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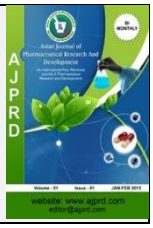

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Research Article

Polymerase Chain Reaction Analysis of Pork DNA in Unauthorized Jamu and Fish Oil Capsule Shells

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A B S T R A C T

Capsules for pharmaceutical dosage forms are generally manufactured from beef gelatin. However, several reports claimed the alleged misuse of pork gelatin was improperly used in producing illegitimate capsules for the Muslim community. This study aimed to analyze pork DNA in unregistered jamu and fish oil capsules using the Polymerase Chain Reaction (PCR) method. The sample was isolated to obtain pure pork DNA and amplified using a specific primer to generate millions of DNA using the multi-step PCR method. The PCR product was analyzed to identify the pork DNA's existence via gel electrophoresis in 1.5% agarose. The analysis showed that several samples were positive at 132 bp compared to reference pork DNA. The result of this study revealed that unregistered products should be evaluated and followed up on to prevent product abuse.

Keywords: Capsule, DNA, PCR, Pork

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INTRODUCTION

Deoxyribose Nucleic Acid (DNA) is a hereditary material found in almost all living things. Most of the DNA is located in the cell's nucleus, but a small amount can also be found in the mitochondria. DNA can replicate or make copies of it. DNA contains genetic information related to the appearance of the phenotype.DNA is a carrier of heredity from parents to offspring and also functions in protein biosynthesis[1-3].

Gelatin is a protein with water-soluble properties from natural collagen derived from cattle, pork, and fish skin and bones.To beconsidered halal, gelatin must be produce dusing only halal ingredients and must not containanytraces of non-halal ones.Producers prefer pork gelatin due to its low cost and ease of preparation[4-7]. Gelatin is typically made from animal parts like cow bones, cow skins, and pig skins. Gelatin made from cowhide is known as type B gelatin, while gelatin porkis commonly known as type A gelatin. In general, the halal status of most capsules is questionable because their shells are typically made of gelatin, the majority of which comes from pork[8].

Indonesia is a country that is predominantly Muslim. Muslim consumers must consume halal products, which must be labelled with a halal logo[9-11].The government should provide protection and guarantee the halalness of products consumed by the public. This is based on the Law of the Republic of Indonesia No. 33 of 2014 concerning Halal Product Guarantee and Government Regulation of the Republic of Indonesia No. 39 of 2021 concerning implementing the Halal Product Assurance Sector[12].

Therefore, the Muslim community in Indonesia needs to pay attention to whether the authorized institution has registered the drugs to be consumed or not.In determining the halalness of a pharmaceutical and food product, analytical methods with high accuracy and sensitivity can be used to detect the presence or absence of pork

ingredients in food or gelatin, such as the Polymerase Chain Reaction method. The procedure begins with the chemical isolation of DNA from the sample, which is then subjected to polymerase chain reaction (PCR) processing to generate millions of copies of DNA, followed by electrophores is to determinethe DNA bands and the number of basepairs, and finally a comparison with positive controls.

METHODS

Sampling

The samples used were herbal capsule shells (hard capsule shells) and fishoil (soft capsule shells) that were not registered. Sample collection was carried out on five herbal capsules marked with samples A, B, C, D, and E with different uses and manufacturers. Four samples were taken for fishoil capsule shells spreading in e-commerce and pharmacieson the market, which were marked with samples 1, 2, 3, and 4.

Sample Preparation

The capsule shell was weighed, and then the herbal capsule shell and fish oil sample were broken into smaller pieces so that the shell dissolved more quickly. The body was freed from any remaining herbs or fish oil by wiping the inside of the cover with a tissue and then placing each sample into a 1.5 mL micro centrifuge tube. A 25-mg weight for pork was used as a positive control. The meat should be cut into smaller pieces using sterile scissors to facilitate the dissolution process during incubation. Then transfer the mixture to a 1.5 mL micro centrifuge tube.

