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Analysis of Pork DNA in Processed Meat by Polymerase Chain Reaction

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Abstract: Meatballs and kebabs are processed meat in great demand by the public, made from beef, chicken, or lamb. PCR is an enzymatic method for amplifying DNA in vitro. This study aims to determine whether pork DNA in meatballs and kebabs is analyzed using the PCR method. Sampling was conducted at three meatball outlets and three kebab outlets selected by simple random sampling. The research phase includes the isolation and purification of DNA samples and positive control (pork) using cytochrome b primer and amplification using electrophoresis. The results showed that from the three samples of meatballs, all were positive for pork DNA and from the three samples of kebabs, there was one positive sample containing pork DNA marked with the same band as the positive control (pork) at 132 bp. Therefore, it can be concluded from the six samples of processed meat examined that four samples were positive for pork DNA.

Keywords: DNA, electrophoresis, pork, meatballs, kebabs.

1. Introduction

Processed meat is all meat that has been processed by being salted, preserved, smoked or added with certain food additives to improve the taste or increase the durability or durability of the meat (Asuransi, 2020). Meatballs and kebabs are processed meat products that are in great demand by the public, made from beef, chicken or lamb. Mixing pork in processed meat products is intended to get a big advantage because the price is cheaper than beef or lamb (Nakyinsige *et al.*, 2012). One method that can be used to detect the presence of pork in processed meat products is the Polymerase Chain Reaction (PCR) method. PCR is an enzymatic method to amplify DNA in vitro (Yusuf, 2010). PCR technique can be used to detect pork contamination in food products because this method is fast, specific, and sensitive (Margawati *et al.*, 2010; Tanabe, 2007).

2. Material and Method

2.1. Material

The samples used in this study were 3 meat kebabs, 3 meatballs and one positive control (pork). Sampling is based on a simple random sampling technique, namely sampling by drawing all members of the population. Thus, the numbers that appear in the lottery will be selected as the research sample (Mulyatiningsih, 2011).

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2.2 Method

1. DNA Isolation

A total of 100 mg of the sample was mashed, then put into a tube and added 200 l of digestion solution and then vortexed for 5 seconds. Add 20 l of proteinase K to the tube then votex for 5 seconds and incubate for 1 hour at 56 C at 120 rpm. Discard the pellet and take the supernatant. The supernatant was put into a new tube. Add RNAse 20 l vortec for 5 seconds and incubate for 10 minutes at room temperature. Then add 200 l of Lysis Solution, vortex 15 seconds and add 400 l of 50% ethanol and vortex. Next, transfer the lysate to a new spin column and centrifuge at 6000 rpm for 1 minute. Discard the collection tube and take the spin column and transfer it to a new collection tube then add 500 l of Wash Buffer I, centrifuge 8,000 rpm for 1 minute, add 500 l of Wash Buffer II, centrifuge 12,000 rpm for 3 minutes and discard the spin column. Then add 200 l of elution buffer, let stand for 2 minutes at room temperature, then centrifuged 8000 rpm for 1 minute. Check the DNA template obtained using the Nanodrop Spectrophotometer (Thermo Scientific, 2016).

2. DNA amplification by PCR

The isolated DNA template was mixed with PCR components, namely GoTaq Green Master Mix reagent, forward primer, reverse primer, Nuclease Free Water and DNA template. For each GoTaq Green Master Mix reagent 12.5 l, 1 l forward primer, 1 l reverse primer, 6.5 l Nuclease Free Water and 4 l DNA. Where the positive control DNA template isolated was done the same thing. The total content of the PCR mix was 25 L. Then the tube was placed into the PCR machine and set the PCR program, initial denaturation at 95°C for 3 minutes, followed by 35 cycles consisting of denaturation of 95°C for 30 seconds, annealing of 60°C for 30 seconds, and extension of 72°C for 30 seconds. 30 seconds. Ended extension 72 °C for 5 minutes. The PCR process lasted for 1 hour 25 minutes.

