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Article Review: Testing for Detection of Low Pig (Porcine) DNA in Cosmetic Products and Health Supplements

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Abstract

The increase in the Muslim population worldwide has led to rapid growth in the halal product industry. Currently, halal food holds the largest proportion in the global market for halal products, followed by cosmetics and health supplements as one of the main sectors. In the raw materials used for capsule shells and the manufacture of cosmetics, many ingredients come from non-halal sources, such as gelatin, fatty acids, glycerin, and collagen, which are derived from pigs. However, the presence of pork ingredients in cosmetic products and health supplements is prohibited in certain religions, such as Islam. Therefore, the detection of lard in cosmetic and pharmaceutical products plays a crucial role for the benefit of Muslim consumers. Many methods have been developed to detect pork content in highly processed products, one of which is the DNA-based method. This literature review was conducted to explain DNA isolation methods and detection techniques that can be used for pig DNA analysis. A literature search was performed through online databases, and 14 research journals were found that met the inclusion criteria. Based on the collected research, DNA-based methods, including the Polymerase Chain Reaction (PCR) and its modifications, have demonstrated the ability to detect porcine DNA in products like cosmetics and health supplements. The selection of DNA detection and isolation methods depends on the type of

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sample under examination, thus enhancing the accuracy and success of porcine DNA detection.

Keywords: DNA analysis, Pig DNA, Pig Content, Cosmetics, PCR, Health Supplements.

1. Introduction

The global halal product market has seen rapid growth, driven by the increasing Muslim population. Halal food dominates the market, followed by cosmetics and pharmaceutical products as key sectors (1). In Islam, a product is considered non-halal if it contains ingredients derived from pigs, dogs, animal blood, alcohol, and other animals that are not slaughtered in accordance with Islamic guidelines such as pigs, carnivorous animals (lions, tigers, leopards), birds of prey (eagles, hawks, vultures, and falcons), insect and animals found dead. (2). Several Islamic countries such as Indonesia have established strict regulations for producers and importers in product labels, which is halal certificates in order to distinguish them from non-halal products (3). Halal certification is required so that a product can enter the halal market. Each product must go through a rigorous qualification process to ensure that it does not contain haram elements (4).

Gelatin is one of the raw materials that is often used in the manufacture of soft capsule shells for medicinal products and health supplements. Gelatin is a very popular ingredient used in various food and pharmaceutical products (5). In large-scale gelatin manufacture, generally the main raw material used is collagen found in cattle and pigs (6). In some cosmetic products, ingredients derived from pigs such as fatty acids, glycerin and collagen can be used in the production of body lotions, creams and masks (7). In most countries, lard substituents in cosmetic formulations are commonly used as an emulsifying agent, and viscosity increasing agent (8). However, the detection of lard in cosmetic products and other pharmaceutical products plays an important role for the benefit of Muslim consumers.

Many methods have been developed to detect pig content in highly processed products, including physicochemical properties-based methods, and DNA based methods.

One of the physicochemical properties-based methods that can be used is Fourier transform infrared spectroscopy (FTIR). However, the FTIR method has limitations in detecting pork content due to low sensitivity, sample purity requirements, and the inability to differentiate gelatin mixtures from different sources due to similar structures and properties (9–11).

Protein/antigen-based methods such as high-performance liquid chromatography (HPLC) and *Enzyme-Linked Immunosorbent Assay* (ELISA) can also be used to detect pig content. However, the HPLC method has limitations in distinguishing gelatin mixtures due to similar chemical properties (9). Meanwhile, antigen-antibody interaction-based methods like ELISA have limitations, including cross-reactions, low sensitivity, false positives at low ionic strengths, and interactions in the presence of inhibitors (9,11,12).

DNA-based detection methods for pork content in highly processed products are known to be specific, reproducible, sensitive, have fast processing times and require low costs (13). DNA is unique and specific to each species, allowing for accurate detection of pork content. Its uniqueness and specificity enable differentiation between species and individuals, making it a reliable method for detecting pork in various products (14). *Polymerase Chain Reaction* (PCR) is a method that can be used to detect pig DNA in cosmetics and health supplements.

