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DETECTION OF PORK DNA CONTAMINANTS IN GROUND BEEF AT GANG BARU MARKET, SEMARANG CITY USING CONVENTIONAL POLYMERASE CHAIN REACTION TECHNOLOGY

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ABSTRACT
This research aims to determine the presence of pork contaminants and the specificity of the primer of the gene cytochrome B in ground beef originating from the meat grinders service at Gang Baru Market, Semarang City. This research used a quantitative descriptive method with a purposive sampling technique, 4 samples were tested through a few steps such as DNA isolation, spectrophotometric testing, PCR amplification and visualization using agarose gel electrophoresis, the amplification process is carried out using pig and cattle primers. The results of agarose gel electrophoresis visualization showed that all ground beef samples with codes S1, S2, S3 and S4 which are amplified using cattle primer show bands with a base pair of ±285, while samples with codes B1, B2, B3, and B4 which used pig primer do not occur amplification. The visualization results show that there is no pork DNA contamination in the ground beef samples studied and no pig amplicon bands forming.

Keywords:
Conventional; DNA; PCR; Pig; Primer.

Introduction

Humans need food to carry out the body's biological functions, many nutritional foods can be obtained from animal and vegetable sources such as protein and fat which are easily obtained from animal sources (Awwaly, 2017). Many animal meat and milk can be consumed for human benefit, however, consumption of animal-based food must be taken into consideration because not all types of animals can be consumed. Consuming nutritious food in daily life cannot be separated from the halal principle, because halal nutritious food in Islam has a big influence on faith and life, both directly and indirectly (Sri Mulyati et al., 2023).

As many as 87.2% of the Indonesian population adhere to Islam as their religion (Portal Informasi Indonesia, 2022). As a follower of the Islamic religion, applying halal principles in consuming food is something that must be done. In the Qur'an Allah SWT said, *"O humanity! Eat from what is lawful and good on the earth ...."* (The Qur'an, 1:168). This verse shows that the Islamic religion requires its adherents to consume halal and good food, halal food is food that is halal in its substance and method of obtaining it (Ali, 2016). Thayyib (good food) is proportional food, has good nutritional value for the body, is free from toxins and if consumed does not cause harm (Setiawan, 2020).

Gang Baru Market is located in an alley in the middle of the Chinatown area, namely on Gang Baru Street, Chinatown area, Semarang City (Murtini & Wahyuningrum, 2017). At night, the area around Gang Baru market (Chinatown) will be filled with traders selling food such as traditional snacks, meatballs, kebabs, fried rice, etc. The interesting thing about the Chinatown

area is the sale of pork-based food such as roast pork, Singapore pork satay, pork meatballs and other processed pork (Widjajanti, 2015). The sale of pork in general can be a concern for Muslims so they do not carelessly consume food from this culinary area.

The presence of pork-based processed foods indicates the potential for contamination of meat-grinding machines in the Gang Baru market. Cross-contamination can occur when the same grinding machines are used for both pork and beef without proper cleaning, leading to the inadvertent mixing of pork residues with halal meat. Meat grinding services are businesses that use tools to grind meat to make it softer and smoother to be reprocessed into certain foods or to be cooked directly (Saputra, 2017). The presence of processed pork in the Chinatown area indicates the potential for contamination of meat milling machines in the Chinatown area, Gang Baru market, thus special attention is needed for meat grinding in this area.

Pig is a dirty animal so their consumption is prohibited. The meaning of the word dirty here means *najis*, *najis* is a dirty object that every Muslim must clean, both on himself and the object affected by *najis*. Pig is highly *najis* animal (*mughaladzah*), anything touched by them must be washed 7 times and one of them is with water, because of their impurity the law on consuming them is haram (Qardhawi, 2007).

Based on the background, research related to pork contamination of ground beef samples at Gang Baru market, Semarang City is something important to do. Detection of pork contaminants can be done easily using Polymerase Chain Reaction (PCR). PCR is highly sensitive and specific, making it an ideal method for detecting even trace amounts of pork DNA contaminants in ground beef samples (Indriati, 2021). Compared to other methods such as immunoassays, PCR provides more accurate and reliable results, especially in complex food matrices. This research was conducted to determine the safety and halalness of ground beef at meat grinding services provided at Gang Baru market, Semarang City. The findings from this study could significantly impact the Muslim community by enhancing consumer trust and ensuring that halal standards are rigorously maintained. Additionally, this research could provide valuable insights for policymakers and market regulators to implement stricter controls and prevent cross-contamination.

Materials and Methods

Sampel Preparation

The sample used was fresh ground beef from the meat grinder at Gang Baru Market. A total of 4 samples were taken and placed in different plastic containers and then carried in a cooler bag containing dry ice to the laboratory. Samples were stored at -20°C until the samples were used for research.

