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Unveiling Porcine Contamination in Halal-uncertified Cosmetics: Detection through Existing Procedure using DNeasy Mericon Food Kit DNA Extraction and Real-time qPCR

Intan Sartika Risky Sampurna[1*], Suharti Suharti[2*], Arief Hidayatullah[3], and Nur Faridah[4]

[1]Central Lab MMM, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang
[2]Department Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang
[3]Health Governance Initiative, United Nations Development Programme Indonesia, ERISCM Building, Jakarta, Indonesia
[4]Integrated Laboratory Universitas Negeri Malang

Abstract. The surge in cosmetic product consumption in Indonesia has spurred the growth of the local cosmetic industry. However, not all cosmetics in circulation adhere to the halal standards mandated by Indonesian authorities through the Law on Halal Product Guarantee. This study endeavors to establish a method for identifying pork-derived content in cosmetic cream products lacking the halal logo. The approach involves total DNA isolation using the Qiagen DNeasy Mericon Food Kit, followed by detecting the NDH5 gene using qPCR. Results indicated that the isolation kit effectively extracted DNA fragments from cosmetic products, yielding 0.6–0.8 ng/μL concentrations, with A260/280 ratios ranging from 0.90 to 1.76 and A260/230 ratios from 0.12 to 0.17. Analysis of five cosmetic cream samples revealed that three of them exhibited no contamination by pig-derived elements, with a cut-off value of 23.7×10^{1}. Consequently, the isolation method utilizing the kit and the subsequent detection of the NDH5 gene through qPCR emerge as a robust approach for identifying pig-derived ingredients in cream-based cosmetics.

1 Introduction

Halal is a fundamental concept in Islam that is generally associated with the permissibility of consumption of a product by a Muslim because the product does not originate or interact with anything forbidden [1–3]. Halal encompasses all aspects of Muslim life, including dietary choices, behavior, and cosmetic products [2]. When referring to cosmetics, "Halal" means that they don't contain any traces of substances that are listed explicitly as "Haram" or "Forbidden," verified through rigorous testing and scrutiny by the relevant regulatory authorities [3–5].

The thorough assessment guarantees that cosmetic formulations align with Islamic dietary and ethical principles, meticulously excluding any components deemed

* Corresponding author: intan.sartika@um.ac.id, suharti.fmipa@um.ac.id

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impermissible. Maintaining cleanliness and pure conditions is imperative at every stage of the preparation, processing, manufacturing, storage, and transportation of halal cosmetic products. A key focus is placed on ensuring the absence of any impurities. Certifying products as halal aligns closely with the objectives of standard quality assurance procedures [6]. Subsequently, upon successful evaluation, the regulatory authority issues a certificate of compliance, permitting the brand to affix a recognizable Halal logo to its products [4,6]. While this logo serves as a clear indicator for customers seeking Halal-certified items, the protracted certification process and the associated high annual fees pose significant challenges for manufacturers [7,8]. This extended timeline can impede efficient product development and manufacturing processes, ultimately contributing to an increase in the final cost. The complexities associated with obtaining and maintaining Halal certification highlight a delicate balance between religious adherence and practical considerations within the cosmetics industry. Consequently, some manufacturers opt to market their products without the Halal logo because the certification is pending or has not yet passed the requisite tests.

The development of halal cosmetics poses various challenges, particularly in verifying ingredients sourced from animals like gelatin, lecithin, glycerol, fatty acids, and collagen, which prove challenging in ascertaining their halal status. Notably, pigs (*Sus domesticus*) serve as a prominent source of these materials, and their use is strictly prohibited for Muslims in any form [4,9]. But because of its easy accessibility, affordable price, and desirable qualities like being safe for human skin and causing fewer allergic reactions, it is also one of the most often utilized components in cosmetic products [10–12]. Therefore, all cosmetic products in Muslim-majority nations like Indonesia and Malaysia are required to go through a stringent halal certification process to guarantee that all of the ingredients are traceable and that there are no residues of non-halal materials in any product [13–15].

To identify any presence of porcine contaminants, our focus should center on a fundamental aspect inherent to every known species on Earth—their unique DNA. Beyond serving as the blueprint for biological processes, the distinctive sequence of DNA can pinpoint the specific species it originates from [16–18]. Its double-stranded structure is biologically stable to withstand rigorous stress, including mechanical and chemical stress, during manufacturing and storing, and enough to be recovered later for identification processes [19,20]. Among the genes frequently employed for this purpose, the mitochondrial nd5 gene stands out. Responsible for encoding the core subunit of the mitochondrial membrane's respiratory chain, known as NADH dehydrogenase [21,22]. It is preferred over nuclear DNA due to its resistance to damage and deficiency, higher copy number per cell, and relatively high mutation rate, which is particularly beneficial for identifying closely related animal species, rendering it ideal for halal identification [23–25].

