DETECTION OF CYTOCHROME B PORK IN PACKAGED MEATBALL FROMTRADITIONAL MARKETS AROUND JAKARTA

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Abstract

Meatballs are one of the favorite foods for Indonesian people. Meatball products are generally known to be based on beef. But there are cases that changed the basic ingredients of meatballs, beef into pork. This research was conducted to detect the presence of pork in packaged meatball products sold at Traditional Markets. Identification is carried out with the principles of molecular biology in the form of the PCR process. Meatball samples were previously subjected to DNA extraction. PCR was performed to determine pork genes using cytochrome b primers with an amplicon length of 130 bp. The cytochrome b gene can be found in every animal cell, but it has a specific characteristic that is only present in certain animal cells. Based on PCR results it is known that most of the samples did not contain pork marked by the absence of a DNA band that appeared and parallel to the 130 bp band on the ladder. However, there are two samples that appear DNA bands and are thought to contain pork. These results are followed by a sequencing process to clarify the results of the PCR process.

Keywords: Halal, Meatballs, Cytochrome b

1. INTRODUCTION

Meatballs are one of the meat-based processed foods that are popular in Indonesia. However, until now still found the use of pork for making meatballs. Some sellers mix beef and pork in meatballs to get around the high price of beef. This is done by traders in the Citereup area, Bogor, West Java. The high price of beef, makes the perpetrators make pork-based meatballs so they can sell it at a cheaper (Bempah, 2017).

Research on halal food by identifying the presence of the cytochrome b pig gene has been carried out. The cytochrome b gene is widely used because there are mutant variations so that in the same gene there are regions that are almost similar for each animal type and a more specific area for one type of animal. This shows the special characteristics possessed by cytochrome b, so that many of them are used as markers of certain animals (Primasari, 2011). Therefore, this study was conducted to determine the presence of pork in packaged meatball products sold in traditional markets. The identification process was carried out by conducting PCR to detect the presence of the pig Cytochrome b (Cyt b) gene in meatballs.

2. MATERIAL AND METHODS

2.1. Sample Preparation

This research was implemented at Molecular Laboratory, Al Azhar University in Indonesia, South Jakarta. This activity is carried out in February to December 2019. Meatballs was obtained from three market in Jakarta and Bekasi.

Table 1. List of meatball sample

Market	Sample
Market 1	P1B1
	P1B2
	P1B3
Market 2	P2B1
	P2B2
Market 3	P3B1
	P3B2
	P3B3
	P3B4

2.2. Tools and Materials

The tools used in this study are micro pipettes [PIPETMAN] (1-10 µL, 20-200 µL, and 100-1000 µL), microcentrifuge, vortex, shaking waterbath, electrophoresis, PCR, DNeasy Mericon Food Kit [QIAGEN] , UV transluminator.

Materials used include fresh beef, fresh pork, meatballs, liquid nitrogen, loading dye, ladder [GENEAID], proteinase K [BIOLINE], agarose, TAE buffer, buffer lysis (Tris-HCl pH 8, EDTA pH 8, NaCl 0.4 M), NaCl 6M, SDS 20%, isopropanol, ddH2O, pork cyt b primer, chloroform, 70% ethanol, MyTaqTM HS Red Mix [BIOLINE], Vi Safe Red Gel Stain.

2.3. Pork and Beef DNA Extraction

Meat that has been cut by ± 1 cm, crushed by adding liquid nitrogen. Lysis buffer 400 mL was added (10 mM Tris-HCl pH 8, 2 mM EDTA pH 8, 0.4 M NaCl) and homogenized using vortex. After that, 40 µL of 20% SDS and 20 µL of Proteinase K were added, then incubated in a water bath for 1 hour at 65 0C. 300 M NaCl of 300 µL was added and vortexed for 30 seconds. Samples were centrifuged for 30 minutes at 10,000 g. The supernatant was transferred to a new centrifugation tube and added 400 µL of isopropanol and then vortexed. Then, incubated at -20 ^{0}C for 1 hour and then, centrifuged for 20 minutes at 10,000 g. Pellet is taken and added 300 µL of 70% ethanol. The ethanol was removed and the pellet was dried, then added 100 µL of ddH2O.

