Food Research 8 (3) : 235 - 241 (June 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

METHOD DEVELOPMENT

Application of direct PCR technique using specified mitochondrial DNA-encoded ND4 and D-Loop genes on detecting pork DNA in processed meat products

[1,*]Kusnadi, J., [1]Natsir, R.F. and [2]Al Awwaly, K.U.

[1]*Department of Agricultural Products Technology, Faculty of Agriculture Technology, Brawijaya University, Malang 65145, East Java, Indonesia*
[2]*Faculty of Animal Science, Brawijaya University, Malang 65145, East Java, Indonesia*

Article history:
Received: 1 October 2022
Received in revised form: 14 November 2022
Accepted: 6 December 2023
Available Online: 26 May 2024

Keywords:
Adulteration,
DNA,
Direct PCR technique,
Pork,
Meat product

DOI:
https://doi.org/10.26656/fr.2017.8(3).362

Abstract

Adulteration or counterfeiting of food products raises public concerns about the halal issue. Many issues related to food products such as meatballs, sausages, and corned beef use pork for counterfeiting. This study aimed to determine a direct polymerase chain reaction (dPCR) technique for pork DNA detection in processed meat products such as meatballs, sausages, and corned beef in a short time and at a low cost using specific primers, ND4 and D-Loop. The study used a direct PCR technique with variations in the incubation time and temperature of the lysis process on the sample. The concentration of DNA obtained from the lysis results with variations in incubation time and temperature in fresh meat and processed meat products was 66.17-469.23 ng/µL, and the purity of DNA in fresh and processed meat products was 1.70-2.25. ND4 primer was more sensitive to DNA amplification than D-Loop in processed meat products using direct PCR. Meatballs, sausages, and corned beef products written on the packaging using beef did not detect any mixing or addition of pork. Direct PCR can detect pork DNA in processed meat products such as meatballs, sausages and corned beef.

1. Introduction

Adulteration or counterfeiting of food products is an unnecessary and dangerous ingredient mixing procedure in food products that leads to the product's quality reduction. The decrease in food product quality occurs due to substituting other cheaper ingredients to reduce production costs or sell price elevation so more profit can be obtained (Widyanto *et al*., 2021). This issue raises public concern against halal, such as the concern of pork substitution for meatballs, sausages, and corned beef with the aim of adulteration. Therefore, detecting food products to evaluate other unnecessary and dangerous ingredients in food products needs to be done (Sharma *et al*., 2017).

Pork contamination detection is classified into 3 techniques: DNA, protein, and fat-based techniques. This technique is done because every organism has a specific DNA, protein, and fat arrangement. Protein and fat-based detection were mostly done in fresh meat rather than processed food products. The production process can cause protein denaturation and fat modification by high temperature and high pressure. Otherwise, the DNA-based technique is more stable and difficult to denature at high temperatures and pressure. Since then, DNA-based detection can be done in processed food products. In addition, the existence of DNA can be found in almost all parts of the cell organism that can be detected easily on a tested sample (Rahmati *et al*., 2016).

Generally, DNA detection is carried out using polymerase chain reaction (PCR), amplifying nucleic acid technique in vitro. This technique amplifies the number of DNA targets by analyzing new DNA molecules complementary to target DNA through enzymes and oligonucleotides as primers in a thermocycler (Widayat *et al*., 2019). One of the most important materials in DNA amplification is primer. In a previous study, several primers were used to detect processed food products (meatballs, sausages and corned beef), such as ND4 and D-loop primers, which have been proven to detect pork DNA in the products (Kusnadi *et al*., 2020). The previous study detected DNA in food products using the PCR technique, but it was time and cost-consuming. Therefore, improving the PCR technique, such as using direct polymerase chain reaction (dPCR) is necessary. The dPCR is a DNA amplification technique without DNA purification steps (Guan *et al*.,

*Corresponding author.
Email: *jkusnadi@ub.ac.id*

eISSN: 2550-2166 / © 2024 The Authors.
Published by Rynnye Lyan Resources

2018) with the following advantages: rapid process, less material needed, cheaper cost, and high DNA concentration can be obtained. Less material is required because this technique only needs lysis material for the cell membrane, so the cost is cheaper. However, the absence of a DNA purification step can become the source of RNA, protein, or other compound contamination. Besides, dPCR also allows PCR inhibitors because, in this technique, there is no material for PCR inhibitor removals such as protein and other materials (Li *et al*., 2011; Kitpipit *et al*., 2014; Guan *et al*., 2018; Mokhtar *et al*., 2020).

