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Molecular Detection of Halal Status of Local and Imported Processed Food Products from E-commerce Using Real-Time PCR

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Abstract

Processed food products are susceptible to contamination by non-halal ingredients such as porcine DNA, which can be difficult to detect visually. Many processed beef products sold on e-commerce platforms do not meet food safety standards and can be sold freely without proper halal labelling. This study aims to detect porcine DNA content in locally produced and imported processed beef products based on molecular analysis. Sampling was conducted using purposive sampling techniques, with the criteria being processed beef products without halal labels from one of the e-commerce platforms in Indonesia. As many as 10 Samples were analyzed using real-time PCR with two DNA template concentrations: 0.25 ng/μL and 1 ng/μL. The negative control was a no-template control (NTC), and the positive control was porcine DNA. The presence of porcine DNA in the samples was determined from the amplification curve and cycle threshold (Ct) value. The lowest Ct value was 25.6 in the imported salami product, and the highest Ct value was 38.98 in the local meatball product, both at a concentration of 1 ng/μL. Two local processed food products (rollade, meatballs) and five imported processed food products (dendeng, steak bar, burger meat, ham, salami) were detected to contain porcine DNA. This study is expected to raise public awareness about the halal status of processed beef products sold through e-commerce.

1. INTRODUCTION

The halal status of food products is very important for Muslims. The concept of halal in Islam is to consume food that does not contain non-halal ingredients, one of which is

pork [1]. In QS. Al-Baqarah verse 173, which means Allah has forbidden the consumption of carrion, pork, and the meat of animals slaughtered in a name other than Allah's. However, whoever is forced to eat it, not out

of desire and without exceeding the limits, then there is no sin upon them. Verily, Allah is Most Forgiving and Most Merciful.

Pork is a potential contaminant in food products [2]. The relatively low price of pork causes traders to mix pork into their processed food products [3]. Processed products, especially processed meat products, are susceptible to contamination with non-halal components [1].

Local and imported processed food products are sold online in e-commerce at 40.80% [4]. Many consumers prefer online shopping due to the practicality and convenience [5]. According to data from the Badan Pusat Statistik 2025, the consumption of processed meat products increased from 0.52 in 2022 to 0.60 commodity units in 2023 [6].

Many processed food products circulating on e-commerce platforms do not meet food safety standards, and legal regulations are challenging to enforce against e-commerce sellers, allowing anyone to sell freely [7]. Guidance on selling products online is relatively weak because the production and distribution processes are not directly supervised by the relevant authorities, resulting in a higher potential for contamination with non-halal ingredients [8]. Several local and imported processed food products are sold on e-commerce platforms without halal labels, for example, instant noodles and sweets [9]. Processed meat products, especially those without halal labels, still have unclear halal status [10]. Many consumers do not consider halal labels as a primary factor when purchasing products, but rather prioritise price and trends [11].

The halal status of processed meat products is difficult to detect visually. Detection based on fat and protein has limitations in heated food products, namely changes in fatty acid position and protein denaturation [12]. DNA-based molecular detection can be performed on processed

products to detect non-halal ingredients such as porcine DNA [13]. The Real-Time PCR method is the primary method for detecting porcine DNA for halal detection [14-15]. The Real-Time PCR method has high sensitivity and is fast and reliable. It can be applied for analysis of in natura or processed meat. Real-Time PCR can play an important role in controlling the origin of meat products [16]. Research by Rahmania et al. [17] detected porcine content in samples from the Semarang market using Real-Time PCR, and some samples tested positive. Processed meat products, such as meatballs and sausages, taken from local markets in Surabaya [17] and Jakarta [18] were found to contain porcine DNA using Real-Time PCR. Based on research by Pathaburee [19], halal-labelled meat products sold in local markets in Thailand were analyzed using the Real-Time PCR method, and it was found that seven samples tested positive for porcine DNA.

Research on the molecular detection of the halal status of processed meat products has been conducted extensively. However, samples in previous studies were obtained directly from markets or supermarkets. Research focusing on molecular detection of the halal status of local and imported products obtained from e-commerce shops.

