



DETECTION OF PORCINE SPECIES DNA ON MEAT PROCESSED FOOD SAMPLES (SHREDDED MEAT) USING REAL-TIME PCR

DETEKSI DNA SPESIES PORCINE PADA SAMPEL PANGAN OLAHAN DAGING (ABON DAGING) MENGGUNAKAN REAL-TIME PCR

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ABSTRACT

DNA detection of porcine species in processed meat samples (shredded meat) was carried out as an effort to enrich the literature and reference alternative testing methods in the control of halal products circulating in Indonesia. The purpose of this study was to detect the DNA of porcine species in samples using real-time PCR. The research method used is a qualitative technique. This technique analyzes the CT value of the sample compared to the positive control by looking at the formation of the sigmoid curve if amplification occurs. The controls used consisted of negative control, positive control, other DNA control, and LOD control. The extraction technique used is the column centrifuge technique. The extraction results were then measured using a nano photometer to see the purity and concentration of the extracted DNA. Based on the research, it is known that the extraction results read on a nanophotometer at a wavelength of A260 / A280 show a concentration of 24,350 with a purity value of 21,164. For sample testing, the results of real-time PCR analysis showed the sample was detected at CT 39.77, positive control at CT 31.66, and LOD at 28.32. for negative control and specificity using other DNA was not detected. The conclusion of this research is that the sample used can be detected by the presence of porcine DNA.

Keywords: DNA, PCR, Porcine, Spesific, Species

ABSTRAK

Deteksi DNA spesies porcine pada sampel pangan olahan daging (abon daging) dilakukan sebagai salah satu upaya memperkaya literatur dan rujukan metode pengujian alternatif dalam pengawasan produk halal yang beredar di Indonesia. Tujuan dari penelitian ini adalah mendeteksi DNA spesies porcine pada sampel yang digunakan menggunakan real-time PCR. Metode penelitian yang digunakan adalah teknik kualitatif. Teknik ini menganalisis nilai CT sampel yang dibandingkan terhadap control positif dengan melihat terbentuknya kurva sigmoid jika amplifikasi terjadi. Kontrol yang digunakan terdiri atas kontrol negatif, kontrol positif, kontrol DNA lain dan kontrol LOD. Teknik ekstraksi yang digunakan adalah teknik *centrifuge column*. Hasil ekstraksi kemudian di ukur menggunakan nano photometer untuk melihat kemurnian dan konsentrasi DNA hasil ekstraksi. Berdasarkan penelitian diketahui bahwa hasil ekstraksi yang dibaca pada nanophotometer pada panjang gelombang A260/A280 menunjukkan konsentrasi 24.350 dengan nilai kemurnian 21.164. untuk pengujian sampel, hasil analisis real-time PCR menunjukkan sampel terdeteksi pada CT 39.77, control positif pada CT

31.66 dan LOD pada 28.32. untuk kontrol negative dan spesifitas menggunakan DNA lain tidak terdeteksi. Kesimpulan pada penelitian ini adalah sampel yang digunakan dapat dideteksi adanya DNA porcine.

Kata kunci: DNA, PCR, daging babi, spesifik, spesies

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INTRODUCTION

As a country with the largest Muslim population in the world, testing of pork DNA-based food ingredients to detect halal food from processed meat is a challenge in opening free trade that makes foreign food easy to enter. Therefore, many testing techniques are needed to detect the DNA of porcine species on the halalness of a product.

The porcine-specific DNA testing technique have different challenges. One of them is the use of different basic materials that will affect the selection of the instrument to be used. In some analyzes with low target DNA concentrations, good results depend on the extraction step. In addition, there are several popular testing techniques used in the detection of pig DNA that are often used. Several studies on the detection of porcine DNA have been carried out using various instruments and methods, such as gas chromatography mass spectrometer with headspace analyzer (GCMS-HS) (Nurjuliana *et al.*, 2011), polymerase chain reaction (PCR) (Soares *et al.*, 2013; Kim *et al.*, 2016; Al-Kahtani *et al.*, 2017; Perestam *et al.*, 2017; Cai, *et al.*, 2012; Erwanto, *et al.*, 2018; Soedjono, 2004), and liquid chromatography-mass spectrometry (LC-MS) (Kleinnijenhuis *et al.*, 2018).

With so many studies to carry out porcine DNA detection tests, this study aims to enrich porcine testing references using real-time PCR on samples of processed meat food (shredded meat), so that in the future these various test references can be used as needed in similar studies.

