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Analysis Pork Contamination in Beef Meatballs through Polymerase Chain Reaction in Palu City

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Abstract: The need for halal products is essential, particularly among various processed beef products like meatballs, which are popular among nearly the entire community. These products are susceptible to issues of meat counterfeiting for greater economic gain. This study aims to determine the smallest DNA concentration that can still be detected and to assess whether there is any pork contamination in meatballs circulating in Palu City using Polymerase Chain Reaction (PCR). This test uses four DNA samples obtained from fresh meat to test the specificity of the primer: wild boar, rat, and goat DNA samples as comparisons, and pig DNA as a positive control. The presence of DNA bands in the agarose gel electrophoresis visualization results indicates that DNA amplification has occurred, meaning that the sample can be detected. The analysis results showed that the primer used (Cyt-b) had good specificity for detecting pork DNA, while the absolute pork DNA concentration of 0.01 ng/µL and the reference meatball DNA with a concentration of 0.01% are the smallest limits that can still be detected using PCR. Of the four meatball samples from Palu City, one sample produced a DNA band, indicating that the sample contains or is contaminated with pig DNA. This method is valid as it meets the specificity test and demonstrates a good detection limit.

Keywords: Beef Meatballs; DNA; Halal; Polymerase Chain Reaction

Introduction

The halal aspect is very important to protect buyers, especially Muslims, from counterfeit products containing pork (Wisker, 2021); (Ambali & Bakar, 2014). Pork is the second most common meat in the world after poultry (Lagarde et al., 2024); (Vicente & Pereira, 2024). Meat combination because meat is often crushed and mixed with various ingredients, it is difficult to distinguish with the naked eye (Kim et al., 2023); (Dabija et al., 2021). Food as one of the basic human needs to sustain life must be guaranteed to be safe starting from chemical aspects, cleanliness, healthy, does not contain ingredients that are harmful to health, and also the integrity of halal product (Aminah et al., 2019); (Mabkhot, 2023). One of the important elements in the Muslim's life around the world is the integrity of halal products, one of the products that is vulnerable to halal issues is processed meat products such as meatballs (Windarsih et al., 2022); (Muflihah et al., 2023a).

Beef meatballs are one of the most popular processed meat products in Indonesia (Pranata et al., 2021). This is possible because the price of pork is relatively cheaper and more versatile than beef or chicken (Hermanto et al., 2022); (Magqupu et al., 2023). The importance of halal certification for every product to protect the interests of Muslims in Indonesia (Zulfa et al., 2023)Halal food is food that is good for Muslims

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based on Islamic law, it is stated that the food is permissible and good to be made into a product (Mariyam et al., 2022). Packaged meatball products sold in supermarkets are more likely to have halal labels applied but often customers counterfeit other unwanted meats (Watkins, 2018).

Most of these counterfeiters do not have religious awareness with a business model driven by non-ethnic profits (Shen et al., 2022). Now there have been many ways or methods developed to analyze species contamination in food products. One of them is the Polymerase Chain Reaction (PCR) technique which is precise, specific, and fast (Artika et al., 2022); (Kadri, 2020). PCR is the most commonly used method to detect the presence of species-specific ingredients in food products because of its high sensitivity and specificity. This method is a useful technique for detecting animal products in food (Muflihah et al., 2023b).

Method

This research was conducted experimentally, using reference primers from the PCR method that had been tested. This research was conducted in May-July 2024. The research was conducted at the STIFA Pelita Mas Palu Research Laboratory and the Research and Testing Laboratory of the Faculty of Pharmacy, Muslim University of Indonesia, Makassar. One method that used for DNA-based analysis is Polymerase Chain Reaction (PCR), the final stage of this analysis, where the results of DNA amplification can be seen through agarose gel electrophoresis, while Real Time PCR results can be seen directly on the monitor while the analysis is taking place (Mariyani et al., 2023). Although Polymerase Chain ReactionConventional is usually qualitative and only gives positive or negative results, but has high sensitivity and is believed to be able to detect target DNA. While quantitative analysis can be done with Real Time PCR.