DNA isolation& purification

The DNA purification process was carried out using a Thermo Scientific GeneJET Genomic DNA Purification Kit® involving four methods: lysis, binding, washing, and elution. The DNA purity, therefore, was evaluated by using a nanodrop spectro photometer.

DNA Amplification and Visualization

PCR amplification was performed with the GoTaq® Green PCR Mix procedure, as shown in Table 1

Tabel 1: PCR reaction composition

No.	PCR Components	Initial Concentration	Final Concentration	Volume (µL)
1.	Go Taq Green Master Mix Promega	2x	1x	12,5
2.	Primer Pork-F	10µM	0,4µM	1
3.	Primer Pork-R	10µM	0,4µM	1
4.	ddH_2O	-	-	6,5
5.	DNA	-	-	4
Final Volume				25

The PCR process in this study was set up for 35 cycles via a multi-step amplification process, including: initial denaturation (95^{o}C3 minutes); further denaturation (95^{o}C, 30 seconds); annealing (60^{o}C 30 seconds); elongation (72^{o}C 30 minutes); final elongation (72^{o}C 5 minutes). The amplified PCR results were mixed with 5µL GelRed 1x and electrophoresed using 1.5% agarose gel in 0.5x TBE buffer at 110 volts for 50 minutes and eventually visualized via gel documenter to identify the DNA migration.

RESULT AND DISCUSSION

Five herbal and four fish oil capsules without a halal logo and not certified halal were studied to evaluate pork DNA contamination in capsule shells. This study initially isolated the corresponding DNAs from capsule shells following the Gene JET Genomic DNA Purification Kit® by Thermo Scientific. Despite the low isolated DNA purity, the amplification process continued at the PCR stage because impure DNA would not interfere with the amplification process in PCR[13]. This is supported by research conducted by Erwanto and coworkers[14]. There was a smear on the visualization results of DNA extraction from meatball samples on the market, but it was still reliable and did not interfere with the PCR process.

The double-stranded DNA was denatured into single strands by subjecting it to the PCR process, which included the pre-denaturation or initial denaturation, at a temperature of 95^{o}C for 3 minutes, as instructed by the kit. For more uniform and precise outcomes, additional denaturation was performed at 95°C for 30 seconds to convert any remaining double strands into single strands. We used a denaturing temperature of 92^{o}C–95^{o}C because at lower temperatures, the double-stranded DNA might not open, and the primer might bind to the wrong side of the strand. If the temperature is too high, the primer may not bind to the DNA strand, and the amplification process will fail[15].

Denaturation is crucial, but a long denaturation time can harm the enzymes in the master mix, which can affect the success of the PCR[16].Then the temperature was lowered to 60^{o}C for 30 seconds, where the primer was attached to the DNA and specifically cleaved. In the annealing stage, the temperature was reduced, and primers were bonded to the DNA strand at both the beginning (forward) and the end (reverse).

After preheating to a comfortable 21°C, the temperature was raised to 72°C for half an hour. Primers and enzymes in a master mix could then extend the DNA strand to the correct length for the replicated sequence, yielding two new DNA strands. Final elongation at 72^{o}C for 5 minutes was the last step in PCR-based DNA amplification. Itgives the remaining unreacted enzyme time to finish the reaction and ensures that the synthesis of new DNA proceeds without a hitch[16].

In this experiment, a 35-time cycle was used. The amount of DNA that could be replicated on each cycle was 2^n. The DNA doubling process, or the PCR principle, was used as a model here to allow for flexible bicycle selection. Once the doubling of the DNA strand was complete, the sample could be used for electrophoresis immediately, or it could be frozen at -20 degrees Celsius to prevent the DNA from degrading in the interim.

An agarose gel of 1.5 per cent was used to examine the amplification results. The concentration of agarose gel to be used is an essential factor. During electrophoresis, the DNA migration rate was significantly impacted by the agarose gel's focus[15]. The lower the agarose concentration, the more precisely the DNA fragments can be separated by size, and the more compact the gel matrix. This location carried out two iterations of the electrophoresis procedure. Bands with values of 132 bp or higher, such as those seen in models B, C, D, and E, as well as in samples 1, 2, and 4, indicate the presence of DNA from pork; these samples can be compared to the positive control. On the other hand, neither Sample A nor Sample 3 shared the same band as the positive control, which led to the conclusion that the samples contained pork DNA.