There are several types of processed meat products commonly consumed by Muslims that are purchased via e-commerce such as food products with a mix of meat that are sold without halal labeling on the packaging, therefore, further analysis is required to verify the halal status. their packaging, consequently the analysis is required to validate their Halal status. This study aimed to detect the presence of porcine DNA in local and imported processed beef using molecular analysis. The results are expected to contribute to greater awareness and vigilance concerning this study aims to detect porcine DNA content in local and imported processed beef products based on molecular analysis. This research is important

to raise public awareness and vigilance regarding the halal status of local and imported processed food products without halal labels sold on e-commerce platforms

2. MATERIALS AND METHODS

Sample collection and preparation

The samples obtained in this study were locally processed beef products (kebab, corned beef, sausages, meatballs, roulades) and imported products (ham, burger meat, salami, beef jerky, beef steak bars) from one of the most visited e-commerce sites in Indonesia. Sampling was conducted using purposive sampling. The selected samples were processed beef products that were not halal-labelled, obtained from e-commerce platforms. Samples were selected based on the highest ratings, taking into account the largest number of buyers. The porcine DNA used as the positive control was provided with the test kit.

DNA extraction, quality assessment, and real-time PCR analysis

The DNA extraction process follows the Monarch T3010 New England Biolab® kit extraction protocol. A 25 mg sample is placed in a 1.5 mL microcentrifuge tube, 10 µL of proteinase K and 200 µL of tissue lysis buffer are added, then homogenized and heated in a thermo shaker at 56°C for 60 minutes. The resulting supernatant is transferred to a new 1.5 mL microcentrifuge tube, and 400 µL of gDNA binding buffer is added, then homogenized and transferred to a spin column.

The sample was centrifuged, the supernatant was discarded, and washing was performed twice with the wash buffer. The spin column was transferred to a 1.5 mL microcentrifuge tube, then 50 µL of elution buffer was added and centrifuged at 14,000 x g for 30 minutes. The extracted DNA sample was measured for DNA concentration according to the Qubit 1x dsDNA HS Kit 4

fluorometer (Invitrogen®) instrument protocol. The purity of the extracted DNA sample was measured using a Nano Spectrophotometer N50 (Implen®) with an A260/280 wavelength ratio [20].

Porcine DNA in the samples was amplified by Real-Time PCR following the AccuPower® pork Real-Time PCR kit procedure. The negative control was a no-template control (NTC), and the positive control (PC) was pork DNA. Each sample (L1, L2, L3, L4, L5, L6, L7, L8, L9, L10) and positive control (PC1 and PC2) underwent DNA amplification using Real-Time PCR with two DNA template concentrations in accordance with SNI 9278:2024.

The DNA concentrations of the samples were 0.25 ng/µL and 1 ng/µL, while the positive control concentrations were 1 ng/µL (PC1) and 3x10⁻⁵ ng/µL (PC2), respectively. A 20 µL PCR Mix solution was added to each 5 µL control and sample DNA to be tested in a PCR tube. Real-time PCR amplification was performed with an initial denaturation at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 5 s and annealing/extension at 55°C for 5 s. The annealing/extension temperature was recommended by the AccuPower® Pork Real-Time PCR kit (Bioneer).

Data analysis

Data analysis was performed based on cycle threshold (CT) values. The presence of target DNA in the sample was determined based on the amplification curve with a cycle threshold (Ct) value below the Ct cut-off value of 39.88 as specified by the AccuPower® Pork Real-Time PCR Kit. The tested sample was declared positive for target DNA if all controls were valid and at least one of the sample DNA concentrations (0.25 ng/µL and 1 ng/µL) was detected [21].

3. Results

All DNA samples had concentrations above 10 ng/μL, ranging from 10.9 ng/μL to 118 ng/μL. DNA purity analysis results ranged

from 1.68 to 1.9. Samples L2 (local kebab meat) and L4 (local sausage) had the purest DNA, with standard DNA purity values of 1.82 and 1.82, respectively (Table 1).

Table 1. Extraction Result of Beef Processed Produce

Sample code	Sample	Qubit Conc. (ng/μL)	260/280 Abs ratio
L1	Local corned beef	64.6	1.9
L2	Local kebab meat	13.2	1.82
L3	Local roulade	31.8	1.86
L4	Local sausage	118	1.82
L5	Local Meatballs	70.2	1.87
I6	Imported beef jerky	10.9	1.68
I7	Imported beef steak bar	14.5	1.86
I8	Imported burger meat	75.8	1.78
I9	Imported ham	52.8	1.89
I10	Imported salami	112	1.84

The results of porcine DNA amplification testing using Real-Time PCR, negative control (NTC), positive controls PC1 and PC2 showed valid porcine DNA amplification (Table 2). There were 7 samples (L3, L5, I6, I7, I8, I9,