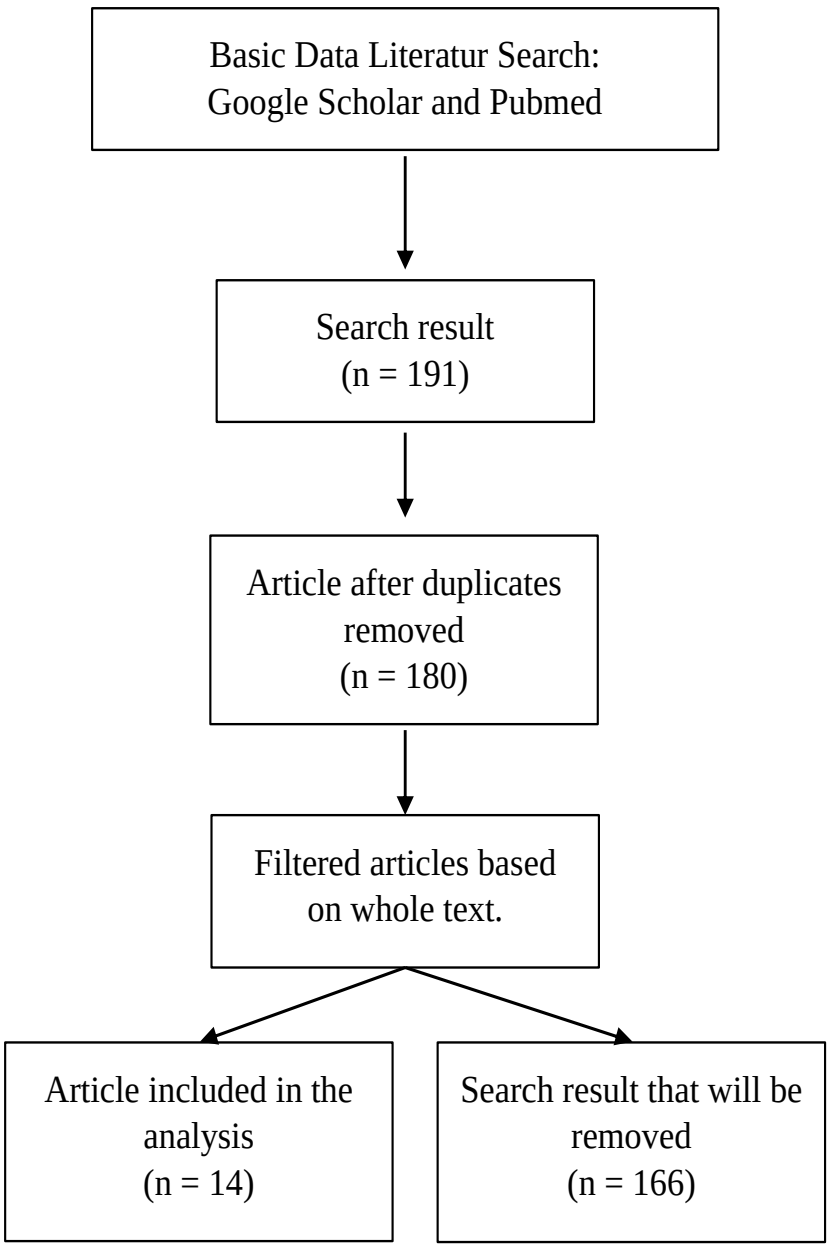
Detecting porcine DNA in cosmetics and health supplements is challenging due to the high processing involved, which leads to DNA degradation into short fragments. This fragmented DNA poses difficulties in detection (12,15). In addition, some of the excipients used in health supplements can absorb DNA, making the detection of DNA in capsule shells made of gelatin more difficult (9). This literature review focuses on methods for isolating and detecting DNA in processed products, such as gelatin capsule shells and cosmetics, to optimize the detection of porcine DNA. It provides insights into various DNA isolation and detection methods, as well as their detection limits for porcine DNA analysis.

2. Method

Literature searches were conducted using keywords such as DNA,

porcine, dietary supplement, cosmetics, gelatin, and halal on online databases including Google Scholar and PubMed. The reference journals for this review are from the last 10 years (2013 – 2023). The inclusion criteria are journals regarding testing for the detection of porcine DNA in samples of health and cosmetic supplements. Exclusion criteria involve journals with incomplete or restricted access to relevant data.

Tabel 1. Methods performed for review.



3. Result

Based on literature searches, data were obtained from several journals that tested pig DNA on cosmetic samples and health supplements using the Polymerase Chain Reaction (PCR) detection method with different DNA extraction methods as well as primers and gene targets. There are 14 journals used for this literature review which are summarized in the following Table 2 and Table 3.

Table 2. Scientific Journal Data related to DNA Analysis in Cosmetics and Health Supplements (Gelatin)

Test Sample	Analysis Method	*Primers* and Gene *Probes* /Targets	Detection Limit	References
Cosmetics (Cream)	Detection method: PCR MyTaq DNA Polymerase DNA Extraction Method: Wizard Genomic DNA Purification Kit	Primer: 12SP and 12SFW (Porcine Specific) from Mt-DNA Gene targets: 12S rRNA	0.001ng/µL	(16)
Cosmetics (Cream)	Detection method: Real-time Polymerase Chain Reaction DNA Extraction Method: CATB, Power PrepTM DNA extraction from food and feed kit (KogeneBiotech), QIAamp DNA stool mini kit (Qiagen, Hilden, Germany), TIANamp Genomic DNA kit (Tiangen Biotech, Beijing, China), and Wizard Genomic DNA purification kit (Promega, Madison, WI, USA)]	Primer: • Sus 2 (Porcine Specific) from Mt-DNA • Sus NDH5 (Porcine Specific) from Mt-DNA Gene targets: ndh5	Liquid preparation: 2.28 x 10^0 copies Powder dosage: 2.28 x 10^1 copies Cream preparation: 2.28 x 10^0 *Threshold cycle (Ct)	(7)

