 µL with nuclease free water and the sample readings were noted at Relative Fluorescence Units (RFU) 565-650 nm. The results were further confirmed by agarose gel electrophoresis performed at 2% in 1X Tris-Acetate-Ethylenediamine tetra acetic acid (TAE) Buffer. The agarose gel electrophoresis (2%) was performed at 80 V for 1 hr. Betaine concentration Tubes 1 to 5 contained betaine concentrations of 4.2, 4.4, 4.6, 4.8 and 5 M respectively in the PSR reaction mix. The colour turned green in all the tubes from 1-5 containing different concentrations of betaine. The intensity of colour developed was similar in all the tubes with minimal variation. Additionally, the ladder pattern was observed for all the concentration of betaine on agarose gel electrophoresis. The ladder pattern was similar for all the concentrations of betaine. Based on the fluorometer reading the betaine concentration was standardised at 5 M for efficiency. Magnesium sulphate stabilises the primer template complexes and binds to dNTPs, primers and template DNA to increase product yield. Low yield and nonspecific binding can result from either too high or too low MgSO4 concentration.

In light of the fact that this is the first time a PSR assay has been performed to detect meat species origin, there is limited amount of information and resources available for comparison and discussion. Culture, religious beliefs and customs play an important role in preferences for particular type of meat and animal products worldwide [20-24]. Along with it, the globalization of the food industry and heightened consumer knowledge have raised awareness of the issue and drawbacks of food adulteration [23, 25]. Therefore, the information on the declaration, food safety, nutritional value and quality attributes has an impact on consumer satisfaction. Proper labelling is one of the significant variables influencing consumer choice. Therefore, there is a need for rapid and robust technique for meat species identification.

DNA-based molecular techniques are now the method of choice for identifying the species of meat throughout the world. Polymerase chain reaction (PCR)-based techniques are frequently used. However, a variety of DNA-based techniques have been created and applied for meat speciation. In recent years, the Polymerase spiral reaction assay (PSR) has been created to address some of the limitations of PCR and other isothermal amplification methods in terms of time, equipment and cost. In the current study, an attempt was made to develop and standardize specific PSR assay for chevon detection by employing a pair of PSR primers oriented to target the mitochondrial DNA (mtDNA). In addition, this technique combined with simple DNA extraction method *viz*., Alkaline lysis (AL) method is rapid and efficient in identification of presence of chevon in meat samples.

PCR is a DNA based method which is very commonly used due to higher specificity, sensitive, reliability and suitability. Various DNA amplification techniques for mutton/ sheep identification were used, such as Arbitrarily Primed (AP) PCR, DNA sequence analysis [8], Random Amplified Polymorphic DNA (RAPD) for finger printing [7], Restriction Fragment Length Polymorphism (RFLP) [6], Species-specific PCR [2, 10], duplex/multiplex PCR [9], Real-time PCR [12] and Touchdown-Duplex PCR assay [11]. The PCR based DNA amplification tools are even though conclusive in species identification, require costly thermal cycler and post-PCR signal visualization instruments, such as electrophoresis assembly and gel documentation system [13]. Isothermal amplification technique to detect goat meat was also reported by Loop mediated isothermal amplification (LAMP) technique [26]. In LAMP, there is a need of multiple primers and stringent optimization and also there is possibility of chance of leftover contamination leads to false-positive results [14].

