GLOBAL ACADEMIC RESEARCH INSTITUTE

COLOMBO, SRI LANKA

GARI International Journal of Multidisciplinary Research

ISSN 2659-2193

Volume: 06 | Issue: 03

On 30th September 2020

http://www.research.lk

Author: De Silva J. L. I. M

(Business Management School, Sri Lanka)

GARI Publisher | Microbiology | Volume: 06 | Issue: 03

Article ID: IN/GARI/ICID/2020/102 | Pages: 31 - 42 (12)

ISSN 2659-2193 | ISBN 978-955-7153-02-5

Edit: GARI Editorial Team | Received: 20.08.2020 | Publish: 30.09.2020

DEVELOPMENT AND VALIDATION OF A RAPID TEST METHOD FOR DETECTION OF PORK MEAT WITH PROCESSED MEAT PRODUCTS

De Silva J. L. I. M

Department of Biotechnology, Business Management School, Sri Lanka

jlishinidesilva1997@gmail.com

ABSTRACT

Meat is one of the major dietary components to human and it mainly provides nutrients such as proteins, amino acids, unsaturated lipids, microelements, vitamins and minerals. Pork adulteration in beef is mainly found in Japan, Korea and china and so on because beef meat is expensive and pork is cheap. This fictitious case not only harms for the allergic consumers but also religious behaviors (Muslims and Jews). Therefore detection of pork adulteration in processed meat products is important. In this project, DNA based PCR method was used because DNA structure is more stable and highly rationed allowing identification and discrimination of species not only in raw meat but also in processed meat samples. Repeatability was performed ten times for the same Lingus sample for detects the proximity of the agreement between the results of successive measurements and it was 100% accurate. Secondly, Recovery was performed for the concentrations of 0%, 0.1%, 0.5%, 1%, 5% and 10% for pork sausage and beef sausage mixtures and PCR was followed using bovine and porcine species specific oligonucleotide primers. DNA was extracted using DNeasy Mericon Food Kit, Qiagen (Germany) and limit of detection 5% pork concentration. PCR products of extracted DNA were subjected to the simplex and duplex polymerase chain reaction (PCR) using porcine species specific oligonucleotide primers and bovine species specific oligonucleotide primers respectively. Finally, detect the pork adulteration in processed meat products available in local market targeting the 289bp porcine and 251bp bovine mitochondrial DNA.

Keywords: bovine, porcine, simplex PCR, duplex PCR, adulteration, repeatability, recovery.

INTRODUCTION

Nowadays, food adulteration is major problem in the world and which began in 13th century A.D. at Florence in Italy with wine was adulterated by sugar to get sweet taste (Bhat et al., 2016). Adulteration of food can define as adding extraneous substances to food products and it reduces the essential nutrients from food (Aysa and Belete, 2015). This adulteration process occurs for various types of reasons for example, financial gain, enhancing taste, carelessness and lack in proper hygienic condition of processing, storing, transportation and selling (Sharma et al., 2017). For example, pork adulteration in beef is mainly found in Japan, Korea and China and so on because beef meat is very expensive and pork is very cheap in those countries (Ha et al., 2017) and also Pork is often added to other processed meat products because pork has a color and texture similar to beef and lamb. Different

type of food adulteration percentages can be varied according to the demand of consumers and profits of manufacturers and adulteration of meat has been increased.

In Sri Lanka, farm pork is adulterated by adding wild boar which is which is restricted by the Fauna and Flora Ordinance in Sri Lanka. (Samaraweere et al., 2011). Identifying the pork adulteration of processed meat products is one of the most important food- quality issue because of it will be allergenic and increased the level of risk of colon cancer for some consumers and as well as some group of people does not consume pork because of religious food ethics and preference (Ha et al., 2017). For example, many Hindus do not eat beef, Islam and Jews prohibited the pork and the halal authentication is credited to purity of meat by Islam and Kosher by Jews (Yang et al., 2018). The relative investigation of the real example examines in the laboratory and the label unveiled in the package of chicken burgers sold in the state of Kuwait uncovered a dissention of labeling. (Bourguiba-Hachemi and Fathallah, 2016). And also pork adulterations in halal beef burgers were discovered in some European countries (Yang et al., 2018). Those types of counterfeit cases are testified by European criminal police organization (EUPOL) and international criminal police organization (ENTERPOL) and they have confirmed in monitoring food security, resulting in 2500-ton illegal and fictitious food (EUROPOL, 2015).