I10[Y1.1]) detected to contain porcine DNA (positive) and 3 other samples (L1, L2, L4) not detected to contain porcine DNA (negative) (Table 2)

Table 2. Threshold cycle (Ct) value of porcine DNA amplification

No	Sample code	Sample	Ct value		Interpretation
			Concentration 0.25 ng/μL	Concentration 1 ng/μL	
1	L1	Local corned beef	Undetermined	43.59	Undetected
2	L2	Local kebab meat	Undetermined	41.98	Undetected
3	L3	Local roulade	38.17	37.31	Detected
4	L4	Local sausage	Undetermined	Undetermined	Undetected
5	L5	Local Meatballs	Undetermined	38.98	Detected
6	I6	Imported beef jerky	40.3	38.42	Detected
7	I7	Imported beef steak bar	37.24	35.71	Detected
8	I8	Imported burger meat	35.26	33.04	Detected
9	I9	Imported ham	30.67	28.55	Detected
10	I10	Imported salami	27.95	25.6	Detected
11	NTC	- control	Undetermined		Valid (-)
12	PC1	+ control (conc.1 ng/μL)	20.42		Valid (+)
13	PC2	+ control (conc. 0.25 ng/μL)	39.84		Valid (+)

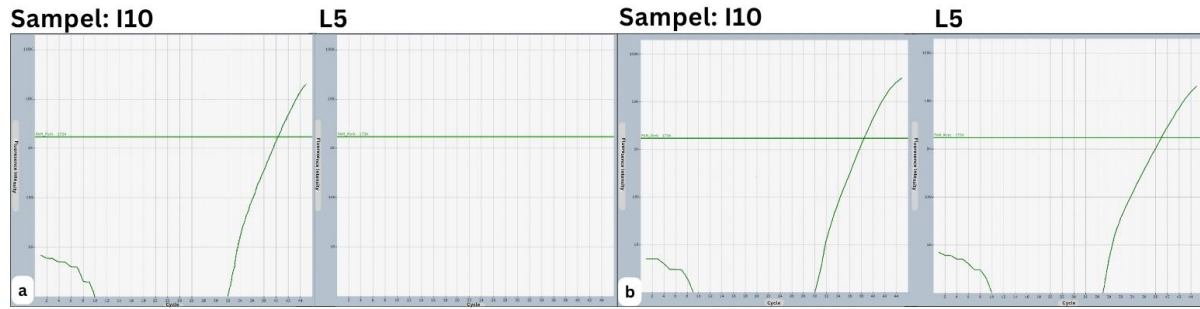


Figure 1. Real-Time PCR amplification curves of porcine DNA samples I10 and L5 at different DNA template concentrations. (a) 0.25 ng/μL and (b) 1 ng/μL. The horizontal line represents the threshold.