Quantitative Polymerase Chain Reaction (qPCR) emerges as the gold standard for halal identification, precisely detecting traceable DNA presence, especially from non-halal components [26,27]. Obtaining high-quality and quantity DNA input through the extraction procedure is crucial to ensure reliable qPCR results. However, a suitable DNA extraction kit is critical to recovering DNA fragments from cosmetic products. Unfortunately, no specific kits are available on the market for this specific purpose [28]. Hence, we adopted our existing method, utilizing a food extraction kit, specifically the Qiagen DNeasy Mericon Food standard input procedure, along with the QuantStudio 3 qPCR as our primary detection instrument. The primary objective of this study is to assess the efficacy of the current procedure for detecting and addressing the challenges related to the identification of non-halal components in cosmetic products.

2 Material and Method

2.1 Sample type

Cosmetic samples were procured from a local e-commerce platform, with specific criteria that included being locally produced creams, devoid of ingredients derived from pigs and their derivatives, possessing a composition in compliance with these criteria, and holding a distribution permit from the Indonesian Food and Drug Supervisory Agency (BPOM) but lacking certification from the Indonesian Ulema Council (MUI). Based on these criteria, three cream products, coded as A, B, and C, were selected as samples for this study. The positive control (K+) consisted of cosmetic products containing ingredients derived from porcine, while the negative control (K-) was a cosmetic sample certified by both BPOM and MUI.

2.2 DNA extraction and primer design

Total DNA was extracted using the Qiagen DNeasy Mericon Food isolation kit (lot No.: 166048917) with the standard protocol and 200 mg sample input. The quantification and assessment of DNA purity were carried out using a Thermo Scientific NanoDrop One Microvolume UV-Vis Spectrophotometer. Subsequently, qPCR analysis was conducted utilizing the NDH5 primer specific to Sus domesticus, the sequence of which is detailed in Table 1. The primer was procured from a reference and submitted to Integrated DNA Technology, Singapore [28].

Table 1. The oligonucleotides of Sus NDH5's primer

Primer Name	Nucleotide
Sus NDH5	F: GCC TCA CTC ACA TTA ACC ACA CT
	R: AGG GGA CTA GGC TGA GAG TGA A

2.3 Real-time qPCR quantification

The Thermo Scientific QuantStudio 3 served as our primary instrument for real-time qPCR analysis. Each sample reaction comprised 20 μl aliquots, encompassing 10 μl of 2× SensiFAST SYBR® No-ROX Mix, 0.8 μL of 10 μM forward primer, 0.8 μL of 10 μM reverse primer, and 8.4 μL of the DNA template. A 40× 3-step PCR stage was employed with a cycle consisting of 95°C for 1 second, 60°C for 20 seconds, and 65°C for 20 seconds. To ensure consistent results, all samples were tested in duplicate.

3 Results and Discussion

The results of DNA isolation using the Qiagen DNeasy Mericon Food kit with cosmetic cream samples revealed a total DNA yield of less than 1 ng/μL (Table 1). Comparable research on another cosmetic type, specifically lipstick, subjected to intensive processing during manufacturing, demonstrated DNA extraction yields as low as 4 pg/μL [29]. The extraction kits play a critical role in the molecular workflow as the primary tools to extract the DNA from each sample. Thus, the kit quality also determines the quality and quantity of DNA as the input material for the qPCR procedure, as shown by a similar study [26]. The underlying fact that there is no commercially available extraction kit for cosmetic products leaves researchers with some options, such as using a general or food DNA extraction kit. However, they were not designed to extract DNA from cosmetics. Another study also

indicates similar results with different yields for each type of extraction kit [30]. Several critical factors contribute to the observed low DNA yield, including processed raw materials, intricate manufacturing processes involving high temperatures and pressures, rigorous procedures like homogenization, exposure to potentially DNA-damaging chemicals, and suboptimal storage conditions [31].

On the contrary, utilizing a spectrophotometer-based instrument like Nanodrop for measuring low-yield DNA extraction results is cautioned against due to the instrument's limitations, rendering the measurement method unreliable [32,33]. Instead, a fluorescent-based instrument such as Qubit Fluorometer would provide a more accurate result [34-36]. The Qubit uses fluorescence-based quantification, which relies on the interaction between the fluorescent dye and the actual bases in the nucleic acid, resulting in the amplified fluorescent signal being directly proportional to the DNA concentration in the solution [37]. It can accurately quantify DNA as low as 10 pg/µL, surpassing Nanodrop, which can only accurately measure concentrations as low as 10 ng/µL. Additionally, the Qubit measurement is less affected by contaminants such as proteins, salts, and organic solvents, which can interfere with absorbance measurements on the Nanodrop [34,37].