In this study, DNA extraction was done using an AL protocol [27]. The AL method is cost-effective and takes about 20-30 min to complete the protocol. The AL method is more economical and less laborious when compared to Phenol: Chloroform: Isoamyl alcohol (PCI) and commercial kit methods [27]. The AL method has several advantages over the other DNA extraction methods because it is affordable, uses less expensive chemicals, including Sodium hydroxide and Tris-hydrochloride that are readily available in labs, takes less time and labour to complete, requires only a single tube and minimal pipetting, and prevents cross-contamination issues [27]. Therefore, chosen for this study. DNA was isolated from various meat samples of different meat animals *viz*., buffalo, cattle, chicken, pig, sheep and ostrich [13]. Similar to this study, DNA was extracted from raw/cooked mutton, pork and carabeef [13, 28, 29]. The DNA concentration for fresh raw goat meat samples using AL method was 113.39±21.77 ng/ µL while the concentration for mutton was in the range 110.73±14.40 and 169.77±11.19 ng/µL [13]. The DNA concentrations produced in this study are in accordance with DNA concentration values obtained for mutton by AL method [13]. When compared to goat meat, which has a lower fat content, sheep meat has a higher concentration of fat and marbling, which makes it more difficult to isolate DNA.

The result for mean DNA concentrations for various processed pork products extracted by AL method was 83.29 ng/µL [28]. However, the concentrations of extracted raw buffalo meat DNA was 93.89±54.21 ng/µL [29]. The concentrations for goat meat obtained in the present investigation were slightly higher in value compared to DNA concentrations obtained by AL method for pork [28] and carabeef [29]. This could be due to the multiple factors that affect DNA isolation such as successful removal of contaminants and environmental conditions (temperature, humidity, sun exposure). The DNA isolation by AL method for cooked chevon samples conducted in this study yielded concentration of 100.44±14.46 ng/µL. The concentration obtained in this study are in accordance with the study conducted for cooked pork samples 87.65 ng/µL [28] and for heat-treated carabeef samples 109.06±19.57 ng/µL [29]. The mean DNA concentrations was 144.623±12.60 ng/µL for heat-treated mutton samples in a study [13], which is higher in value compared to cooked chevon samples in the present study. The purity obtained at OD260/280 was 1.6 to 2.0 for raw and cooked chevon samples. Similar results were obtained in a study [13]. Purity obtained for raw and cooked mutton samples was in the range of 1.6-1.8 [13]. However, the

purity of raw/cooked buffalo meat samples was 1.4-1.5 [29] and raw/cooked pork samples was 1.6-1.9 [28] which are lower in purity compared to the purity obtained in the present study.

For the purpose of identifying the species of meat, mitochondrial genes, particularly cytb, 12S rRNA, 16S rRNA, and D loop region of mitochondrial DNA are frequently employed [30]. The mtDNA sequence is highly conserved in different species of animals [31]. In prior work, LAMP primers targeting the D loop region were created to identify goat-specific meat using the isothermal approach, LAMP [26]. Presently, there are no reports of the PSR technique used for meat species identification. As a result, primers were designed to specifically target mtDNA to identify chevon.

The amino acid analogue, betaine, which has both positive and negative charges and has a pH- Magnesium sulphate concentration was optimised at 1 µL of 100 mM for chevon-specific PSR reaction mix (12.5 µL) in the current study as it was found efficient to observe positive results in goat meat samples when compared to other concentrations. Whereas, the PSR mixture (25 µl) for detection *Salmonella* spp. in pork contained 2.5 µL of 8 mM Magnesium sulphate ($MgSO_4$) [32]. The current study contains higher concentration of MgSO4 compared to the study by Momin [32]. Similar to study [32], 2.5 µL of 6 mm MgSO4 is standardized in the reaction mix of Reverse Transcriptase - Polymerase Spiral Reaction (RT-PSR) to detect Chikungunya virus [33] and *Staphylococcus aureus* in meat contained 2.5 µL of 4 mM $MgSO_4$ [34].

The amplification temperature is affected by nucleotide content of primers, target and polymerase enzyme present in the reaction [13]. The standardized goat-specific PSR assay was performed at 62 °C for 60 min. The amplification temperature 62 °C standardized in this study is lower than the amplification temperature 63.5 °C standardized for goat meat identification by LAMP method [26]. Similarly, the amplification temperature standardized by to detect *Salmonella* spp. in pork was 64 °C [32] and to diagnose *S. aureus* was 66 °C [34] employing PSR were higher than that of goat specific PSR in the current study.