3. Electrophoresis

The PCR results were visualized using electrophoresis. A total of 1.5 g of agarose was dissolved in 100 ml of TBE, then heated in the microwave for 2 minutes until all the agarose was dissolved and the color was clear. Pour into the tray or chamber and insert the electrophoresis comb, let stand until solid then the comb is released. Add TBE buffer until the agarose gel is submerged to 1 mm above the surface of the gel. 5 l ladder was inserted into the first well and 20 l of sample DNA and positive control PCR results were mixed with 5 l of red gel, then the solution was inserted into the agarose gel well (each well was one DNA solution). connected to a power supply with a voltage of 110 volts for 50 minutes. The gel was placed on top of a UV transilluminator and documented with a digital camera. Agarose gel can be visualized using a gel documentation (GelDoc) with a UV transilluminator used to observe the electrophoretic DNA bands (Nurhayati & Darmawati, 2017).

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3. Result and Discussion

3.1 *Result*

The results of the measurement of the purity and concentration of DNA measured by a spectrophotometer can be seen in table 1.

Table 1. Value of Purity and Concentration of DNA

Sample	DNA Concentration	Unit	Purity
B1	48,8	ng/ul	1,98
B2	66,6	ng/ul	1,97
B3	114,7	ng/ul	1,9
K1	42,6	ng/ul	2,00
K2	37,7	ng/ul	2,04
K3	49	ng/ul	2,00
pork	5,5	ng/ul	3,42

The three meatball samples B1, B2, and B3 and the kebab meat samples K1, K2, and K3 showed good purity, while pork (positive control) showed poor purity but could be continued to the PCR stage because seen from the results of electrophoresis there was no smear. The results of detection of pork DNA, followed by electrophoresis, showed that there was pork DNA in the four samples, namely samples B1, B2, B3 and K2 seen from the DNA bands of the samples which were the same as the positive control DNA bands (pork) with a size of 132 bp (Figure 1).

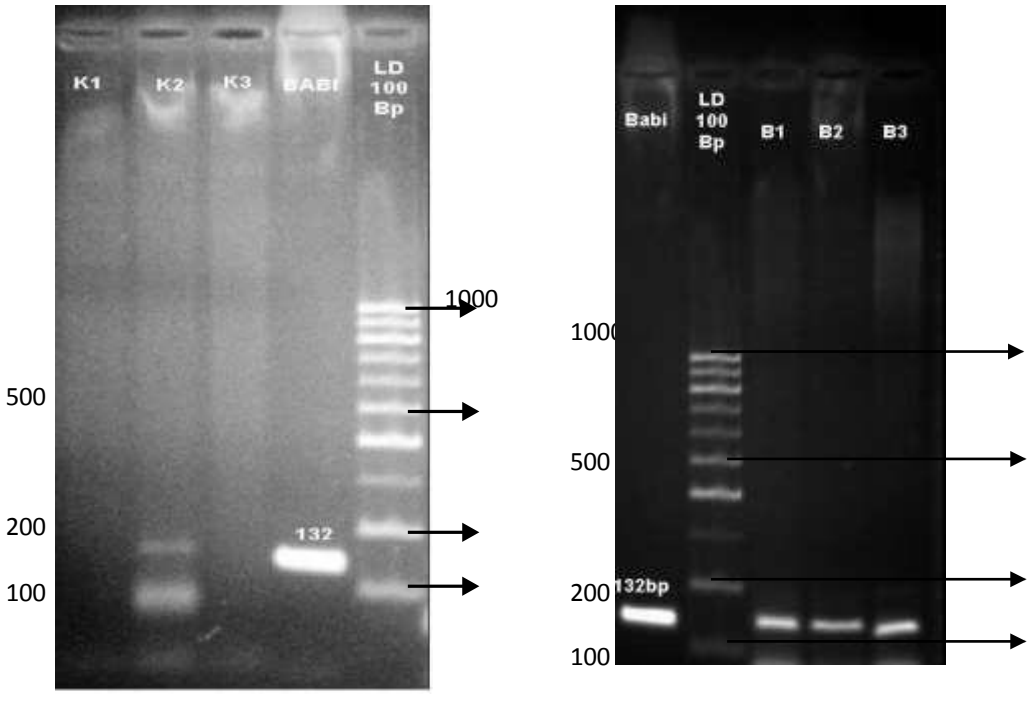

Figure 1. Results of PCR visualization with electrophoresis, (a): sample of kebabs; (b): sample of meatballs