DNA Isolation

DNA extraction was performed using the Geno Plus Genomic DNA Extraction Minirep System Kit. 30 mg of ground beef is put into a 1.5 mL tube, the first step is to add 180 µL LYS Buffer and 20 µL Proteinase K into the test tube and homogenize with a vortex, incubate the sample solution at 60˚C for 60 minutes, total 300 µL of FX Buffer is added and homogenized then incubated at 70°C for 20 minutes. A total of 200 µL of isopropanol is added and mixed thoroughly, place a B/T Genomic Mini Column onto a Collection tube, pipet all the mixture into the column. Centrifuge at 6.000 x g for 2 minutes than place the column onto a new collection tube. Shake the sample solution micture twice with 0.5 ml of WS Buffer solution by centrifuging at 6.000 x g for 2 minutes and discard the flow-trhough. Centrifuge the sample mixture at full at 1.000 x 9 for 2 minutes to remove residual ethanol. Place the sampel mixture into a new 1.5 mL tube and elute the DNA with 200 µL of preheated elution buffer, place the solution in a standing position for 1-5 minutes the centrifuge for 1-2 minutes to elute the DNA. Store the eluted DNA at -20°C in freezer. The purity of the isolated DNA is seen using a Nanodrop AE-200 Nano Nucleic Acid Analyzer spectrophotometer at wavelengths of 230, 260 and 280 nm.

DNA Aplification Using the PCR Conventional

The primers used in this study were cytochrome B gene primers originating from mitochondria, the pig primers had the basic sequence F 5'-CTT GCA AAT CCT AAC AGG CCT G-3' and R 5'-CGT TTG CAT GTA GAT AGC GAA TAA - 3', while for cattle use the primers cattle F 5'-CCC GAT TCT TCG CTT TCC AT-3' and cattle R 5'-CTA CGT CTG AGG AAA TTC CTG TTG-3' (Tanabe et al., 2007). Prepare a PCR mixture solution with a total volume of 25 µL consisting of PCR master mix (Taq Polymerase enzyme, Taq polymerase buffer, Taq buffer, $MgSO_4$, and DNTP) 12.5 µL, forward and reverse primers (10 µM) 1 µL, DNA template 2 µL and ddH_2O 8.5 µL. (Fadlurrahman et al., 2015). The PCR solution is mixed thoroughly then the amplification process is carried out for 35 cycles, the amplification reaction runs in 6 stages with 3 main stages in each cycle, the reaction begins with a pre-denaturation process at 95°C for 3 minutes, denaturation at 95°C for 30 seconds, annealing at 56°C 30 seconds, elongation at 72°C for 50 seconds, post-elongation 72°C for 5 minutes and end at 4°C (Effendi et al., 2020; Fadlurrahman et al., 2015; Irwandi et al., 2020)

Identification Using Agarose Gel Electrophoresis Method

Make a 1.5% agarose gel solution by mixing 45 mg agarose and 30 ml TBE buffer 1x then heating, after cooling, add 4 µl DNA Dye. Pour the solution mixture into the gel mold then install the electrophoresis comb on the gel then let it sit until it hardens, make DNA marker 100 bp in the first well and PCR amplification samples in the other 8 wells, run electrophoresis at 100 V for 40 minutes and visualize using a UV transilluminator to show the thickness and clarity of DNA base pairs, then document the visualization results (Effendi et al., 2020).

Results and Discussions

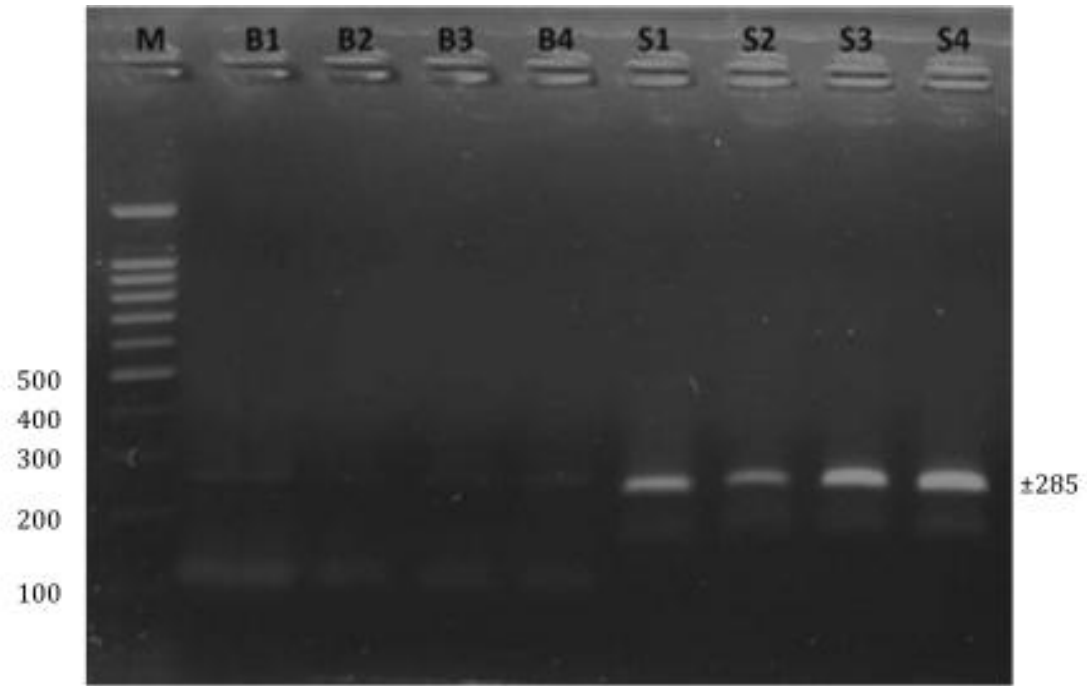


Figure 1. visualization of PCR product results on the electrofotogram

M = Marker/DNA Ladder 100 base pair;
B1-B4 = Ground beef samples with pig primer;
B1-B4 = Ground beef samples with cattle primer.