There are various types of analytical methods that have been utilized to detect pork adulteration in processed meat products. For example, high-performance liquid chromatography, enzyme-linked immunosorbent assays, Fourier transform infrared (FTIR) and electrophoretic techniques are used as protein based methods (Al-Kahtani et al., 2016). But in recent years, most attention has been turned towards DNA-based analytical methods as it is very reliable, sensitive and rapid. DNA analysis is more applicable than protein analysis because proteins lose their biological activities after animal death and denature the proteins during processed (Calvo, Osta and Zaragoza, 2002). DNA analysis can be detected through polymerase chain reaction (PCR) such as duplex droplet PCR, PCR- RFLP, real time PCR, or species specific PCR (Tanabe et al., 2007). Mitochondrial DNA is a good target for phylogenetic reconstruction at various taxonomic levels. There are 1000 of copies and therefore mitochondrial DNA has high variability compared to nuclear DNA. This allows the differentiation of closely related species. Cytochrome b is a functional gene which located between mitochondria and it used for molecular marker in detection of pork adulteration. This gene contains species specific information and it has been used in an extensive number of studies on phylogenic and dealing with forensic science and food inspection (Hassan and Tauma, 2014).

Main aim of this research is detection and validation of pork adulteration in commercially available processed meat products in local markets. Those processed foods are determined by DNA extraction using DNeasy Mericon food kit, Qiagen. It extracts total nucleic acids from range of food sample types (Sample and Assay Technologies, 2014). An extracted DNA is amplified using conventional PCR method because it is simple, rapid and specific nucleic acid amplification method for the identification of pork species. Duplex PCR is amplifying two different DNA sequences at one PCR reaction (Tang, 2009). Therefore it can distinguish pork which adulterated in other processed meat items. Finally, gel electrophoresis is used to visualize the amplified PCR product for the detection of pork adulteration.

Method validation is used for take clear and high sensitive results of meat samples and confirm the reliability of a method that is resolve by validation results, limit of detection and sensitivity are reported for recognize the least amount of DNA that the primers will be sensitive for (Ozkan, 2018). In this project, repeatability is performed for the Lingus sample which contains both pork and beef meat to validate the method. Recovery for pork DNA will be evaluated at a concentration of 0%, 0.1%, 0.5%, 1%, 5% and 10% for pork beef mixtures using conventional PCR for detection of limit of detection (LOD) of pork.

METHODOLOGY

Collection of samples

Positive meat samples (Pork, beef) and processed meat samples were purchased from the Arpico super center, Hide Park. Those products stored under frozen condition -18±1 OC to prevent from enzymatic degradation of DNA in meat samples.

DNA Extraction

Repeatability

Genomic DNA was extracted from 200mg of Lingus using DNeasy Mericon food kit, Qiagen (German) (Ref No: 69514) and some steps were optimized to get high yield of DNA. Firstly, Lingus sample was homogenized using sterile mortar and pestle. Then 200mg of homogenized meat samples were transferred in to 10 numbers of 2ml micro centrifuge tubes and added 1ml food lysis buffer and 5µl Proteinase K solution. That sample was vortexed for 30s. After that samples were incubated in the shaking incubator GFL for 30 minutes at 60oC with constant shaking with 180rpm and after the incubation kept on ice for 5 minutes. After incubation, the sample was centrifuged for 5 minutes at 7500 x g. 500 µl of chloroform was pippeted to another fresh 2 ml eppendorf tubes. Next 700 µl of supernatant was pippeted out and added to the tube which containing chloroform. Again the samples were vortexed for 15s and centrifuged at 14,000 x g for 15 minutes. 1ml of PB buffer was added into a fresh 2ml micro centrifuge tubes (10X) and 250 µl of upper aqueous phase was added to the tubes containing buffer PB and vortexed thoroughly. Then 600 µl of the mixture was added into QIAquick spin columns and placed in a 2ml collection tube. And sample was centrifuged at 17,900 x g for 1 minute and discarded flow-through. Again the same step was repeated and discarded the flow through. Then 500 µl AW2 buffer was added to the QIAquick spin column and centrifuged at 17,900 x g for 1 minute and discarded the flow through. Again sample was centrifuged at same conditions to dry the membrane. Finally spin column was transferred to a 1.5ml eppedorf tube and pippeted 100µl of EB buffer and incubated for 1 minute at room temperature and the samples were centrifuged at 17,900 x g for 1 minute to elute.

Recovery

Beef and Pork Sausages were Homogenized separately using mortar and pestle and measured precisely using an analytical balance to get the following beef pork concentrations (Table 1).

Table 1. *Pork beef concentrations*

	Pork	**Beef**
A	0g (0%)	10g (100%)
B	0.01g (0.1%)	9.99g (99.9%)
C	0.05g (0.5%)	9.95g (99.5%)
D	0.1g (1%)	9.9g (99%)
E	0.5g (5%)	9.5g (95%)
F	1g (10%)	9g (90%)

After the measuring of pork sausage and beef sausage samples, mixed to get 0%, 0.1%, 0.5%, 1%, 5%,and 10% pork, beef sausage mixtures and homogenized into a fine paste using separate mortar and pestle (figure 1).