METHODOLOGY

Materials

The material in this research was processed meat food products made from pork. The genome DNA was extracted using DNeasy mericon Food Kit (Cat.No./ID: 69514) [Qiagen] and the master mix for PCR proses was used Mericon Pig Kit (paint.No.292013) [Qiagen].

Sample preparation

A total of 0.5 gram of the sample was added 700 μ l of Food Lysis Buffer. Then 20 μ l of proteinase K was added and homogenized with a vortex for 10 seconds. Sample was incubated at 70°C for 60 minutes and centrifuged at 1400 rpm. The stage was continued by lowering the sample temperature by leaving it at room temperature for 30 seconds. After cooling, the sample was centrifuged at 1400 rpm for 10 minutes. Sample that has been centrifuged will form 2 phases. Carefully pipette 500 μ l of chloroform into a new 2 mL tube. Then remove 700 μ l of clear layer without touching the precipitate at the bottom of the tube. Put it in a tube containing 700 μ l of chloroform and vortex for 15 seconds then centrifuged at 14000xg for 15 minutes. Take 350 μ l of the clear layer and place it into the Qiacube using the standard method with 100 μ l of EB elution buffer. The eluted DNA can be used directly for real-time PCR processing or stored at -20°C or -80°C for extended storage (Sophian, 2021).

Qiacube setup

The initial stage starts from inputting the Qiacube protocol for the Dneasy Mericon Food Kit. All systems are carried out automatically using a robotic system so that people's involvement is only during the initial lysis stage. The protocol used is the extraction of total DNA from raw or processed food material using standard methods. The 350 μ l sample was pipetted into a 2 mL tube and placed into the



Qiacube. After that, proceed with arranging the kit that will be used according to the protocol map. After all the results are appropriate, then the tool is run (Sophian, 2021).

Purity and concentration analysis

Analysis of purity and concentration was performed using a nano photometer NP80 (IMPLEN). Method setting; Nucleic acid, dsDNA type, nano volume mode, 2 µL sample volume, nucleic acid factor 50.00, background correction 320 nm, air bubble recognition off, manual dilution factor 1.000 (Sophian, 2021; Sophian and Syukur, 2021).

Master Mix Setup

The master mix used is a type of mericon pig (Qiagen). This type of kit is a commercial kit that has mixed the master mix and primer at the same time so that it can be used immediately. Instructions for mixing the master mix are presented in table 1.

Table 1. Setup of sample and control reaction (Qiagen, 2013).

Component	Sample	Positive PCR Control	Negative PCR Control	Specificity	LOD
Master Mix	10 µl	10 µl	10 µl	10 µl	10 µl
Template DNA	10 µl	-	-	-	-
Positive Control	-	10 µl	-	-	10 µl
RNA Free Water	-	-	10 µl	-	-
Other DNA	-	-	-	10 µl	-
Total Volume	20 µl	20 µl	20 µl	20 µl	20 µl

Note: For LOD using a positive control that has been diluted 10 times than the standard positive control used

Negative Control / No Template Control (NTC)

The negative control used is NTC. This stage is carried out to see the performance of the master mix used. If NTC is implied, a sigmoid curve will appear. If this happens, there is a possibility that the master mix used is contaminated. And if NTC is not detected, it can be concluded that the master mix used is not contaminated.

Positive Control

The positive control used is the positive control that comes from the master mix package. This stage will be used as a comparison to the sample to show a positive interpretation result. Positive controls that are the target of the test should be amplified.

Specificity

The specificity in this test aims to see the suitability between the kit used and the expected target. Specificity is created by replacing the template used with a template derived from another DNA. In this test, other DNA used is bovine DNA.

LOD

LOD was made by replacing the sample DNA template with a positive control that had been diluted 10 times. The purpose of this stage is to see the sensitivity of the tool in detecting target DNA in low concentrations (Sophian *et al.*, 2020).

Data analysis

Data analysis was performed based on the CT (Cycling Threshold) value obtained during amplification using real-time PCR. CT values detected in the sample will be compared with positive controls. Samples that were detected positive were indicated by the formation of a sigmoid curve. (Sophian *et al.*, 2021).



RESULT AND DISCUSSION

DNA Extraction

The results of DNA extraction on the samples showed that the extracted DNA had an average concentration of 24.35. The purity that was read on the nano photometer at a wavelength of A260/280 showed a concentration value of 2.164. DNA extraction data can be seen in table 2.