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3.2 *Discussion*

The first procedure performed was the isolation of DNA samples and positive controls. The main purpose of DNA isolation is to separate DNA from other materials such as proteins, fats, and carbohydrates (Nurhayati & Darmawati, 2017). The principle in the DNA isolation process is centrifugation which is the main principle for separating substances based on molecular density (Faatih, 2009). Isolation begins with the refinement of each sample, and positive control. It aims to break the tissue mechanically so as to free the cells contained in the sample. After the mechanical breakdown of the tissue, a chemical lysis process is carried out on the tissue with the addition of a digestion solution solution which functions to decompose the sample (Nayasilana *et al*., 2010). Next, a deproteinization process is carried out using the proteinase K enzyme which aims to purify DNA from protein contaminants. To remove the RNA contained in the isolated sample and positive control, RNAse solution can be added (Nurhayati & Darmawati, 2017). Then add a lysis solution that serves to destroy cell walls or membranes (Harisah, 2017). The addition of ethanol serves for the DNA washing phase to remove the salt contained in the sample (Nurhayati & Darmawati, 2017). Wash buffer functions for the DNA washing process which aims to allow DNA that is still attached to the spin column to reach the bottom of the tube. Furthermore, an elution buffer solution is added which functions to produce DNA purification (absolutely pure DNA) (Theodorus, 2021). So that the DNA template is obtained.

After obtaining the isolated DNA template, it can be checked against the concentration and purity of the DNA obtained using a Nanodrop Spectrophotometer. The working principle of the Nanodrop Spectrophotometer is that pure DNA is able to absorb ultraviolet light due to the presence of purine and pyrimidine bases. The results of the nano drop test are the value of DNA purity on A260/A280 and the value of DNA concentration. Nanodrop results can be seen in Table 1. The purity of the six samples of meatballs and kebabs showed good results because they were in the range of 1.8-2.1 (Hikmatyar *et al.*, 2015). Meanwhile, the positive control sample (pork) showed poor purity, which was above the range of 2.1 but could proceed to the PCR stage which was marked by the absence of a smear on the electrophoresis results. Smears can be caused by the presence of contaminants such as protein or carried away by the rest of the solution in the isolation process (Iqbal *et al.*, 2016). A DNA purity value below 1.8 indicates that isolated DNA still contains contaminants in the form of protein compounds. Contamination in the form of protein compounds in DNA can be caused by the absence of the addition of protease enzymes in the DNA isolation protocol. While the DNA purity value above 2.0 indicates that there are still contaminants in the form of RNA. This could be due to not adding ribonuclease (Harahap, 2018).

The next step is to mix the isolated DNA template with PCR components, namely GoTaq Green Master Mix reagent, forward primer, reverse primer, Nuclease Free Water and DNA template. The PCR results were visualized using electrophoresis. The purpose of electrophoresis is to separate cellular molecules based on their size, using an electric field that is applied to a medium containing the sample to be separated (Nurhayati dan Darmawati, 2017). Electrophoresis using agarose gel is the standard method used to separate, identify and purify nucleic acids. The advantages of this gel are that it is easier, simpler and the rate of separation is faster to form fragments and is not toxic (Iqbal *et al*., 2016). The percentage of agarose used depends on the size of the fragment to be examined, normally the concentration of agarose gel is in the range of 0.2% - 3% (Nurhayati dan Darmawati, 2017). In this study, 1.5% agarose gel was used because the concentration commonly used to visualize using agarose gel . The greater the concentration of agarose gel, the sample will migrate more slowly than the lower concentration because the pores of the gel are getting smaller (Sambrook *et al*., 2001).

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Agarose gel can be visualized using a gel documentation (GelDoc) with a UV transilluminator used to observe the electrophoretic DNA bands (Nurhayati dan Darmawati, 2017). From the results of the study the positive control band was found at 132 bp and samples B1, B2, B3, and K2 were seen in the same DNA band as the positive control, namely at 132 bp, it can be concluded that samples B1, B2, and B3 were positive for pig DNA (Fig. 1). The band produced by the K2 sample was not as good as the positive control band, samples B1, B2, and B3, because the K2 sample contained smears. This smear can be caused by the presence of residual solution carried during the isolation process.

4. Conclusion

From six samples was examined, it can be concluded that the three samples of meatballs and one meat kebab were positive for pork DNA. Where samples B1, B2, B3, and K2 were seen in the same DNA band as the positive control at 132 bp.

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