In the previous study, dPCR was used along with centrifuge, proteinase-K addition, and long incubation time as the function of lysis buffer to lyse the cell membrane (Li *et al*., 2011). However, this study did not use centrifugation, proteinase-K, and long incubation time and expected to get similar results as the previous one. This study aimed to evaluate the dPCR technique for pork DNA detection on processed meat products in a short time and cheaper cost. The study also assessed the time and temperature of incubation used for the lysis process on pork DNA detection using the dPCR technique.

2. Materials and methods

The study was conducted in Laboratorium Sentral Ilmu Hayati, University of Brawijaya (LSIH). The study was a qualitative descriptive study. The samples used in this study were pork, meatballs, sausages, and corned beef. The time incubation used in this study was 5, 10, and 15 mins, while the temperature was 85°C and 95°C. ND4 and D-Loop were the primers designed by Haunshi *et al.* (2009) and Kusnadi *et al.* (2020), respectively.

2.1 Lysis process with lysis buffer addition

The samples were weighed at 2.5 mg, put into a 1.5 mL tube, and added 200 µL lysis buffer to each sample. The samples were divided into several categories based on the temperature and time of incubation. Each sample was then incubated in a thermomix with a predetermined temperature and time. After that, 45 µL tween-20 (0.7%) was added to the tube.

2.2 Polymerase Chain Reaction

The amplification of the PCR process was done simultaneously in a PCR machine with 10 µL of solvent. The sample and PCR material were inserted into a sterile thin wall and put in a PCR machine. The temperature and time of the PCR were set according to the predetermined stage (denaturation, annealing, and extension). The temperature was used according to the primer ND4 and D-Loop. The pre-denaturation step was set at 95°C for 5 mins, which was then continued for 30 s denaturation. The annealing step was done at 54°C for ND4 primer and 55°C for D-Loop primer for 45 s. After that, the extension step was done at 72°C for 30 s, and the last step was at 72°C for 5 mins. The PCR process of ND4 primer was 32 cycles and 30 cycles for D-Loop. The storage process was done at 4°C for 5 mins. The result confirmation was obtained using electrophoresis (Guan *et al*., 2018).

2.3 Purification and DNA concentration test

The sample port was cleaned using tissue with sterile aquadest. An elution buffer was used as the blank. About 1 µL DNA sample was added in the sample place and measured in spectrophotometry-UV with 260 nm and 280 nm wavelengths. The DNA concentration data was obtained in µg/µL, and DNA purification data as the ratio of A280 per A260.

2.4 Electrophoresis

The electrophoresis in this study was done using agarose gel with a concentration of 1.5% (0.45 g of agarose with 30 mL TBE 1x solvent). The agarose was heated in a microwave until the agarose powder dissolved and became clear. The agarose solution was then waited and added with ethidium bromide (EtBr) carefully and homogenized. The agarose mixture was poured on the mold and a comb was placed to form a well or a hole for the sample placement. When it was cold, the comb was removed slowly and the agarose was soaked in TBE 1× until the agarose state was completely submerged. Besides, the sample was added by loading dye 1 µL, sample and DNA ladder were put into each well carefully. A 50-volt voltage was set up to start the electrophoresis process. The process was about 1 hr, and the electrophoresis result was visualized using Gel Doc Transilluminator (Magdeldin, 2012).

2.5 Sampling test

The sample used in this study was pork and processed meat products, such as meatballs, sausages, and corned beef, indicating mixed or no indication of mixed pork. The sample used three brands of each product which was obtained from peddlers, traditional and modern markets. The sample DNA was obtained from lysis buffer addition with the material concentration, time incubation, and temperature that had been optimized and predetermined. The time and temperature for incubation used in the lysis process of the sampling test were 5 mins and 95°C.

https://doi.org/10.26656/fr.2017.8(3).362
© 2024 The Authors. Published by Rynnye Lyan Resources

3. Results and discussion

3.1 Lysis process with the addition of lysis buffer

The lysis buffer components such as NaOH, EDTA (Ethylene Diamine Tetraacetic Acid), NaCl, SDS, and Tris:Cl have several functions. EDTA could inhibit DNA degradation by binding with divalent cation required by endogenous nuclease inactivation. SDS could release chromatin-bound DNA, while NaCl can provide Na+ ions, which form ionic bonds with negatively charged phosphates to neutralize the negative charges and allow DNA molecules to stick together. Besides, Tris-Cl was used to maintain pH conditions, while NaOH damaged cell walls if it was in contact with strong bases (Li *et al*., 2011). The lysis process result from the DNA concentration and purification test is shown in Table 1.