PCR is a very sensitive method of molecular biology analysis, the sensitivity of PCR is able to detect DNA from a small amount of a sample and then multiply it, the PCR method is a technique

for obtaining a large amount of a particular DNA sequence from a DNA sample by multiplying it into more quantities in a short time (Kadri, 2020). Conventional PCR is a method that uses gel electrophoresis to read the amplification results. The band thickness shown in gel electrophoresis does not prove an accurate amount of DNA, this is because this test only proves the presence of contaminants qualitatively, not quantitatively (Rachmawati et al., 2018). The results of agarose gel electrophoresis showed the appearance of bands which were indicators of the presence of contaminants in ground beef samples.

Based on the results of the electrophoresis test as in Figure 1, samples of ground beef to which cattle primer was added showed the appearance of very clear bands with a base pair length of ±285 bp for codes S1, S2, S3 and S4, while for codes B1, B2, B3 and B4 sample of ground beef mixed with pig primer did not show clear bands. Samples with codes S1 and B1 are the same beef samples, but both were amplified with two different primers, as were the other samples. In this study Codes 1-4 indicate that there were 4 samples of ground beef, then every fourth sample was amplified using pork primer and cow primer to form sample codes S1, S2, S3, S4, B1, B2, B3 and B4. This research shows that there is no pork contamination in the ground beef samples obtained from the Gang Baru Market, Semarang City.

Based on the results obtained, it can be seen that ground beef with the addition of pig primer does not occur annealing. This is because the primer used is not specific so the amplification process does not occur perfectly. the annealing process is the process of attaching primers to DNA isolates, if the primer used is not suitable then the amplification process will not occur (Rahayu & Nugroho, 2015). Research conducted by Zulfahmi (2018) also reported the same results, samples of food products originating from chicken and beef did not occur amplification because the primer used in his research was a specific primer specifically for pigs so the results of electrophoresis visualization did not show bands in samples that did not use specific primer. The next similar research was conducted by Indriati (2021), 5 samples of beef meatballs which were amplified using beef primers in multiplex PCR showed clear band thickness, this means that all beef samples were amplified well using cattle primer while pig primer did not show the presence of amplicon bands.

The primers used in this research are pig and cattle primers derived from the cytochrome B gene. The cytochrome B gene has a diverse DNA sequence so it can be used to differentiate species. The advantages of the mitochondrial cytochrome B gene make it more efficient to use compared to other nuclear DNA, the results obtained from attaching the cytochrome B gene primer are also more specific, thereby reducing mispriming (Zulfahmi, 2018). DNA originating from mitochondria has a high copy number in cells, the primary type of cytochrome B gene has a DNA sequence with large variations so it is suitable for detecting DNA differences between an individual living creature and another individual (Effendi et al., 2020; Tanabe et al., 2007).

The use of conventional PCR does not qualitatively prove the presence of pig DNA contaminants, but this method is quite efficient and sensitive in detecting existing contaminants even though the purity of the sample obtained is below 1.8 (Effendi et al., 2020), the type of primer and optimization of the annealing temperature has an important influence. In the DNA amplification process from ground beef, the types of primers used refer to Tanabe et al. (2007) because this primer is quite accurate in detecting the presence of pig DNA in various types of food (Effendi et al., 2020), the optimal temperature used is the modified temperature referred to Fadlurahman et al. (2015), Irwandi et al. (2020), and Effendi et al. (Effendi et al., 2020), starting from pre-denaturation with a pre-denaturation temperature of 95°C for 3 minutes, denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, elongation at 72°C for 50 seconds, post-elongation 72°C for 5 minutes and end at 4°C. In general, the temperature used in this research

has a range of ±5°C from other research. The annealing temperature, which is the process of attaching the DNA template to the primer, is set at 56°C because this temperature is the optimum temperature for the amplification of pig DNA and cattle DNA.

Conclusion

This research proves that ground beef obtained from the Gang Baru market in Semarang City is not contaminated by pork DNA. The use of two primers for each sample in this study proves that the pig and cattle cytochrome B gene primers are amplified at specific DNA sequences so that no DNA bands are formed if the DNA does not match. This method is good enough to prove the presence of contaminants by utilizing primary sensitivity if there is no positive control of the DNA of the contaminant whose presence is being investigated.

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Conflicts of interest

I have no conflicts of interest to disclose.

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