Based on this, a study was conducted using a smaller concentration, namely an absolute sample of 0.001 ng/µL and a reference sample of 0.01% to see the detection limit, resulting in the method having high sensitivity. In addition, an analysis was also conducted on processed meat products circulating in the market, the result of household to medium-scale production, to provide a guarantee of the integrity of halal products circulating in the market as public consumption products using Polymerase Chain Reaction.

Result and Discussion

DNA Isolation Results

DNA isolation was carried out on fresh meat (pork, wild boar, goat, rat), reference meatball samples, and four meatball samples from Palu City using conventional methods (phenol-chloroform extraction). The isolation process begins with cell lysis using a mortar and prestel, followed by incubation at 60°C for one hour. The main principle of isolation involves cell lysis, precipitation with chloroform and ethanol, and purification using Ribonuclease to remove RNA, which produces pure DNA without contaminants (Heikrujam et al., 2020); (Umesha et al., 2016). The success of PCR-based detection depends on the quality of DNA isolation, which is then analyzed for quantity and quality (Aboul-Maaty & Oraby, 2019); (Sophian et al., 2021).

DNA Concentration and Purity Measurement Results

Table 1. Fresh Meat DNA Concentration and Purity

DNA Isolate	Concentration (ng/ µl)	Purity (λ260/λ280)
Lamb	82.82	1.32
Rat Meat	81.85	1.31
Wild Boar Meat	82.30	1.31
Pork	81.62	1.29

Table 2. Meatball DNA Concentration and Purity Reference

DNA Isolate (%)	Concentration (ng/µl)	Purity (λ260/λ280)
0.50	83.37	1.35
0.10	84.22	1.37
0.05	83.40	1.37
0.01	83.35	1.35

Table 3. Concentration and Purity of Meatball DNA in Palu City

DNA Isolate	Concentration (ng/ µl)	Purity (λ260/λ280)
District A	82.25	1.30
District B	85.85	1.37
District C	83.92	1.34
District D	83.02	1.36

Based on Tables 1, 2, 3, the purity and concentration levels of the DNA isolates produced show that the purity of the DNA isolates is below the purity range, which is around 1.3. Good DNA purity is when the comparison value is in the range of 1.8-2.0 (Dewanata & Mushlih, 2021); (Utaminingsih & Sophian, 2022). If the purity value obtained is <1.80, it indicates that contaminants in the DNA are still present in the form of proteins and polysaccharides (Demkina et al., 2023); (Barta et al., 2017); (Zulkarnain et al., 2021). The DNA concentration obtained ranged between 81-85 ng/µL, which is below the normal value for DNA concentration, so the concentration was standardized. With DNA dilution according to the optimal concentration in the PCR

amplifier. To produce a thick DNA band, the optimal concentration for PCR amplification at 30 cycles is 50 g/L (Abdel-Latif & Osman, 2017) ;(Liu et al., 2022). both in comparison pork, wild boar meat, goat meat, rat meat, and reference meatball samples and beef meatballs in Palu City.

DNA analysis by PCR

DNA analysiswith the PCR method is carried out at this stage with the annealing temperature used with the real-time PCR method. The ideal temperature for annealing is 61^0C. At this temperature, the target DNA of pigs, wild boars, goats, mice, and pigs can be identified precisely, for this study, the temperature *annealing* used at 61^0C for 45 seconds, the success of the PCR product depends on the annealing. If the annealing temperature is too low, the primer will attach to the other side of the DNA, causing the resulting DNA to have low specificity. If it is too high, the prime will not attach, which can cause amplification failure (Cheng et al., 2023); (Wang et al., 2024). Next, in the extension phase, the formation of new DNA strands occurs through a polymerization process at a temperature of 72^0C. At this stage, the polymerase process occurs to form new DNA strands, which are produced and used as templates for DNA.