The detection time was 60 min for PSR to detect *Salmonella* spp. [32] and *S. aureus* [34] similar to the detect time 60 min employed in the current study for detection of chevon. Whereas, the detection time for goat specific LAMP assay was faster (30 min) [26].

The specificity test was performed with buffalo, cattle, chicken, pig, sheep and ostrich in the current study. Ostrich meat, which was earlier infrequent but is now becoming more popular due to its high nutritive value, and it presents a few similarities to goat meat. Ostrich meat is similar to goat meat in terms of its dark red cherry colour, myoglobin content, ability to hold water and collagen content [35] but there was no evidence of cross-amplification with ostrich meat in this study. Further, the cross- reactivity for LAMP assay for goat meat in raw and cooked meat was performed with cattle, pig, horse, sheep, chicken, duck and turkey [26]. To eliminate cross-amplification with goat-specific primers in duplex PCR assay, specificity test with DNA extracted from sheep and buffalo meats was analysed [9].

The Limit of Detection (LOD) is the lowest quantity or concentration of a component that can be reliably detected with a given analytical method. The LOD of the chevon-specific PSR was 0.5 ng/µL in the present study. Similar sensitivity of detection (LOD – 0.5 ng/µL) by LAMP assay was also observed for beef [29], pork [28] and mutton [13] whereas sensitivity was 1 pg for goat specific LAMP assay [26]. Also, PSR to diagnose parvoviral DNA had a detection limit of 5 x 10-6 ng [19].

A recent study reported that 11 (52.37%) of the 21 mutton-labelled goods were found to be mislabelled and ten items (47.6%) displayed 99-100% identity to *Capra hircus* (goat) [36]. Thereby, highlighting the use of goat meat to adulterate mutton. When evaluated utilizing meat mixtures, the LOD reported using extracted template DNA varies [37] because the template is used as input DNA weight when estimating LOD using target DNA. For meat mixtures, however, variables like DNA extraction effectiveness and the proportion of meat weight to DNA weight in various species may impede any such direct comparison. Additionally, it was discovered in the investigation[11] that the concentration of DNA increased with an increase in goat meat compared to sheep meat in the current study. The tissue breakdown may influence by meat structure, and sheep have more fat than goats, which may also be an impediment [38]. In the LAMP assay the goat meat was detected up to 0.001% level in goatsheep admixtures [26], duplex PCR assay was able to detect the presence of chevon up to 1% level in goat-sheep admixture meat [9] and touchdown duplex PCR, detected up to 10% of goat DNA in the analysed meat mixture percentages [11]. Whereas, in the present study by employing PSR assay the LOD for detection of goat meat in goat-sheep binary meat mixtures detected up to 0.1%.

Results from the present study indicated that a combination of the AL method of DNA extraction and PSR technique targeting mtDNA can aid in rapid and efficient identification of goat meat in both fresh and processed meats. AL method requires only a water or dry bath and easily available chemicals for DNA extraction. PSR amplification was undertaken at constant temperature which precludes the requirement of expensive instruments such as a thermocycler for the amplification of the target gene. Results of the reaction can be interpreted by colour change with addition of SYBR green dye which obviates the requirement of electrophoresis unit and gel documentation system. Overall, PSR method is a quick and economical technique for species identification of chevon which can be undertaken with minimum instrumentation which makes the technique suitable at both laboratory and field level for detection of presence of chevon in meat samples.

Conclusion

The present study elucidates a novel chevon-specific PSR assay that can detect chevon in not only raw meat but also in cooked and adulterated meat. The PSR assay is standardised at 62 °C for 60 min, including optimization for betaine concentration at 5M, magnesium sulphate concentration at 100 mM at a total volume of the PSR reaction mix of 12.5 µL. The extraction of DNA is followed by PSR assay and the amplified product, after addition of SYBR Green I stain to product, are detected visually

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