2.4. Meatball DNA Extraction with Kit

Approximately 200 mg of sample is crushed by adding liquid N2 to freeze the sample, so that it is easy to smooth. The refined sample was put in a 2 ml microtube and added 1 ml Food Lysis Buffer and 2.5 µL Proteinase K solution, then vortexed. Then, incubated for 30 minutes in a shaking waterbath at 60 ^{0}C. After that, centrifuged for 5 minutes at 2,500 g. 700 µL supernatants were mixed with 500 µL chloroform and vortexed for 15 seconds, then centrifuged for 15 minutes at 14,000 g. The top phase of centrifugation results are taken and mixed 1 ml of PB buffer then vortexed. A total of 600 µL of the mixture was put in a QIAquick spin column and centrifuged for 1 minute at 17,000 g, then the liquid was discharged. The tube was refilled as much as 600 µL from the rest of the previous mixture and centrifuged at 17,000 g for 1 minute, then the liquid was discharged. AW2 buffer was added 500 µL and centrifuged at 17,000 g for 1 minute, then the liquid was discharged. The tube was centrifuged again with the same time and speed to dry the membrane. Then, the tube was added with 100 µL EB buffer and incubated for 1 minute at room temperature 15 to 25 ^{0}C then centrifuged for 1 minute at 17,000 g to elute.

2.5. PCR reaction

The isolated DNA was amplified by PCR. The total PCR reaction volume was 33.5 µL consisting of 20 µL DNA templates, 0.5 µL forward primers, 0.5 µL reverse primers, and 12.5 µL MyTaq HS Red Mix. The primers used in this study were the specific primers of the pig cyt b gene (Tanabe, et.al 2007) with the forward sequences 5'-AAC CCT ATG TAG GTC GTG CAT-3 ' and reverse sequences 5'-ACC ATT GAC TGA ATA GCA CCT-3 '. The conditions of the PCR stage used are pre-denaturation for 3 minutes at 95 ^{0}C, and denaturation for 10 seconds at 95 ^{0}C, annealing for 15 seconds at 62 ^{0}C, extension for 30 seconds at 72 ^{0}C in 35 cycles .

PCR products were subjected to electrophoresis in 1% agarose gel (w / v) using 1X TAE buffer for 30 minutes, 100 V and stained by ViSafe Red. The gel electrophoresis results were then observed under UV light. The positive control used in this amplification is the result of isolation of pig DNA (*Sus* sp.) as reference DNA.

3. RESULTS AND DISCUSSION

3.1. Results of DNA Extraction of Meatballs

Figure 1 shows the results of DNA isolation from meatballs. All DNA bands was thin and smear. The results of meatball extraction DNA electrophoresis in (Figure 1) showed a smear of DNA bands. Smear results that occur allegedly because during the processing of meat made into meatballs and the presence of additional ingredients such as flour and seasonings contained in meatballs cause DNA quality to decrease (Fibriana, *et.al* 2012).

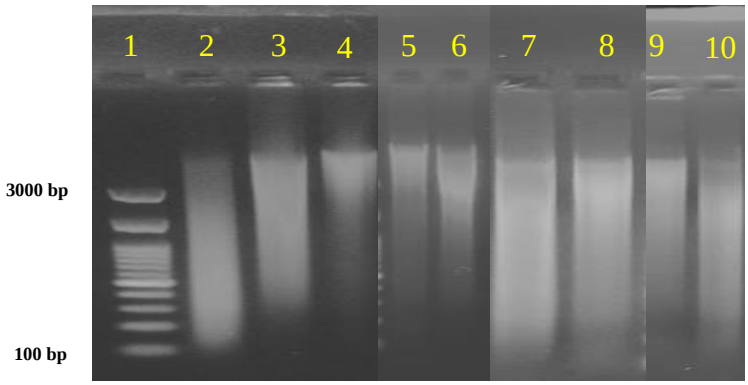

Figure 1. Meatball extraction results; 1. ladder; 2. P1B1, 3.P1B2, 4.P1B3, 5.P2B1, 6. P2B2, 7. P3B1, 8.P3B2, 9.P3B3, 10.P3B4.

3.2. PCR Optimization Results

The isolated DNA was then carried out by PCR. PCR uses specific cytochrome b pig primers (Tanabe, et.al 2007) with a base length of 130 bp. PCR optimization needs to be done before isolated DNA samples are amplified. This optimization process is carried out on the temperature and time of amplification and the number of PCR cycles. The PCR stage is performed based on MyTaq Mastermix by adjusting the annealing temperature, the length of time at each stage, and the number of cycles to be performed. The results of PCR optimization can be seen in (Figure 2).