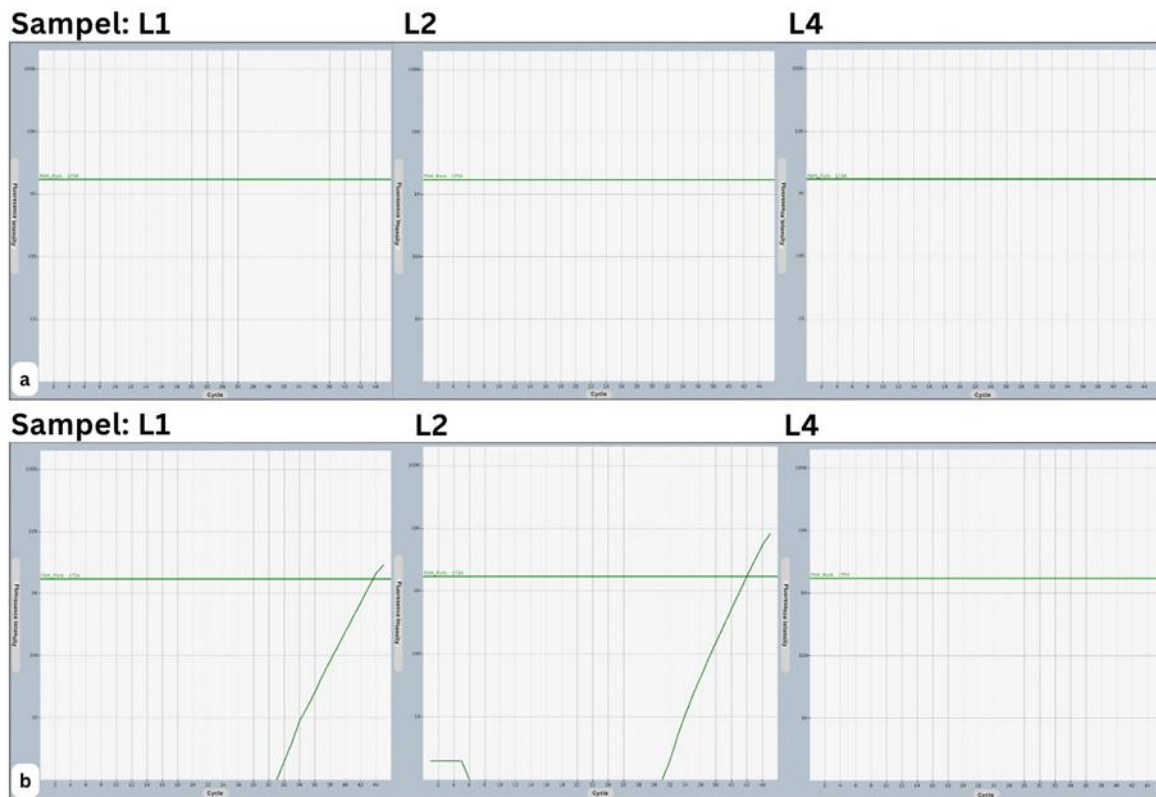


Figure 2. Real-Time PCR amplification curves of porcine DNA samples L1, L2, and L4 at different DNA template concentrations. (a) 0.25 ng/μL and (b) 1 ng/μL. The horizontal line represents the threshold

The results of porcine DNA amplification testing using Real-Time PCR, negative control (NTC), and positive controls PC1 and PC2 showed valid porcine DNA amplification (Table 1). There were 7 samples (local roulade, local meatballs, imported beef jerky, imported beef steak bar, imported ham, and

imported salami) detected to contain porcine DNA (positive) and 3 other samples (L1, L2, L4) not detected to contain porcine DNA (negative) (Table 2).

4. DISCUSSION

DNA extraction

Based on the DNA concentration measurements, all DNA sample extractions could undergo DNA amplification using Real-Time PCR. The DNA concentrations obtained were classified as good because good DNA extraction yields DNA concentrations above 10 ng/ μ L [22].

A good DNA purity ratio to protein contamination is in the range of 1.7-2.0 [23-24]. All samples had a DNA purity ratio below 2.0 (Table 2), indicating that there was no RNA contamination in the DNA extract [20]. Sample I6 had a DNA purity value of 1.68 (Table 2), indicating the presence of protein contaminants [24]. Protein contamination in DNA samples occurred during the cell lysis stage of DNA extraction, where some cell components are not lysed, resulting in proteins that are not completely degraded [25].

Based on the results of DNA concentration measurements and purity analysis, sample I6 (beef jerky) was the sample with the lowest DNA concentration (10.9 ng/ μ L) and a purity value below the category of good DNA (1.68). The low concentration and quality of sample I6 were caused by a longer processing time and higher temperature compared to other samples [26], which can reduce DNA concentration and purity.

The concentration and quality of processed product DNA are influenced by the level of processing, such as heating at high temperatures, which can cause DNA damage and degradation, resulting in lower concentration and quality [22]. Previous studies have also reported that meat DNA heated at 120°C experiences damage due to high temperatures [19].

Porcine DNA amplification

The specificity of the probe-based Real-Time PCR method was assessed based on amplification in the controls. If there was no increase in the curve in the negative control, this indicated that there was no mispriming or primer dimer [27]. Valid results from all controls indicated that the PCR system used was functioning properly and that the detection results in the samples were valid.

The amplification curves of all samples detected with porcine DNA are valid, with Ct values not exceeding the Ct cut-off value (39.88) (Table 2). The sample detected with porcine DNA with the smallest Ct value was sample I10, while the sample with the largest Ct value was sample L5 (Table 2). There was a difference in the increase in the porcine DNA amplification curve in samples I10 and L5 (Figure 2).