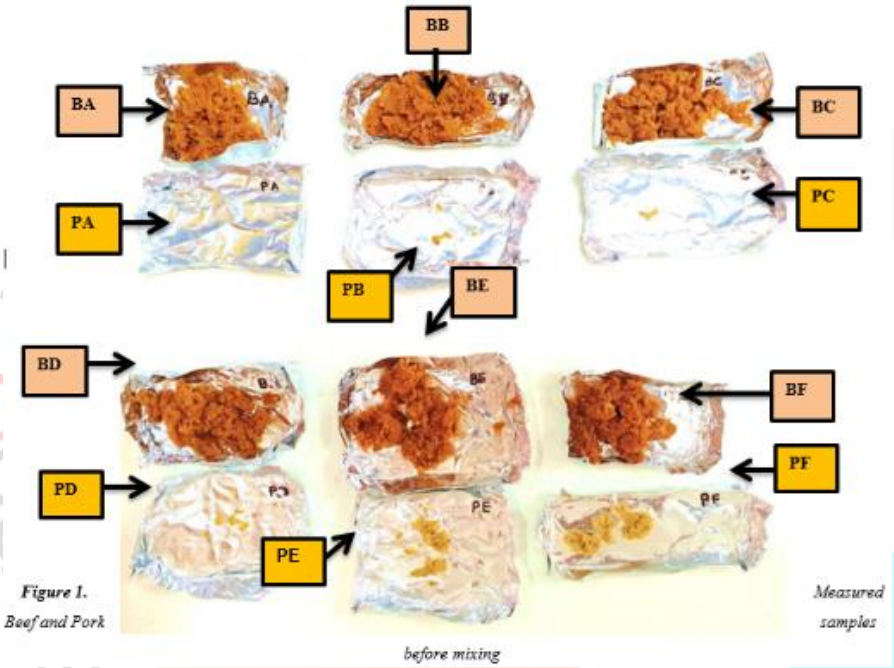

Figure 1. Beef and Pork Measured samples before mixing

BA + PA – 100% of beef sausage sample + 0% Pork sausage sample, BB + PB – 99.9% beef sausage sample + 0.1% pork sausage sample, BC + PC – 99.5% beef sausage sample + 0.5% pork sausage sample, BD + PD – 99% beef sausage sample + 1% pork sausage sample, BE + PE – 95% beef sausage sample + 5% pork sausage sample, BF + PF – 90% beef sausage sample + 10% pork sausage sample.

Those measured samples were extracted according to the 200mg small fragment protocol of DNeasy mericon food kit with some modification, same for the repeatability (Table 2).

Table 2. *DNA extraction protocol for recovery (DNeasy Mericon Food Handbook, 2014) (Adapted).*

Steps	Original Protocol	Optimized steps
Homogenized using sterile mortar and pestle	-	0.5µl of food lysis buffer
Homogenized sample taken into 2ml micro centrifuge tube	200mg	200mg
Volume of food lysis buffer and proteinase K added	1ml of food lysis buffer and 2.5µl of proteinase K	5µl of proteinase K
vortex	-	30 seconds
Incubation	For 30 min at 60^0C with shaking incubator at 1000rpm	For 30 min at 60^0C with GFL incubator at 180rpm
Cool the samples to room temperature (25^0C)	-	5 minutes
Centrifugation	For 5 min at 25,000xg	For 5mmin at 7500xg
Volume of chloroform added into fresh 2m micro centrifuge tube	500 µl of chloroform	500 µl of chloroform
Volume of supernatant transferred into 2ml micro centrifuge tube	700 µl supernatant	700 µl supernatant
vortex	15 seconds	15 seconds
Centrifugation	For 15 minutes at 14000xg	For 15 minutes at 14000xg
Volume of PB buffer added into the fresh micro centrifuge tube	1ml of PB buffer	1ml of PB buffer
Volume of upper aqueous phase transferred into the 2ml micro centrifuge tube	250 µl of aqueous phase	250 µl of aqueous phase
Volume of solution added into QIAquick spin column	600 µl of solution	600 µl of solution
Centrifugation	For 1 minute at 17,900xg	For 1 minute at 17,900xg
Discard flow-through	-	-
Repeat above 3 steps for remaining 600 µl of samples	-	-

Volume of AW_2 added into QIAquick spin column	500 µl of AW_2	500 µl of AW_2
Centrifugation	For 1 minute at 17,900xg	For 1 minute at 17,900xg
Discard flow-through	-	-
Centrifugation	For 1 minute at 17,900xg	For 1 minute at 17,900xg
Volume of buffer EB added to QIAquick spin column	100 µl	100 µl
Incubate	1 minute at room temperature	1 minute at room temperature
Centrifugation	1 minute at 17,900xg	1 minute at 17,900xg

DNA extraction from processed meat samples

Each processed meat samples were homogenized into a fine paste using separate mortar and pestle and DNA was extracted from 200mg small fragment protocol by DNeasy Mericon food kit, Qiagen but some steps were changed to get more DNA yield same as the recovery(Table 2).

DNA Confirmation using spot test

Spot test is performed to confirm the presence of DNA. 0.1mg of agarose powder was measured and dissolved in 10ml of TAE buffer (25X) in a conical flask. After dissolving the agarose completely 1µl of ethidium bromide (0.5µg/ml) was added to the solution. That solution was poured to petri dish and kept to set. 1% of agarose gel piece was cut and placed on the tray. Then 1 µl extracted DNA samples were placed on the gel. DNA confirmation was visualized from Image Lab Software with the gel documentation system.