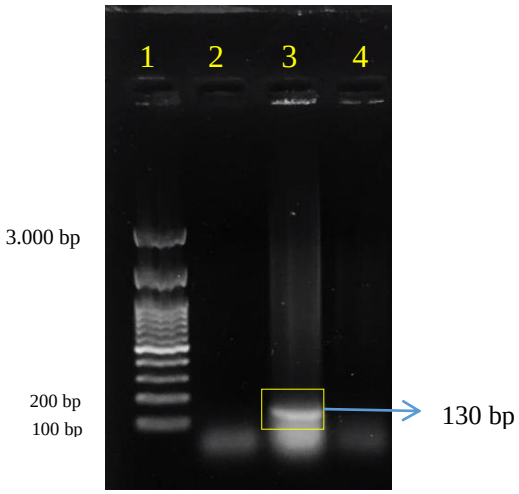

Figure 2. Results of cytochrome b optimization; 1. ladder, 2. non-template control (ntc), 3. fresh pork, 4. fresh beef.

The PCR temperature at the denaturation stage is around 93 - 95 ^{0}C within 30 - 90 seconds depending on the length of the DNA template used and the length of the target DNA. If the denaturation temperature is too high and the time used is too long, it will damage the DNA template while the temperature is too low and the time is too short will cause an imperfect

denaturation process. Annealing temperatures are usually used around 37 - 60 ^{0}C. Annealing temperature is related to primary Tm. The time of annealing stage depends on the length of the primary base. If the primer has a length of 18-22 bases, it can be done within 30 seconds. Whereas primers with a base length of more than 22, are carried out within 60 seconds. The extension stage is carried out at 72 ^{0}C which is the optimum temperature of DNA polymerase in the PCR process. Extensions are generally carried out for 30-60 seconds (Handoyo and Rudiretna 2001).

3.3. Meatball PCR Results

Samples of meatballs that have been isolated DNA, then carried out the PCR process. After the PCR results were visualized using UV light (Figure 3).

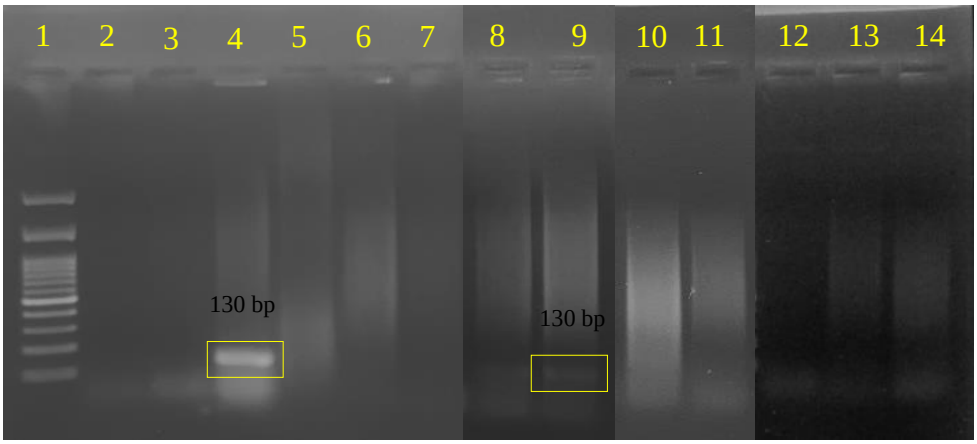

Figure 3. Results of PCR sample of meatballs; 1. ladder, 2. ntc, 3. fresh beef, 4. fresh pork, 5.P1B1, 6.P1B2, 7.P1B3, 8.P2B1, 9.P2B2, 10.P3B1, 11.P3B2, 12. P3B3, 13.P3B4.

It was seen that almost all samples did not contain pork. This is indicated by the formation of the DNA band especially on 130bp bases. However, in P2B1 and P2B2 samples, there was a thin DNA band at 130 bp. These results indicate the possibility of pork content in these meatball products. However, further testing is needed in the form of sequencing to ensure the truth of the PCR results.

4. CONCLUSIONS, SUGGESTIONS, AND RECOMMENDATIONS

The samples tested in this study mostly do not contain pork, but there are two samples that are likely to contain pork, so it is necessary to do sequencing and PCR amplification in Real Time to find out the truth of the results.

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