The results showed the similarity of DNA concentration between pork and processed meat products. Furthermore, sausages were found to have a higher DNA concentration compared to pork. Pork should have a higher DNA concentration than processed meat products. Processed food products have similar or even higher DNA concentrations than pork, caused by other DNA contamination in food production (Yudianto, 2010; Saputro *et al.,* 2015).

DNA purity was obtained from the ratio of 260/280 nm wavelength, where good purity (less or no contaminant) was found when the value was 1.80-2.00 (Vesty *et al*., 2017; Dilhari *et al*., 2017; Uda *et al*., 2020). Lucena-Aguilar *et al.* (2016) showed that the purity value <1.60 was a contaminant from protein, phenol, or another contaminant. A contaminated sample inhibits the PCR process and produces a poor shape of the DNA band (Hartawan *et al.,* 2015). If the purity value >2.00, the sample is contaminated by RNA because 2.00-2.20 is the range for RNA purity (Lucena-Aguilar *et al*., 2016).

Based on the study, the purity of DNA of samples with incubation time and temperature variations showed no contaminant of protein or RNA, except the sausages sample that showed RNA contamination. The DNA isolation process did not use RNAse like other DNA isolation methods because the function of RNAse in the DNA isolation process was to degrade RNA in a sample (Sari *et al*., 2014).

3.2 ND4 and D-Loop primer amplification

The pork ND4 primer and processed food products (meatballs, sausages, and corned beef) were amplified for 5 mins at 85°C and 95°C (A1, A3, A4, B1, B2, B3, dan B4). The DNA amplification of meatballs (A2) was done for 5 mins at 85°C and formed a thin DNA band. While in the D-Loop primer of pork, meatballs, and sausages, the DNA amplification was done for 5 min at 85°C and 95°C (A1, A2, A3, B1, B2, and B3). However, in 5 min incubation at 85°C and 95°C, the DNA band of

Table 1. Concentration and purity of DNA in processed meat products at various incubation times (5, 10, 15 mins).

Sample	Time (min)	Temperature (°C)	DNA concentration (µg/µL)	DNA Purity
Pork	5	85	124.27	1.81
		95	98.07	1.92
	10	85	230.78	1.73
		95	213.79	1.84
	15	85	118.07	1.70
		95	180.01	1.74
Meatballs	5	85	121.94	1.94
		95	66.17	1.95
	10	85	102.62	1.81
		95	101.41	1.83
	15	85	85.95	1.73
		95	180.89	1.73
Sausages	5	85	164.23	2.25
		95	469.23	2.11
	10	85	417.02	2.14
		95	205.77	2.14
	15	85	192.15	2.19
		95	407.79	2.14
Corned Beef	5	85	191.53	1.81
		95	205.16	1.86
	10	85	79.14	1.89
		95	215.81	1.81
	15	85	193.51	1.83
		95	242.39	1.85

https://doi.org/10.26656/fr.2017.8(3).362 © 2024 The Authors. Published by Rynnye Lyan Resources

corned beef samples (A4 and B4) was not formed during electrophoresis (Figure 1).

This study found that the ND4 primer of pork and processed food products such as meatballs, sausages, and corned beef were amplified at 10 mins and 85°C and 95° C (C1, C3, D1, D2, D3, dan D4). However, the sample of meatballs and corned beef (C2 and C4) which were amplified at 10 mins and 85°C formed a thin DNA band. Besides, the corned beef sample with 10 mins incubation at 85°C and 95°C (C4 and D4) did not form a DNA band (Figure 1).

The amplification of ND4 primer in samples using 15 min incubation time and 85°C and 95°C incubation temperature (E1, E3, F1, F2, F3, F4) showed the DNA band formation during electrophoresis. In the corned beef sample with 15 mins and 85°C times and temperature of incubation (E4), the amplification occurred, but the DNA band was thin. However, in the sample of corned beef with the same time and temperature of incubation (E2), the amplification and the DNA band were not formed. Whereas the D-loop primer of pork, meatballs, and sausages was amplified in 15 mins incubation with a temperature of 85°C and 95°C (E1, E3, F1, F2, F3, and F4). However, meatballs with 5 mins incubation time and 85°C temperature (E2) and corned beef E4 and E5 (15 mins with 85°C and 95°C) were not amplified, and the DNA band was not formed during electrophoresis.