In terms of DNA purity, all samples, including both controls, failed to meet the normal DNA purity ratio within the range of 1.8-2.0. The results indicated that the A260/280 ratios for all samples fell significantly below this range. The abnormal A260/280 ratio may be attributed to the presence of contaminants such as proteins, polyphenols, polysaccharides, guanidine, or other chemicals existing in the extraction reagents or initial samples that cannot be completely eliminated during the manual extraction procedure [38]. In addition, the low yield of DNA samples can also cause the value of this ratio to be inaccurate due to instrument limitation [39]. This holds true for an abnormal A260/230 ratio result as well, where contaminants that typically absorb light at a wavelength of 230 nm, such as salts, carbohydrates, peptides, and phenol, are highly abundant in cosmetic products [40].

Table 2. Nanodrop-based quality and quantity measurements results

Sample	Concentration (ng/µL)	A260/280	A260/230
K+	0.7	1.51	0.12
K-	0.8	1.76	0.17
A	0.6	0.90	0.13
B	0.6	1.34	0.12
C	0.7	1.00	0.13

Table 3. qPCR results for porcine DNA detection in cosmetic samples

Phase type	Sample code	CT Value
Cream	K+	21.788
	K-	No Ct
	A	22.49
	B	No Ct
	C	No Ct

The qPCR results presented in Table 3 highlight the performance of the positive control (K+), displaying a relatively low Ct value of 21.788. This outcome signifies the successful amplification of porcine DNA, validating the efficacy of the qPCR assay and its capability to detect a substantial quantity of porcine genetic material. Notably, the positive control serves a crucial role in verifying the experimental methodology and functions as a benchmark for interpreting the results obtained from other samples [41]. Conversely, the negative control (K-) produced a "No Ct" result, aligning with expectations. This particular outcome reinforces the absence of porcine DNA in the negative control, thereby validating the experimental protocol and ensuring that the controls remained uncontaminated. The integrity

of the negative control is paramount, underscoring the reliability of the experiment's setup [28,41].

Intriguingly, Sample A displayed a Ct value of 22.49, indicating the presence of porcine DNA, albeit at a slightly higher cycle threshold than the positive control. This finding raises the possibility of porcine-derived contamination within this cosmetic sample. However, the extent of contamination, whether incidental or intentional, warrants further investigation. The modest increase in the Ct value suggests a potentially similar level of presence of porcine genetic material compared to the positive control, which may necessitate a more detailed analysis to ascertain the origin and implications of this contamination. Conversely, both Sample B and Sample C exhibited "No Ct" values, suggesting the absence of detectable porcine DNA. This absence of amplification aligns with the presumption of non-contamination with porcine-derived ingredients in these cosmetic samples [28,42,43]. The robustness of these results strengthens the case for the absence of porcine contamination in these samples, thus underscoring their potential suitability for consumers with specific dietary and ethical preferences.

The results yield significant insights into the qualitative assessment of porcine DNA contamination. Particularly noteworthy is the finding in sample A, lacking the Halal logo, which exhibits a substantial amount of porcine DNA trace with a Ct value remarkably close to that of the positive control. This strongly suggests the potential use of porcine-derived material in sample A or contamination with such material during its manufacturing process. The negative control exhibited no amplification, as indicated by a "No Ct." It's noteworthy that the Ct value inversely correlates with the amount of target DNA—lower Ct values indicate higher amounts, while higher Ct values indicate lower amounts [26]. Nevertheless, due to the low-quality DNA input, it is strongly recommended to undertake optimization across the entire workflow to ensure the accuracy and reliability of the detection results.

4 Conclusion

The qPCR analysis provided valuable insights into porcine contamination in cosmetics, with the positive control confirming assay sensitivity and the negative control validating experiment integrity. Varied Ct values among test samples suggested potential contamination levels, revealing a substantial degree of contamination in sample A and the absence of detectable contamination in samples B and C. To deepen our understanding of this critical cosmetic safety issue, further investigations, including repeat analyses, alternative methodologies, and comparative studies with established standards, are recommended. The analysis of five samples using qPCR with SusNDH5 primers highlights contamination in sample A, emphasizing the need for consumer caution, especially when choosing local cosmetics lacking MUI halal certification. The use of inaccurate isolation kits can compromise DNA quality, underscoring the necessity for the development of advanced DNA isolation kits to enhance the quality of results in such analyses.

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