Specificity Test Results

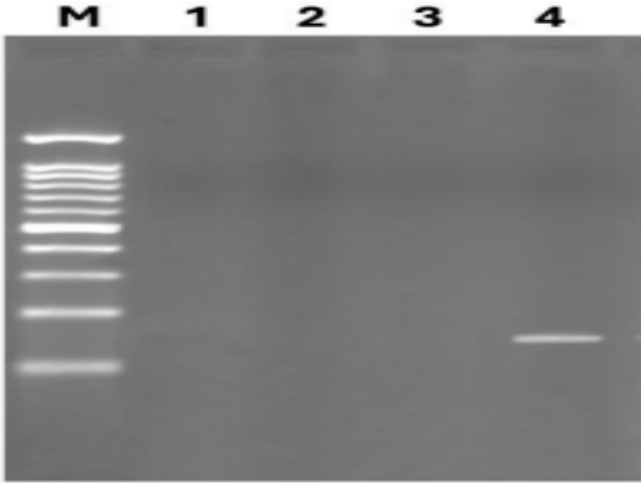

Figure 1. Electropherogram PCR Results Specificity Test Caption: M (Marker); 1. (Goat DNA); 2. (Rat DNA); 3. (Boar DNA); 4. (Pig DNA)

Based on Figure 1**. t**he electropherogram results show the specificity test parameters for the four raw meats, namely pork, wild boar, goat and rat meatvalidated by PCR method using cyt b primersprovide specific primary results where this primer has good specificity, as proven by the fact that only lane 4 (pig DNA) produces DNA bands.

Sensitivity Test Results

In the sensitivity test using absolute pork DNA and reference DNA of imitation meatball samples used one pork DNA diluted in concentrations of 0.05; 0.01; 0.005; and 0.001 µg/µL. This illustrates the ability to determine the amount of template required for the species in question. Based on Figure 2. shown in the four-concentration series that produce bands on column 5withconcentration of 0.05 µg/µL and lane 6 at a dilution of 0.01 µg/µL, and in lane 7 with a dilution of 0.005 µg/µL and lane 8 with a dilution of 0.001 µg/µL no longer produced a band. The band could no longer be seen clearly. This may be because the resulting dilution concentration was too low to be used in the PCR process, resulting in it being unable to amplify (Nwe et al., 2024) ; (Ahmed et al., 2022).

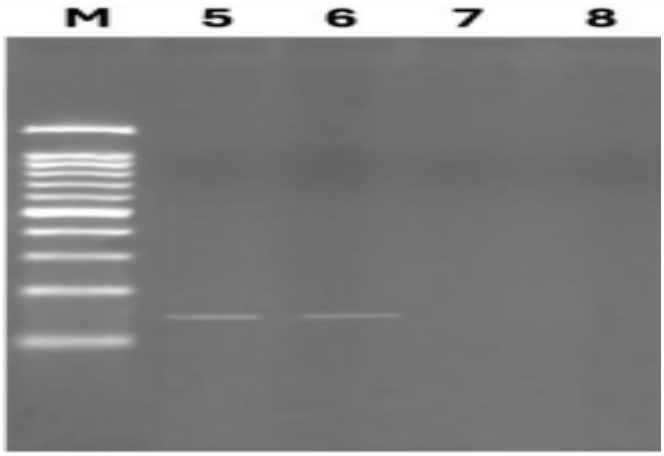

Figure 2. Electropherogram Absolute DNA Detection Limit Test (Pork) Note: M (Marker): 5 (0.05 ng/µL): 6 (0.01 ng/µL): 7 (0.005 ng/µL): 8 (0.001 ng/µL)

After dilution, then make a mixture of pork and beef with a percentage of 0.50%, 0.10%, 0.050%, and 0.01% for the artificial contamination. Artificial contamination is made to the smallest percentage of 0.01%, namely to see the sensitivity of PCR in detecting unintentional cross-contamination (Skouridou et al., 2019); (Hu, 2016); (Rollin et al., 2023). Based on Figure 3. The main sensitivity of the pig primer to identify pig DNA at a concentration of 0.50% to 0.10% is shown in lanes E–H (lanes E and F). While the results of the bands produced from other lanes do not show any DNA bands, because with the reduced concentration of pig DNA contamination, it can no longer be detected.