PCR Assay

The extracted DNA was subjected to PCR amplification using duplex PCR technique for adulteration detection.

Repeatability

The PCR amplification was performed in a final reaction volume of 15µl 4.4µl of PCR water, 7.5 µl of Ceygen master mixture (2X), 0.4 µl of each primers (10µM), 0.5 µl of Taq (1U/µl) solution and 1 µl of DNA samples of Lingus were added respectively to the 10 PCR tube (Table 3).

Table 3. *The components of the PCR master mixture in repeatability*

PCR Mixture	Reaction volume (1x)
Ceygen master mixture(2X)	7.5µl
Pork F_2 (10 µM)	0.4 µl
Pork R_2 (10 µM)	0.4 µl
Bovine F (10 µM)	0.4 µl
Bovine R (10 µM)	0.4 µl
Taq (1U/µl)	0.5 µl
DNA sample	1.0 µl
PCR water	4.4 µl
Total Volume	**15 µl**

Recovery

Simplex PCR

The extracted DNA pork sausage sample was subjected to simplex PCR. In this step, porcine species specific forward and reverse oligonucleotide primers were used (Table 4).

PCR Mixture	Reaction volume (1x)
Ceygen master mixture (2x)	7.5µl
Pork F_2 (10 µM)	0.7 µl
Pork R_2 (10 µM)	0.7 µl
Taq (1U/µl)	0.5 µl
DNA sample	1.0 µl
PCR water	4.6 µl
Total Volume	**15 µl**

Duplex PCR

The extracted DNA (beef sausage & pork sausage) was subjected to duplex PCR. In this step, porcine and bovine both species specific forward and reverse oligonucleotide primers were used as in table 3.

Detection of pork adulteration in processed meat samples

Table 5. *The components of the duplex PCR of detection in processed meat samples*

PCR Mixture	Reaction volume (1x)
5xFIREPOL® Master Mixture	4.0µl
Pork F_2 (10 µM)	0.6 µl
Pork R_2 (10 µM)	0.6 µl
Bovine F_2 (10 µM)	0.6 µl
Bovine R_2 (10 µM)	0.6 µl
DNA sample	1.0 µl
PCR water	7.6 µl
Total Volume	**15 µl**

The amplification conditions for PCR for cyt b gene were given (Table 6).

Table 6. *The amplification conditions for bovine and porcine specific duplex and simplex PCR*

PCR Condition	Temperature	Time	
Initial denaturation	94°C	5 min	
Denaturation	94°C	30 sec	35 cycles
Annealing	59°C	30 sec	
Extension	72°C	60 sec	
Final extension	72°C	5 min	

Agarose Gel Electrophoresis

Gel electrophoresis used to separate DNA and proteins according to their size and charge.

The PCR products of repeatability, recovery and the detection of pork in processed meat sample were analyzed using 1.5% agarose gel electrophoresis techniques and visualized under UV light using the Bio Rad molecular imager gel documentary system to validate the method and detect the pork adulteration respectively.

To prepare 1.5% agarose gel, 1.5g of agarose powder was measured and 100ml of TAE buffer was added to the conical flask. To the melted agarose solution 2.0µl of ethidium bromide (0.5µg/ml) was added. Next the comb was placed on the gel cassette. Prepared solution was poured to the casting tray and left it solidifies and after few minutes comb was removed from gel without damaging wells. All the samples were mixed with the 1 µl gel loading dye (6X) and loaded into the wells according to the below table. (Table 9)

Table 9, Taken Samples and volumes of gel electrophoresis

Well number	Samples	Volumes
1	100bp ladder	4.0 µl
2	Positive sample	2.5 µl
3	PCR Products of the lingus sample	3 µl
4		3 µl
5		3 µl
6		3 µl
7		3 µl
8		3 µl
9		3 µl
10		3 µl
11		3 µl
12	Negative sample	3 µl

Results

DNA quantification using spot gel test.

Spot gel of DNA extracted from the beef and pork mixture in repeatability (figure 2).