The amplification curve of porcine DNA sample I10 with a concentration of 0.25 ng/ μ L (Figure 2a) shows an exponential phase from cycle 24 to cycle 34, which means that the fluorescence signal increases sharply during this period before reaching a plateau phase with a Ct value of 27.95 (Table 2). Meanwhile, sample I10 with a concentration of 1 ng/ μ L (Figure 2c) shows an exponential phase from cycle 22 to cycle 32 with a Ct value of 25.6 (Table 2). The curve for sample L5 (Figure 2b) shows no increase in the amplification curve, and no Ct value appeared until cycle 45. Meanwhile, sample L5 (Figure 2d) showed an exponential phase at cycles 29-42 with a Ct value of 38.98.

The difference in the rate of increase of the curve and the Ct value indicates that the amount of porcine DNA in sample I10 is greater than in the other samples that tested positive. Meanwhile, sample L5 shows that porcine DNA is present in the smallest amount among all samples that tested positive for porcine. This is in accordance with the principle of Real-Time PCR, whereby the greater the amount of porcine DNA in the sample, the faster the Real-Time PCR reaction

will proceed, resulting in the exponential phase appearing at an earlier cycle because the fluorescence signal crosses the threshold line earlier. The greater the amount of target DNA in the sample, the smaller the Ct value and vice versa [28].

Samples L1 and L2 were not detected as containing porcine DNA, even though one of the porcine DNA amplification curves at a concentration of 1 ng/ μ L showed an increase (Figures 3d-e), because the Ct value of the sample was higher than the Ct cut-off value (>39.88) (Table 2). Beef jerky (16) was classified as positive for target DNA based on a Ct of 38.42 at 1 ng/ μ L, below the manufacturer's cut-off of 39.88 for the AccuPower® Pork Real-Time PCR Kit. Although the Ct at 0.25 ng/ μ L exceeded the cut-off, the sample was considered positive because detection at at least one tested DNA concentration meets the criteria of the Indonesian National Standardization Agency [21]. Samples with undetermined results did not show an increase in the porcine DNA amplification curve until the end of the cycle (Figures 3a, 3b, 3c, 3f). If the fluorescence signal has not crossed the threshold line by the last cycle, the Ct value cannot be determined (undetermined), which means that porcine DNA is not detected [29].

Shops selling samples of imported processed beef products, such as beef jerky, burger meat, ham, and salami, are also known to sell processed pork products at the same time. Each shop claims that the products sold are genuine beef, made from 100% beef. However, none of these shops have halal certification, although some of them (the imported ham and salami) have obtained permits from the Badan Pengawas Obat dan Makanan (BPOM).

The samples were detected to contain porcine DNA, indicating that the samples are contaminated with pork. This contamination could have occurred accidentally because some sample producers sell processed pork products at the same time. Positive porcine

DNA samples were caused by accidental cross-contamination at producers who process chicken, beef, and pork simultaneously in the same factory [30]. Contamination occurs due to improper handling using shared equipment to process various meat species on an industrial scale [30-31].

Samples of local meat rolls and meatballs were found to contain porcine DNA, but the producers of the samples do not sell processed pork products. The producers of each shop stated that the products sold were authentic beef rolls and authentic beef meatballs, which was trending in their shop descriptions. However, neither shop had a halal certification and did not list a distribution permit number from the BPOM. This raises the possibility of counterfeiting or mixing pork into beef products for economic gain. Pork or wild boar meat is commonly sold alongside beef or other halal meat as processed foods such as meatballs, sausages, and nuggets using grinding machines in the market [32]. Previous studies have reported that a few businesses counterfeit meatball products using pork mixtures [33].

The processed beef product samples detected to contain porcine DNA did not differ visually from the three other samples that did not contain porcine DNA. The colour, texture, and aroma characteristics appeared similar, making it difficult to distinguish the halal status of a processed food product visually. The halal status of processed beef products is difficult to detect visually and therefore cannot be used as an accurate reference. Therefore, the halal status of processed beef products needs to be detected molecularly using Real-Time PCR..

5. CONCLUSION

Based on molecular detection using the Real-Time PCR method and in accordance with SNI 9278:2024, it was found that 7 out of 10 samples contained porcine DNA. The seven

samples included two locally processed food products (rollade, meatballs) and five imported processed food products (dendeng, steak bars, burger meat, ham, salami) obtained from e-commerce. Based on the results of this study, stricter supervision of meat-based food products sold through e-commerce platforms is recommended, including periodic laboratory testing of

processed food products if they contain meat. Products found to contain porcine DNA should be subject to appropriate regulatory action, including recall where warranted. Marketplaces should also strengthen seller verification by requiring information such as halal certification numbers, ingredient composition, manufacturer identity, and the origin of raw materials.

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