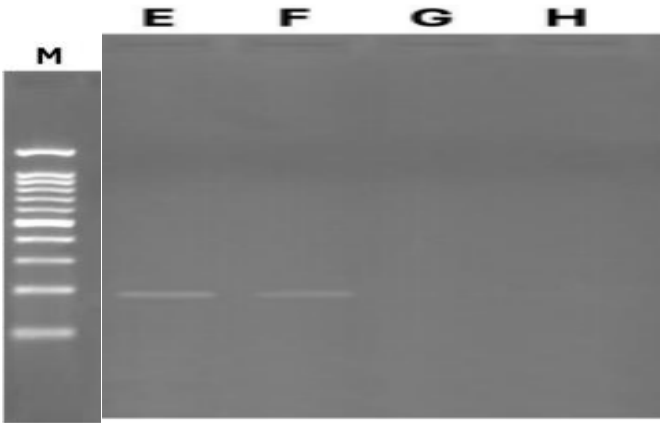

Figure 3. Electropherogram of DNA Detection Limit Test of Reference Meatball (Pork-Beef Mixture) Type: M (Marker) Form. Pig E: 0.5%; F: 0.1%; G: 0.05%); H: 0.01%)

Meatball Sample Test Results

In the examination of beef meatball products taken randomly in the Palu City area from four sub-districts, all test samples were examined using the cyt b primer. Based on Figure 4. shows the test results on meatball samples with electropherograms from the four samples which formed DNA bands, namely in lane A or sub-district A. This shows that the beef meatball sample in Palu City in sub-district A tested positive for pork DNA contamination.

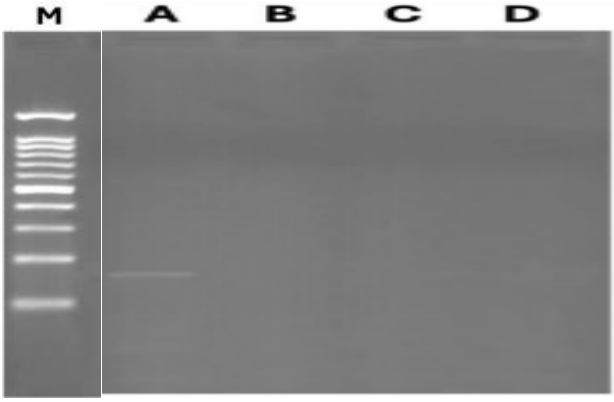

Figure 4. Electropherogram Test for Meatball Sample Contamination Caption: M (Marker) (Meatball sample from District A); District B); District C); District D)

From the analysis that has been carried out, the specificity test showed positive results for the target type and high sensitivity, which means it can detect at small concentrations, indicating that the Polymerase Chain Reaction method is an accurate method and meets validation requirements. The electrophoresis results were visualized using an ultraviolet translaminator. Based on the results the PCR amplification product was observed by agarose gel electrophoresis (Shang et al., 2023), DNA bands were obtained from the DNA isolation results, one band appeared when observed with an ultraviolet transilluminator lamp. The extraction results showed that DNA isolation was successful, so it can be used for further analysis such as PCR amplification.

Conclusion

Based on the results of the research that has been conducted, it can be concluded that: Cyt-b primer has high specificity that can only detect pig DNA. The result of detection limit test is at 0.1% concentration of DNA of meatball reference mixture of beef and pork, and absolute DNA concentration of pork is 0.01 ng/µL as the smallest limit that can still be detected; meatball sample in Palu city was detected to contain pork DNA.

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Author Contributions

Conceptualization, T. J. P, and M; methodology, T. J. P, M, and J. T; validation, R. S. D; data analysis, T. J. P, and M; investigation, T. J. P; resources, T. W. H; data curation, I, and M; writing—original draft preparation, T. J. P; writing—review and editing, T. J. P., M, and J. T; supervision, M. All authors have read and approved the published version of the manuscript.

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Conflicts of Interest

The author declares no conflict of interest.

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