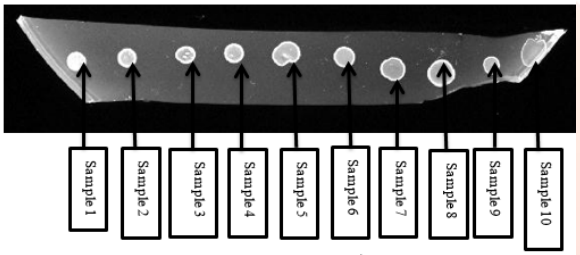

Figure 2. *Spot gel of DNA extracted from Lingus for repeatability.*

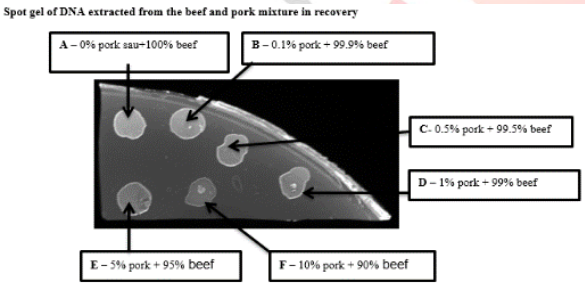

Figure 3: *Spot gel of recovery*

Spot gels of DNA extracted from processed meat products

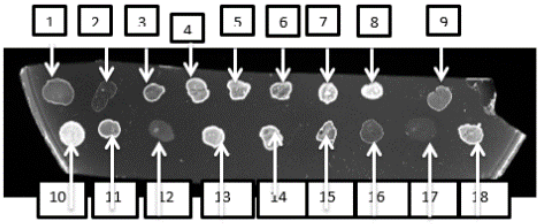

Figure 4. *Spot gel image of extracted DNA from processed meat product.*

1 - Lingus, 2 – Lingus outer cover 1, 3 – Lingus outer cover 2, 4 – pork sausage 1, 5 – pork sausage 1 outer cover, 6 – beef sausage, 7 – Chicken sausage 1, 8 – chicken ham 1, 9 – chicken sausage 2, 10 – chicken sausage 3, 11 – chicken meat bal 1, 12 – corned mutton, 13 – chicken ham 2, 14 – chicken meat ball 2, 15 – pork sausage 2, 16 – pork sausage 2 outer cover, 17 – chicken spread, 18 – fish sausage.

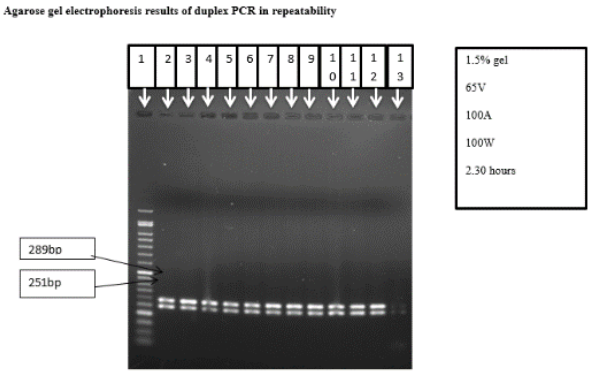

Figure 5. *Agarose gel image of PCR samples from beef pork mixtures in repeatability*

Lane 1- 100bp ladder, Lane 2 – beef and pork positive, Lane 3,4,5,6,7,8,9,10,11,12 – Lingus samples, Lane 13 – Negative sample (PCR water)

Agarose gel electrophoresis results of simplex PCR in recovery

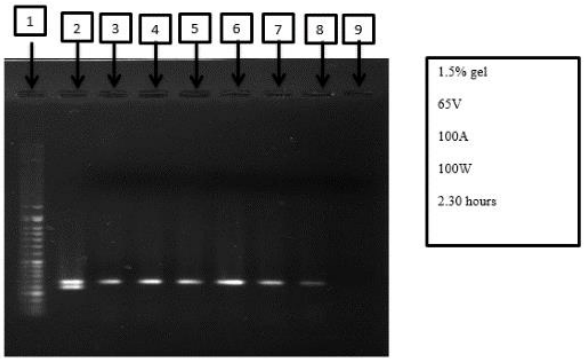

Figure 6. Agarose gel image of extracted DNA from beef pork mixtures in recovery

Lane 1- 100bp ladder, Lane 2- positive sample (pork + beef), Lane 3- 0.1% pork sausage sample, Lane 4- 0.5% pork sausage sample, Lane 5- 1% pork sausage sample, Lane 6- 5% pork sausage sample, Lane 7- 10% pork sausage sample, Lane 8- 100% beef sausage sample, Lane 9- negative sample (PCR water)

Agarose gel electrophoresis results of duplex PCR of recovery

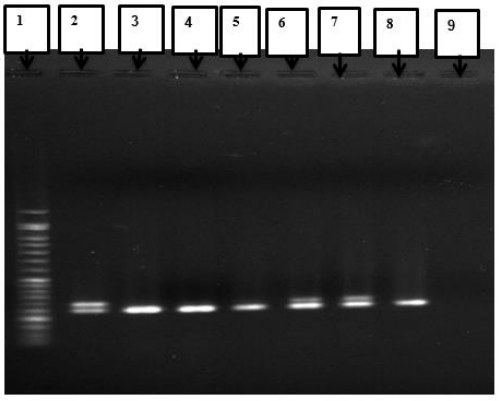

Figure 7. Agarose gel image of extracted DNA from beef pork mixtures in recovery

Lane 1- 100bp ladder, Lane 2- positive sample (pork + beef), Lane 3- 0.1% pork sausage sample, Lane 4- 0.5% pork sausage sample, Lane 5- 1% pork sausage sample, Lane 6- 5% pork sausage sample, Lane 7- 10% pork sausage sample, Lane 8- 100% beef sausage sample, Lane 9- negative sample (PCR water)