A thin DNA band or no DNA band formation was caused by several factors, including primer with the shortest DNA target, which caused the primer to amplify the DNA effectively during PCR due to the higher sensitivity of the primer (Kusnadi *et al.,* 2020). The other factor is the absence of DNA formation caused by the amplification that did not occur due to the incompatible primer against the DNA template (Sinaga *et al*., 2017). Furthermore, DNA degradation caused the primer not to attach and the existence of PCR inhibitor (Bartlett and Stirling, 2003; Saputro *et al*., 2015). Other factors that cause thin DNA or no band formation were the concentration and quality of the DNA, the annealing temperature of the primer (forward and reverse),

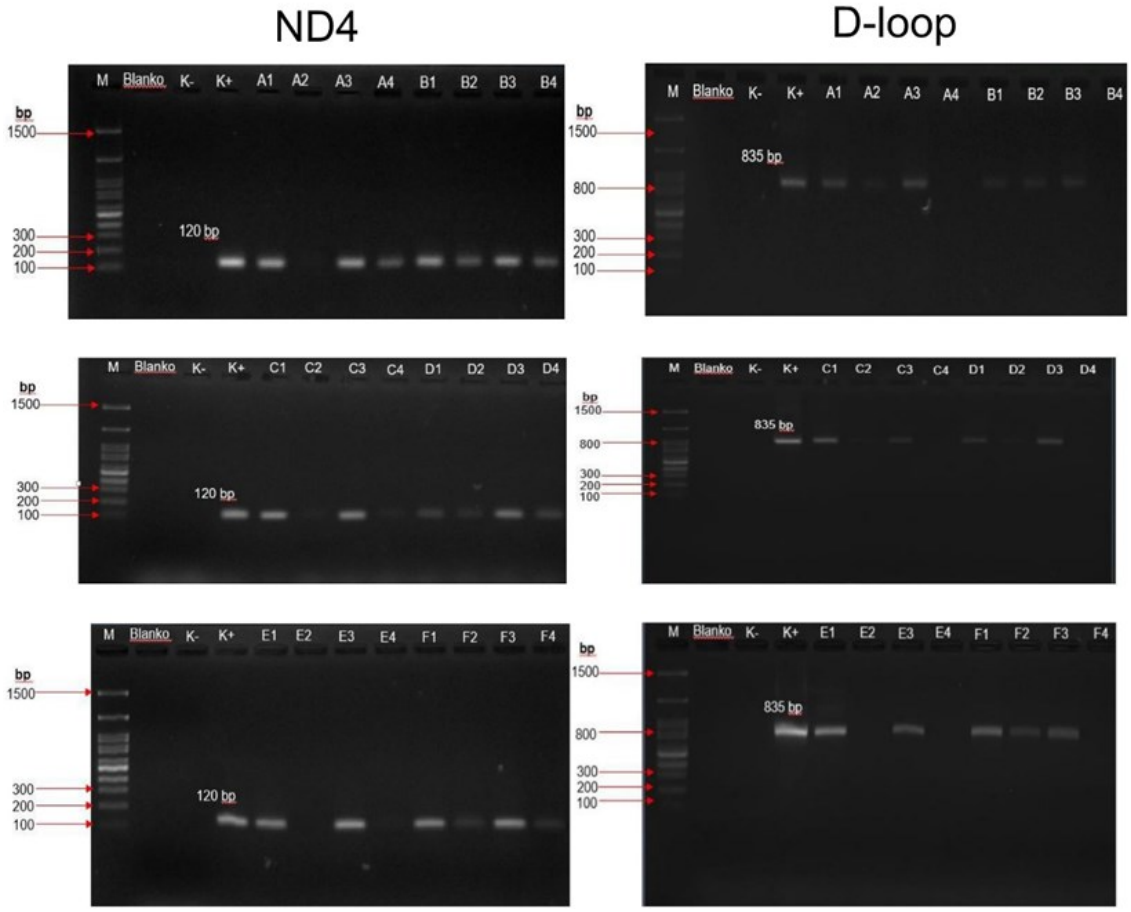

Figure 1. Visualization of DNA bands based on ND4 and D-Loop primer amplification at incubation time of 5, 10, 15 mins and incubation temperature at 85°C and 95°C. Note: 5 mins treatment: K-/K+ = ddH_2O/Pork isolation kit, A1 = Pork (5', 85°C), A2 = Meatballs (5', 85°C), A3 = Sausages (5', 85°C), A4 = Corned beef (5', 85°C), B1 = Pork (5', 95°C), B2 = Meatballs (5', 95° C), B3 = Sausages (5', 95°C), B4 = Corned beef (5', 95°C); 10 mins treatment: C1 = Pork (10', 85°C), C2 = Meatballs (10', 85° C), C3 = Sausages (10', 85°C), C4 = Corned beef (10', 85°C), D1 = Pork (10', 95°C), D2 = Meatballs (10', 95°C), D3 = Sausages (10', 95°C), D4 = Corned beef (10', 95°C). 15 mins treatment: E1 = Pork (15', 85°C), E2 = Meatballs (15', 85°C), E3 = Sausages (15', 85°C), E4 = Corned beef (15', 85°C), F1 = Pork (15', 95°C), F2 = Meatballs (15', 95°C), F3 = Sausages (15', 95°C), F4 = Corned beef (15', 95°C).