The genomic DNA electrophoresis data showed not only the expected DNA bands but also the presence of smears that looked thick and had been carried away. Smears form due to contaminants such as proteins separated from the rest of the solution during isolation and DNA degraded during separation[17,18]. Therefore, improved agarose gel conditions with a greater quantity of each nutrient content are required to repeat the electrophoresis process and ensure better quality DNA results are obtained.

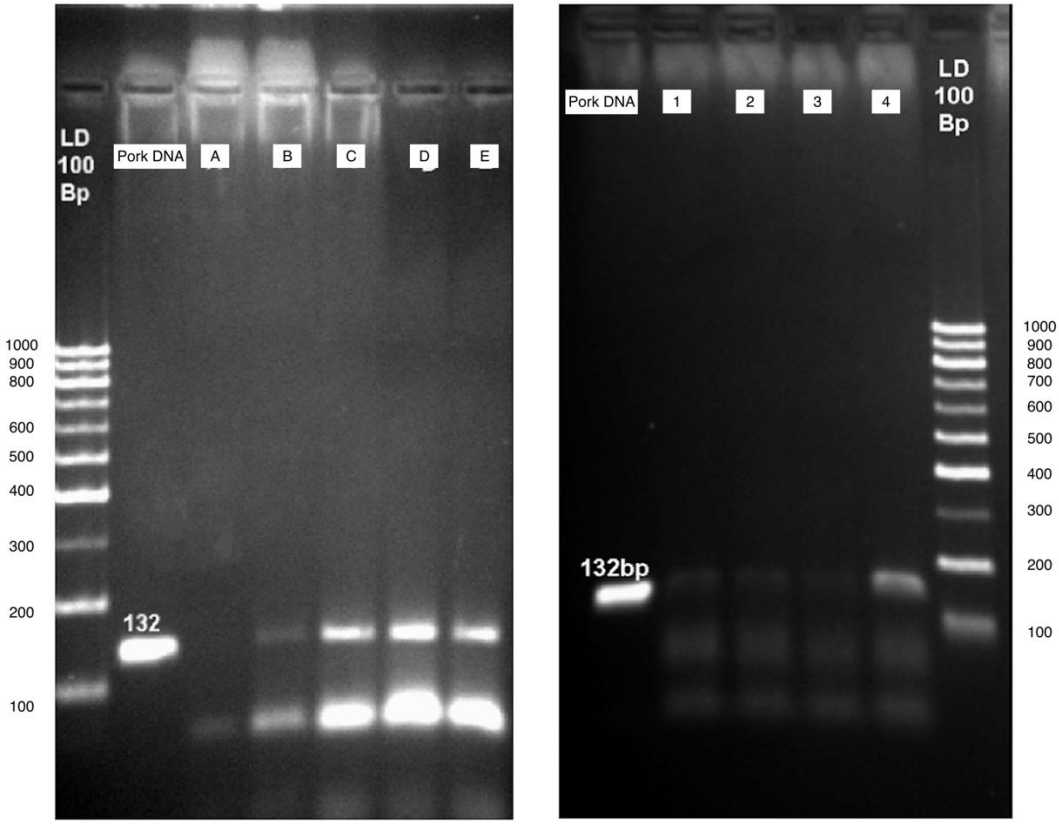

Figure 1: Results of the electrophoresis with 20µL PCR product

CONCLUSIONS

Researchers discovered that some of the capsules used in the study were made with pork gelatin. Bands with values of 132 bp or higher, as seen in models B, C, D, and E, and samples 1, 2, and 4, indicate the presence of pork DNA; these samples can be compared to the positive control. In contrast, neither Sample A nor Sample 3 shared the same band as the positive control, suggesting neither sample contained pork DNA.

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