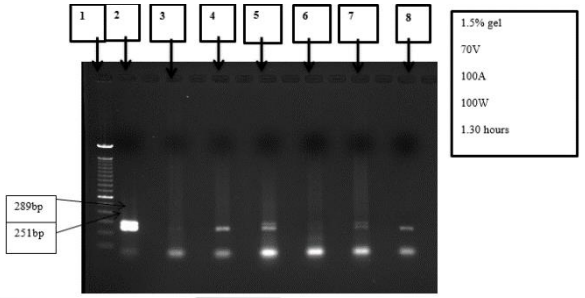

Figure 8. Agarose gel image of DNA extracted from processed meat products

Lane 1- 100bp ladder, Lane 2- positive sample (Pork + beef), Lane 3- Chicken sausage 1, Lane 4- chicken ham 1, Lane 5- Chicken ham 2, Lane 6- chicken sausage 2, Lane 7- Chicken meat ball, Lane 8- Negative sample (PCR water)

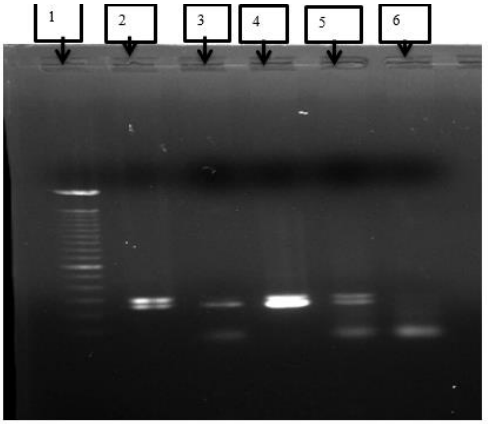

Figure 9. *Agarose gel image of DNA extracted from processed meat products*

Lane 1- 100bp ladder, Lane 2- Positive sample (Pork +beef), Lane 3- Chicken sausage, Lane 4- Minced mutton, Lane 5- Chicken ham, Lane 6- Negative sample (PCR water)

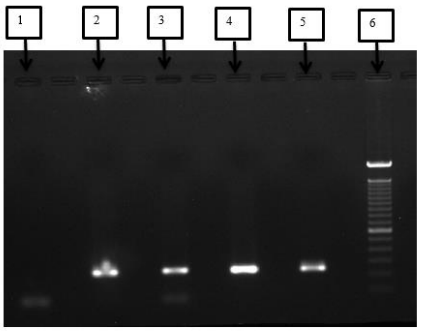

Figure 10. *Agarose gel image of DNA extracted from processed meat products*

Lane 1- Negative sample (PCR water), Lane 2- Lingus, Lane 3- fish sausage, Lane 4- beef sausage, Lane 5-positive sample (pork+beef), Lane 6- 100bp ladder

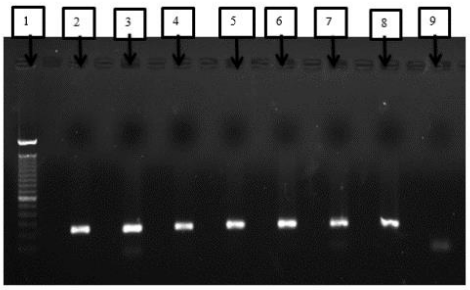

Figure *11. Agarose gel image of DNA extracted from processed meat products*

Lane 1-100bp ladder, Lane 2-Positive sample (pork+beef), Lane 3-lingus, Lane 4-Lingus outer cover1, Lane 5-Lingus outer cover2, Lane 6-pork sausage, Lane 7-pork sausage outer cover, Lane 8-Negative sample (PCR water)

DISCUSSION

Food adulteration is a main issue for many years in processed meat products. Pork meat is often mixed with other meat products intentionally, as well as unintentionally. Pork meat is very cheap; therefore it mixed with other meats like beef (Ha et al, 2017). When discussed about unintentional methods that occur in pork adulteration, main one is unclean grinding and cutting machines utilization. The main aim of the first part of the project (repeatability) is the proximity of the agreement between the results of successive measurements of the same measured carried out under the same condition of measurement (Barry, Taylor and Kuyatt, 2010). The second part of the project is aimed at primer sensitivity consideration of porcine oligonucleotide species specific primers. Recovery is done for recognize the least amount of pork DNA that the primers will be sensitive for (Ozkan, 2018). As a final, detect the pork adulteration in processed meat samples

In repeatability, Lingus were used because it has included both beef and pork meats in the ingredients list. DNA was extracted from the DNeasy Mericon food kit, Qiagen, 200mg small fragment protocol with some modifications. It is used for the processed meat products because it is easy to extract total number of DNA from small scale and processed meat products are highly fragmented because those samples has been subjected to extensive thermal treatments, Irradiation, Drying, high pressure and pH changes. And also this kit is used modified cetyltrimethylammonium bromide (CTAB) extraction (DNeasy Mericon Food Handbook, 2014).