https://doi.org/10.26656/fr.2017.8(3).362
© 2024 The Authors. Published by Rynnye Lyan Resources

concentration and quality of primer, the number of PCR cycles, and the material used in the PCR formulation (Setyawati and Zubaidah, 2021) and the condition or temperature used in the dPCR (Sunarno *et al*., 2014). The time and temperature of incubation used in dPCR were 5 mins and 95°C. Moreover, the study found that 5 min and 95°C was the shortest time for DNA detection in processed food products using dPCR.

3.3 Sampling test

This study found meatballs B, sausages A, and sausages C were contaminated by RNA and showed a purity value of >2.00 (Table 2). This was because the DNA isolation process did not use RNAse, which has the function of RNA degradation in the sample (Sari *et al*., 2014).

Processed meat products such as meatballs A with pork contained on the packaging (A1, A2, and A3) were amplified, and the DNA band was formed in the electrophoresis. It showed that these products (A1, A2, and A3) contain pork. Besides, meatballs B and C that were obtained from the market and peddler showed no amplification of the DNA, and the DNA band was not formed in the electrophoresis. It can be concluded that this product is pork-free and can be consumed by Muslims. The amplification test result of meatball sampling is shown in Figure 2.

Table 2. Concentration and purity of DNA in processed meat products.

Sample	DNA concentration (µg/µL)	DNA purity
Meatballs A*	145.72	1.74
Meatballs B	212.59	2.01
Meatballs C	126.59	1.80
Sausages A*	321.43	2.11
Sausages B	192.54	1.95
Sausages C	274.63	2.08
Corned Beef A*	168.82	1.86
Corned Beef B	223.29	1.62
Corned Beef C	168.43	1.62

*Sample contained pork

Sausages as a processed meat product with pork that was written on the package showed DNA amplification (A1, A2, A3), so the DNA band also formed in the electrophoresis. The results confirmed the pork contained in the products. However, sausages B and C obtained from the traditional and modern markets were not amplified, and the DNA band was not formed, so it was pork-free and safe to consume by the Muslims. The