Based on table 2, the data result is not included in the well-extracted DNA range (1.8-2.0). However, this is not a standard in determining whether the extracted sample can proceed to the PCR or not. According to Qiagen (2020), there is no correlation between the ratio of 260/280 and 260/230 to real-time PCR performance. Even DNA with low concentrations at 260/280 and 260/230 can produce good PCR results.

Table 2. Results of sample DNA extraction

Average Value of Nucleic Acid Concentration (A260/ A280)	Purity					
	A260	A280	A230	A320	A260/A280	A260/A230
24.350	0.497	0.235	0.450	0.010	2.164	1.107

In the extraction stage, Proteinase K functions for cell lysis so that dense tissue and protein residues will settle when centrifuged and DNA will be at the top with the solution. Incubation that is carried out after the addition of Proteinase K to the extraction process serves to assist the cell lysis process after proteinase K is active during the incubation period. Therefore, this incubation process requires an optimization step to find the right extraction technique. In a study conducted by Christensen *et al.* (2011), the optimization found that the incubation temperature was 65°C for 3 hours. This is done because according to Christensen *et al.* (2011), proteins and polysaccharides that interact when lysis are carried out need less than 3 hours to produce a complete denaturation or cell lysis process.

The sample used in this study is a sample of shredded meat, one way to facilitate the lysis process is by destroying the sample to be tested using shredding. The crushing of the sample can be done by crushing or growing so that the sample is destroyed. This crushing will facilitate the lysis process which has an impact on the incubation period of the sample which can be shorter when compared to samples that do not pass the crushing stage.

Real-Time PCR Analysis

Real-time PCR testing itself is known to have two common methods that are often used, namely by using the fluorescent dyes technique (e.g., SYBR green) and sequence-specific DNA probes (e.g., Taqman probe) (Cai *et al.*, 2012). This test using the sequence-specific DNA probes technique. This technique was chosen because it yields better interpretation with specific probes.



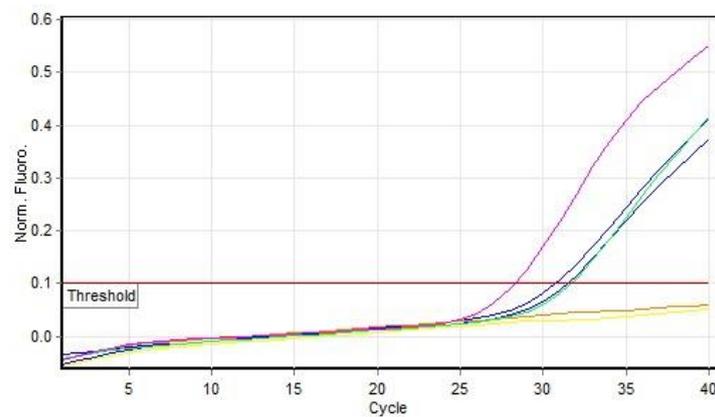


Figure 1. Real-time PCR amplification results. (blue = sample, orange = negative control / NTC, yellow = other DNA control, green = positive control, pink = LOD)

The results of real-time PCR analysis can be seen in figure 1. In the figure 1, it can be seen that the sample tested was amplified with a CT value of 30.77, while the positive control was amplified on CT 31.66, as well as the LOD control on CT 28.32. The difference in CT values is because CT in the real-time PCR analysis is influenced by the concentration of the template DNA used. This is in line with research conducted by Sophian *et al.* (2020), who explained that in real-time PCR analysis, CT was influenced by concentration, while Tm was influenced by GC content that composed the target DNA sequence.

Table 3. Real-Time PCR Amplification Results

	Name	Mean CT Score
	Sample	30.77
	Negative control/ NTC	Undetermine
	Other DNA	Undetermine
	Positive control	31.66
	LOD	28.32

In testing to detect DNA species, the use of test controls is an important step that must be taken to ensure that the resulting test only targets the expected DNA target. In this test, the controls used were four types of controls, namely negative control or NTC, positive control, other DNA control and LOD control. Each of these controls has its function that can contribute information in viewing or investigating if at any time there are questionable test results.

Based on the data presented in table 3, it can be seen that other DNA controls and negative controls were not amplified as indicated by undetectable CT values. Another DNA control is intended to see whether the primer works specifically only detects the target DNA or not, while the negative control is carried out to monitor the test process and whether there is contamination in the master mix or when doing sample testing work.

CONCLUSION

Based on the results of this study, it can be concluded that the specific DNA of porcine species analyzed using real-time PCR can be detected on CT an average of 30.77.



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