In the extraction, all samples were homogenized using mortar and pestle and during the homogenization, food lysis buffer was added for even homogenization of the sample. After that, food lysis buffer was added to break down the cell membrane and proteinase K was added to digest the proteins. The samples were incubated at 600C with constant shaking incubator for enhance the cell disruption by food lysis buffer and enhance the activity of proteinase K. After the incubation, samples were cooled on ice to enhance the inhibitor precipitation. Centrifugation takes place to separate the layers and it was done at the room temperature by spinning the sample at

high speed, the components in the mixture are subjected to centrifugal force. In that process, inhibitors are precipitated (pelleted) and extracted DNA remains in the solution called supernatant liquid (Majekodunmi, 2015). Addition of chloroform is supported to separate the phases furthermore, called aqueous phase and organic phase. Aqueous phase contains DNA and small amount of inhibitors and organic phase contains proteins, lipophilic inhibitors and cell debris. As a trouble shooting, chloroform as an organic solvent started to leak from the pipette tip. By calibrating the pipette by pipetting up and down in the solvent repeatedly before transferring was helped to avoid it. Buffer PB (binding buffer) is added to clean up the DNA and bind DNA to the silica membrane in the QIAquick spin column. QIAquick spin column is a silica membrane which binding on the column and optimized reagent amounts. This allows a high yield of short DNA fragment to be extracted from the samples. Buffer AW2 is a wash buffer and it contains 70% ethanol to wash the salts in DNA. Buffer EB (elution buffer) is added to release pure DNA from silica membrane and store DNA for after use (DNeasy Mericon Food Handbook, 2014).

After the extraction of DNA, a spot gel test was performed to visualize the strength of the extracted DNA and it provide comparison between each extracted DNA to get some idea about how much DNA has been extracted (Dar et al., 2016). According to the figure 3 to 5, bright spots can be visualized and which indicates large amount of DNA, proteins and RNA. Dark spots also visualized and those spots contain least amount of DNA, proteins and RNA (Figure 3 -5).

The PCR products of DNA samples were subjected to agarose gel electrophoresis to prevail the results and those products were run on a 1.5% agarose gel because of good and clear separation of the bands. Porcine species specific oligonucleotide is 289bp and bovine species specific oligonucleotide is 251bp so both species has close base pair length and band separation is very important to take a good results.

Measurements of repeatability refer to the difference in repeat measurements which made on the same subject under same conditions same instrument method, same observer, and the same measurement (Bartlett and Frost, 2008). In repeatability, DNA extraction, PCR, gel electrophoresis was repeated by the same person under the same condition and according to the same procedure for 10 times and results were 100% accurate because both pork and beef bands were line up with correct base pair lane. In primer sensitivity (Recovery), duplex and simplex PCR were used to get clear understanding about samples and duplex PCR product was used as positive sample for gel electrophoresis of simplex PCR for detect the contaminations of primers. However porcine species specific oligonucleotide primer was sensitive to 5% of porcine DNA and 0.1% and 0.5% porcine DNA is not sensitive for adulteration detection.

According to the figure 8, Chicken sausage1, 2 and chicken ham 1 have slight band with beef but not adulterated with pork but chicken ham 2 and chicken meat balls have been adulterated with both pork and beef DNA. In that case PCR water was used as the negative and it also contaminated with the beef primers. According to the figure 9, Chicken sausage, Minced mutton and chicken ham were adulterated with both pork and beef DNA but chicken sausage has very slight band of pork. Negative sample was not contaminated with any DNA or primers and there have been accumulated the primer dimers on the bottom which not utilized. According to the figure 10, Lingus has both pork, beef and pork has slight band of DNA but it was not adulterated because the manufactures have been mentioned on the label that has both

pork and beef meat. Beef sausage was not adulterated with pork and it performed only beef band. However, those adulterated samples were not mentioned in labels. This pork adulteration could be both intentional and unintentional. And slight bands might be performing according to the unintentional adulteration and sharp bands might be performing according to the intentional adulteration. When some gel electrophoresis, Band were taken dumbbell shape due to the high current and voltage. According to the figure 11, there was main issue with pork forward and reverse primers, so pork bands were not observed on the gel. Therefore it can be concluded that pork primers are degraded.

CONCLUSION

DNA of processed meat samples were extracted by DNeasy Mericon Food kit, Qiagen. According to the above results, minimum detection level of pork sausage is 5%. In repeatability, DNA extraction, PCR, gel electrophoresis was repeated for 10 times and results were 100% accurate because both pork and beef bands were line up with correct base pair lane. Pork adulteration in processed meat products were detected in chicken ham, chicken meat balls, chicken sausage and some chicken hams, chicken sausages, chicken meat balls have been adulterated with beef. Beef sausage was not adulterated with pork and it pure beef. Therefore according to validated results, the above mentioned conventional PCR was a cost effective, efficient and reliable method for beef pork adulteration detection in highly processed meat products.