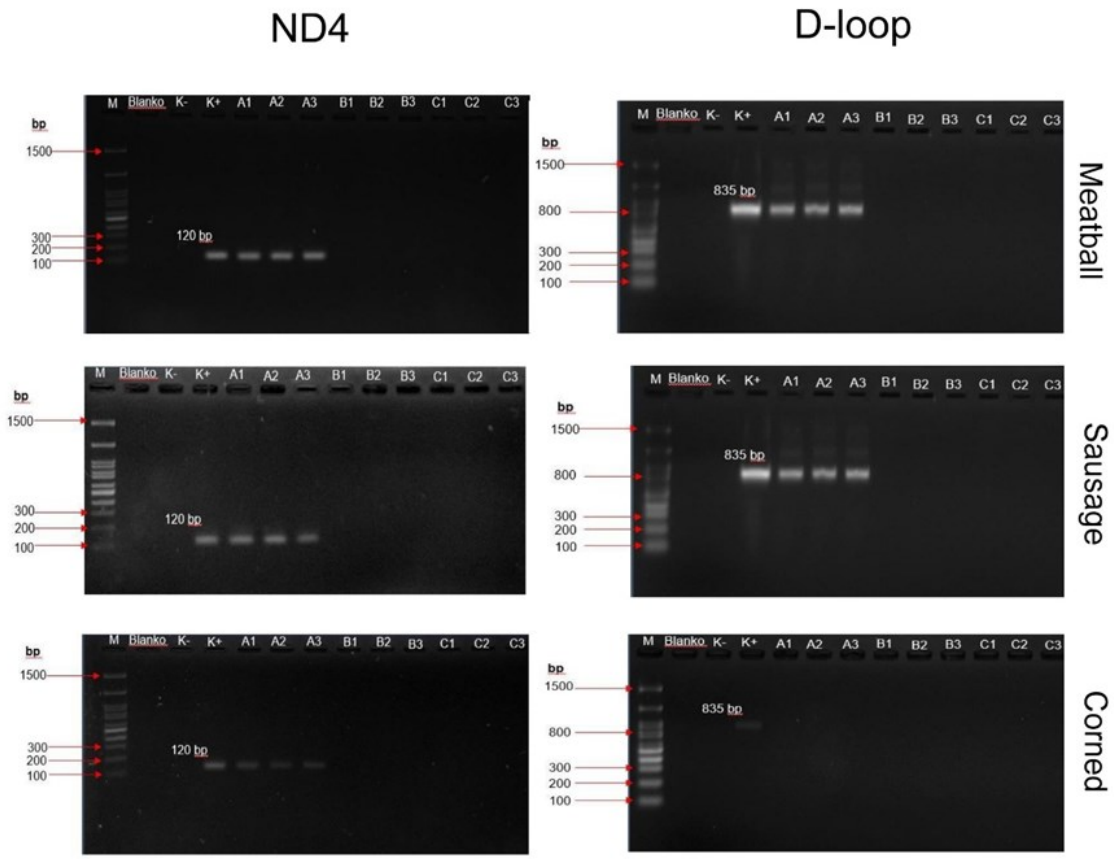

Figure 2. Visualization of DNA bands on 3 meatball brands, 3 sausage brands, 3 corned pork, for triplex, for both ND4 and D-loop. Note: Meatball: K-/K+ = ddH_2O/Pork isolation kit, A1-A3 = Meatball A (contains pork), B1-B3 = Meatball B, C1-C3 = Meatball C; Sausage: K-/K+ = ddH_2O/Pork isolation kit, A1-A3 = Sausage A (contains pork), B1-B3 = Sausage B, C1-C3 = Sausage C; Corned: K-/K+ = ddH_2O/Pork isolation kit, A1-A3 = Corned A (contains pork), B1-B3 = Corned B, C1-C3 = Corned C.

https://doi.org/10.26656/fr.2017.8(3).362 © 2024 The Authors. Published by Rynnye Lyan Resources

amplification test results are shown in Figure 2.

The amplification of the ND4 primer of corned beef A (A1, A2, A3) and the formation of DNA band in electrophoresis showed pork contained in the products. The corned beef B and C obtained from the traditional and modern markets, the DNA was not amplified, and the DNA band was not formed. It can be concluded that corned beef B and C were pork-free and safe to be consumed by Muslims. However, the D-Loop primer of meatballs A did not show DNA amplification (A1, A2, and A3) with no DNA band formation in the electrophoresis. The literature stated that using D-Loop primer needs PCR optimization, especially against the corned beef sample test. The annealing temperature affects the primer attachment on the DNA template, so the optimum annealing temperature needs to be found (Prakoso *et al*., 2016; Rahmadhan *et al*., 2019). The too-low annealing temperature can cause the primer attachment site to be not specific (low specificity), which then amplifies unwanted locus fragments, while the annealing temperature is too high, causing the primer to not adhere well to the template DNA (Pertiwi *et al*., 2015; Zedta and Setyadji, 2019). Determining the factor of temperature and time for primer attachment to the DNA template so the amplification can occur was a non-complementary primary sequence (Bucklin *et al*., 2011; Zedta and Setyadji, 2019).

4. Conclusion

ND4 primer was more sensitive to DNA amplification than D-Loop in processed meat products using direct PCR. Meatballs, sausages, and corned beef products written on the packaging using beef did not detect any mixing or addition of pork. Direct PCR can detect pork DNA in processed meat products such as meatballs, sausages, and corned beef.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank Hilda Maghfirotu Hakiki, S.Biotek, Dini Nurilmi Putri, S.TP, M.Si, and Rr. Fitria Dewi Listiani, S.Si, for assisting with this study. The authors also thank Laboratorium Sentral Ilmu Hayati of Brawijaya University (LSIH) for permitting to conduct of this study.

References

Bartlett, J.M.S. and Stirling, D. (2003). Methods in Molecular Biology. New Jersey, USA: Humana Totowa.

Bucklin, A., Steinke, D. and Blanco-Bercial, L. (2011). DNA barcoding of marine metazoa. *Annual Review of Marine Science*, 3(1), 471-508. https://doi.org/10.1146/annurev-marine-120308-080950.