REFERENCE

Abdul-Hassan, L.A. and Tauma, J.A. (2014) 'Identification of some meat species Using PCR and multiplex PCR of mitochondria Cytochrome B Gene', 9-1(1), [Online] Available at: https://www.iasj.net/iasj?func=fulltext&aId=106229 (Accessed: 20 October 2018).

Calvo, J.H, Osta, R and Zaragoza, P. (2002) 'Quantitative PCR detection of pork in raw heated ground beef and pate', Agriculture and food chemistry, 50(19), pp. 5265-5267, [Online] Available at: https://pubs.acs.org/doi/pdf/10.1021/jf0201576 (Accessed: 30 November 2018).

Daily mirror, (2018) 'Ban on transporting, selling wild boar meat to be lifted', [Online] Available at: http://www.dailymirror.lk/article/Ban-on-transporting-selling-wild-boar-meat-to-be-lifted-155787.html (Accessed: 29 November 2018).

Dar, A., Shafique, U., Anwar, J., Zaman, W.U. and Naseer, A. (2016) 'A simple spot test quantification method to determine formaldehyde in aqueous samples', Journal of Saudi Chemical Society, 20(1), p. 352-356 [Online] Available at: https://www.sciencedirect.com/science/article/pii/S1319610312001913 (Accessed: 15 November, 2018).

Doosthi, A., Dehkordi, P.G. and Rahimi, E (2011) 'Moleculer assay to fraud identification of meat products'. Journal of food science and technology, 51(1), pp.148-152 [ONLINE] DOI: 10.1007/s13197-011-0456-3

EUROPOl. (2015) 'Record seizure of fake food and drink in Interpol-europol operation', [Online] Available at: https://www.europol.europa.eu/newsroom/news/record-seizures-of-fake-food-and-drink-in-interpol-europol-operation (Accessed: 28 November 2018).

Ha, J., Kim, S., Lee,J., Lee, S., Lee, H., Choi, Y., Oh, H and Yoon, Y. (2018) 'Identification of pork adulteration in processed meat products using the developed mitochondrial DNA-Based primers', NCBI, 37(3), pp. 464-468. [Online] Available at: https://www.ncbi.nlm.nih.gov/pmc/a

rticles/PMC5516074/ (Accessed: 27 November 2018).

Kahtani, H. A., Ismail, E. A., & Asif Ahmed, M. (2017). Pork detection in binary meat mixtures and some commercial food products using conventional and real-time PCR techniques. Food Chemistry, 219, 54–60. doi:10.1016/j.foodchem.2016.09.108

Majekodunmi, S.O. (2015) 'A Review on Centrifugation in the Pharmaceutical Industry', American Journal of Biomedical Engineering, 5(2), pp. 67-78, [Online] Available at: http://www.sapub.org/global/showpaperpdf.aspx?doi=10.5923/j.ajbe.20150502.03 (Accessed : 21 February 2019)

Ozkan, S.A. (2018) 'Analytical Method Validation Pharmaceuticals Analysis', Pharmaceutical Sciences, 24, p.1-2. [Online] Available at: https://journals.tbzmed.ac.ir/PHARM/Manuscript/PHARM-24-1.pdf (Accessed: 8 October 2018).

Samaraweera,M.,Himali, S. M.C.,Zeng, S.C.,Jianlin, H.and Silva, P.(2011) 'Development of Molecular Tools to Differentiate Sri Lankan Wild Boar (Sus scrofa affinis) Meat from Exotic and Village Pig (Sus scrofa domestica) Meat',Tropical Agricultural Research,23 (1), pp.11 –20 [Online] Available at:file:///C:/Users/user-pc/Downloads/4627-16474-1-PB%20(1).pdf (Accessed: 19 July 2018).

Sharma, A., Batra, N.G., Garg, A. and Saxena, A. (2017) 'Food adulteration: A review', International Journal for Research in Applied Science and Engineering Technology, 5(3), pp. 686-689 [Online] Available at: https://www.researchgate.net/publication/322577275_Food_Adulteration_A_Review (Accessed: 26 November 2018).

Tanabe S., MiyauchiE., Muneshige A., and Kazuhiro M (2007) 'PCR methods of detecting pork in foods for verification allergen labeling and for identifying hidden pork ingredients in processed foods', Bioscience biotechnology and biochemistry, 71, pp. 1663-1667. [Online] DOI:10.1271/bbb.70075

Yang, L., Wu, T., Liu, Y., Zou, J., Huang, Y., Babu, S and Lin, L. (2018) 'Rapid identification of pork adulterated in the beef and mutton by infrared spectroscopy', Hindawi, 2018, pp. 10. [Online] Available at: https://www.hindawi.com/journals/jspec/2018/2413874/abs/ (Accessed: 26 November 2018).

Bartlett, J.W and Frost, C. (2008) 'Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables', Obstetrics & Gynaecology, 31(4), [Online] Available at: https://obgyn.onlinelibrary.wiley.com/doi/full/10.1002/uog.5256 (Accessed: 21 February 2019)