Dilhari, A., Sampath, A., Gunasekara, C., Fernando, N., Weerasekara, D., Sissons, C., McBain, A. and Weerasekera, M. (2017). Evaluation of the impact of six different DNA extraction methods for the representation of the microbial community associated with human chronic wound infections using a gel-based DNA profiling method. *AMB Express*, 7, 179. https://doi.org/10.1186/s13568-017-0477-z

Guan, F., Jin, Y., Zhao, J., Ai, J. and Luo, Y. (2018). A novel direct PCR lysis buffer can improve PCR from meat matrices. *Food Analytical Methods*, 12, 100-107. https://doi.org/10.1007/s12161-018-1342-7

Hartawan, I.G.N.B.A., Arsa, M., Ariati, N.K. and Wirajana, I.N. (2015). Isolasi DNA metagenomik dari madu dengan dan tanpa pengayaan media LB (Luria-Bertani). *Jurnal Kimia*, 9(2), 189-195. https://doi.org/10.24843/JCHEM.2015.v09.i02.p08 [In Bahasa Indonesia].

Haunshi, S., Basumatary, R., Girish, P., Doley, S., Bardoloi, R.K. and Kumar, A. (2009). Identification of chicken, duck, pigeon and pig meat by species-specific markers of mitochondrial origin. *Meat Science*, 83(3), 454-459. https://doi.org/10.1016/j.meatsci.2009.06.026

Kitpipit, T., Sittichan, K. and Thanakiatkrai, P. (2014). Direct-multiplex PCR assay for meat species identification in food products. *Food Chemistry*, 163, 77-82. https://doi.org/10.1016/j.foodchem.2014.04.062

Kusnadi, J., Ashari, N.A. and Arumingtyas, E.L. (2020). Specificity of Various Mitochondrial DNA (mtDNA), ND5, D-Loop, and Cty-b DNA Primers in Detecting Pig (*Sus scrofa*) DNA Fragments. *American Journal of Molecular Biology*, 10(3), 141-147. https://doi.org/10.4236/ajmb.2020.103010

Li, H., Xu, H., Zhao, C., Sulaiman, Y. and Wu, C. (2011). A PCR amplification method without DNA extraction. *Electrophoresis*, 32(3-4), 394-397. https://doi.org/10.1002/elps.201000392

Lucena-Aguilar, G., Sánchez-López, A.M., Barberán-Aceituno, C., Carrillo-Avila, J.A., López- Guerrero, J.A. and Aguilar-Quesada, R. (2016). DNA source selection for downstream applications based on DNA quality indicators analysis. *Biopreservation and Biobanking*, 14(4), 264-270. https://doi.org/10.1089/bio.2015.0064

Magdeldin, S. (Ed.) (2012). Gel Electrophoresis–Principles and Basics. London, United Kingdom:

 © 2024 The Authors. Published by Rynnye Lyan Resources

IntechOpen E-Book. https://doi.org/10.5772/2205

Mokhtar, N.F.K., El Sheikha, A.F., Azmi, N.I. and Mustafa, S. (2020). Potential authentication of various meat based products using simple and efficient DNA extraction method. *Journal of The Science of Food and Agriculture*, 100(4), 1687-1693. https://doi.org/10.1002/jsfa.10183

Pertiwi, N.P.N., Mahardika, I.G.N.K. and Watininiasih, N.L. (2015). Optimasi amplifikasi DNA menggunakan metode PCR (Polymerase Chain Reaction) pada Ikan Karang anggota Famili Pseudochromidae (Dottyback) untuk identifikasi spesies secara molekular. *Jurnal Biologi*, 19(2), 1-5.

Prakoso, S.P., Wirajana, I.N. and Suarsa, I.W. (2016). Amplifikasi fragmen gen 18S rRNA pada DNA metagenomik madu dengan teknik PCR (Polymerase Chain Reaction). *Indonesian Journal of Legal and Forensic Sciences*, 2(3), 45-47. https://doi.org/10.24843/IJLFS.2017.v07.i01.p03 [In Bahasa Indonesia].

Rahmadhan, D., Sari, R. and Apridamayanti, P. (2019). Pengaruh suhu annealing terhadap amplifikasi gen TEM Menggunakan primer dengan %GC Rendah. *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN*, 4(1), 1-7. [In Bahasa Indonesia].

Rahmati, S., Julkapli, N.M., Yehye, W.A. and Jefrey. W. (2016). Identification of meat origin in food products-a review. *Food Control*, 68, 379-390. https://doi.org/10.1016/j.foodcont.2016.04.013

Saputro, A., Yudianto, A. and Koesbardiati, T. (2015). Pengaruh lama paparan suhu kamar terhadap kualitas DNA pada pemeriksaan SWAB Earphone dalam penentuan jenis kelamin. *Jurnal Biosains Pascasarjana*, 17(1), 33-45. https://doi.org/10.20473/jbp.v17i1.2015.33-45 [In Bahasa Indonesia].

Sari, S.K., Listyorini, D., Mazieda, M.N. and Sulasmi, E.S. (2014). Optimasi teknik isolasi dan purifikasi DNA pada daun cabai rawit (*Capsicum frutescens* cv. cakra hijau) menggunakan genomic DNA mini kit (Plant) Geneaid. *Proceeding Biology Education Conference,* 11(1), 65-70. [In Bahasa Indonesia].

Setyawati, R. and Zubaidah, S. (2021). Optimasi Konsentrasi Primer dan Suhu Annealing dalam Mendeteksi Gen Leptin pada Sapi Peranakan Ongole (PO) Menggunakan Polymerase Chain Reaction (PCR). *Indonesian Journal of Laboratory*, 4(1), 36-40. https://doi.org/10.22146/ijl.v4i1.65550 [In Bahasa Indonesia].

Sharma, A., Batra, N., Garg, A. and Saxena, A. (2017). Food Adulteration: A Review. *International Journal for Research in Applied Science and Engineering Technology*, 5(3), 686-689. https://doi.org/10.22214/ijraset.2017.3129

Sinaga, A., Putri, L.A.P. and Bangun, M.K. (2017). Analisis Pola Pita Andaliman (*Zanthoxylum acanthopodium* D.C) berdasarkan Primer OPD 03, OPD 20, OPC 07, OPM 20, OPN 09. *Jurnal Agroekoteknologi Universitas Sumatera Utara*, 5(1), 55-64. [In Bahasa Indonesia].

Sunarno, S., Rizki, A., Sariadji, K., Malik, A., Karuniawati, A. and Soebandrio, A. (2014). Direct PCR: alternative diagnostic method for diagnosis of diphtheria rapidly, easily and cost effective. *Makara Journal of Health Research*, 17(2), 88-94. https://doi.org/10.7454/msk.v17i2.3031

Uda, M.N.A., Parmin, N.A., Jambek, A.B., Hashim, U., Uda, M.N.A. and Shaharuddin, S.N.A. (2020). Evaluation and optimization of genomic DNA extraction from food sample for microfluidic purpose. *IOP Conference Series, Materials Science and Engineering*, 743, 012031. https://doi.org/10.1088/1757-899X/743/1/012031

Vesty, A., Biswas, K., Taylor, M.W., Gear, K. and Douglas, R.G. (2017). Evaluating the impact of DNA extraction method on the representation of human oral bacterial and fungal communities. *PLoS ONE,* 12(1), e0169877. https://doi.org/10.1371/journal.pone.0169877

Widayat, W., Winarni, T.A., Suzery, M., Al-Baarri, A.N., Putri, S.R. and Kurdianto, K. (2019). Real Time-Polymerase Chain Reaction (RT-PCR) sebagai Alat Deteksi DNA Babi dalam Beberapa Produk Non-Pangan. *Indonesia Journal of Halal*, 2(1), 26-33. https://doi.org/10.14710/halal.v2i1.5361

Widyanto, R.M., Dini, C.Y., Rahmawati, I.S., Putri, S.R., Rozana, A.N., Abida, S.H. and Yunimar. (2021). Uji deteksi adulterasi daging babi (*Sus scrofa* domestica) pada bakso metode Loop-Mediated Isothermal Amplification. *Indonesian Journal of Human Nutrition*, 8(1), 76-87. https://doi.org/10.21776/ub.ijhn.2021.008.01.8 [In Bahasa Indonesia].

Yudianto, A. (2010). Analisis DNA tulang dan gigi pada lokus Short Tandem Repeat-Combined DNA Index System (STR-CODIS), Y-Chromosome STRs and mitochondrial DNA (mtDNA) akibat efek paparan panas suhu tinggi. Surabaya, Indonesia: Universitas Airlangga, PhD Dissertation. [In Bahasa Indonesia].

Zedta, R.R. and Setyadji, B. (2019). Optimalisasi PCR ikan tongkol Krai (*Auxis thazard*) dan Lisong (*Auxis rochei*) pada analisis keragaman genetik. *Bawal Widya Riset Perikanan Tangkap,* 11(2), 95-102. https://doi.org/10.15578/bawal.11.2.2019.95-102 [In Bahasa Indonesia].

 © 2024 The Authors. Published by Rynnye Lyan Resources