Cosmetics (Liquid, powder and cream preparations)	Detection Method: TaqMan Probe real-time Polymerase Chain Reaction DNA Extraction Method: CATB	Primer: • VIC™/MGB probe, limited primer from Mt-DNA • The primer used for the pig species was designed by the Meat Animal Research Centre Porcine Repetitive Element Gene targets: • 18s rRNA gene (Universal) • Specific primers target multiple porcine repetitive element (MPRE) genes	Undetectable	(17)
Gelatin Capsule Shell	Detection Method: PCR-RFLP and Duplex PCR. PCR-RFLP Extraction Method: DNeasy Mericon Food Kit (Qiagen, Germany)	Primer: 3 Oligonucleotide which contains 1 universal primer and 2 porcine-specific primers. Restriction Enzyme: BsaJI	0.01% DNA or 0.01 gram/100 ml DNA	(18)

Gelatin and Gelatin Capsules	Detection method: Real-time Polymerase Chain Reaction DNA Extraction Method: MasterPure DNA Purification Kit	Primer: • 16S (Universal) from Mt-DNA • CBH (Porcine Specific) from Mt-DNA • MPRE (Porcine Specific) from Nuclear DNA Gene Targets • 16S rRNA (Universal) • Cyt-b (Porcine Specific) • MPRE41 (Porcine Specific)	Nuclear DNA: 1 pg of DNA gelatin Mitochondrial DNA: 10 pg DNA gelatin	(19)
Gelatin capsule shell	Detection method: PCR-RFLP DNA Extraction Method: FavorPrep Food DNA Extraction Kit (Favorgen Biotech Corp, Ping-Tung, Taiwan)	Primer: • Eukaryotic primer (Universal) from Mt-DNA • Porcine primer (Porcine Specific) from Mt-DNA Restriction Enzymes: • BsaAI Gene targets: • 18S rRNA (Universal) • Cyt-B (Porcine Specific)	0.001 ng of pig DNA	(20)

Health Supplements	Detection Method: Taqman Qualitative PCR Multiplex Probe DNA Extraction Method: FavorPrep Food DNA Extraction Kit (Favorgen Biotech Copr, Ping-Tung, Taiwan)	Primer: • Eukaryotic 18S rRNA (Universal) from Mt-DNA • Porcine primer Mt-DNA Porcine specific) Gene targets: • 18S rRNA (Universal) • Cyt-B (Porcine Specific)	0.005 ng/µL DNA in gelatin mixture	(21)
Gelatin Raw Materials	Detection Method: qPCR DNA Extraction Method: NucleoSpin® Food (Macherey-Nagel GmbH & Co. KG, Postfach, Germany), DNeasy Mericon Food Kit (Qiagen GmbH, Hilden, Germany), QuickGene DNA Tissue Kit L (Kurabo Industries ltd., Osaka, Japan), and Foodproof® GMO Sample Preparation Kit (Biotecon Diagnostics GmbH, Postdam, Germany).	Primer: • Universal primers used for mammalian species were researcher-designed. • Primers used for the pig species were researcher-designed Gene Targets: • Universal primers target the GH (growth hormone) gene • Specific primers target prion protein genes	1 pg/µL	(11)

Gelatin Capsule Shell	Detection Method: Conventional PCR DNA Extraction Method: DNeasy mericon Food Kit (Qiagen, Hilden, Germany)	Primer: Porcine specific primer used was AF039170 Gene Targets: Cyt-b gene (porcine specific)	0.1% w/w	(15)
Gelatin Capsule Shell	Detection Method: PCR DNA Extraction Method: MasterPure DNA Extraction Kit (Epicentre, Madison, WI, USA) with pH optimization	Primer: Porcine specific primer used is as follows. • SUS_FWD & SUS RVS • SWF & SWR • SWF & SUSC2R • SUS_FWD & SUSN3R Gene Targets: • SUS_FWD & SUS RVS: ND5 • SWF & SWR: Cyt-b • SWF & SUSC2R: Cyt b • SUS_FWD & SUSN3R: ND5	0.1 pg	(12)

Gelatin powder & gelatin capsule shells	Detection Method: PCR with Loop-Mediated Isothermal Amplification (LAMP) DNA Extraction Method: DNeasy Mericon Food Kit (Qiagen, Hilden, Germany)	Primer: • Universal primer used were researcher-designed. • The primers used for the pig species were researcher-designed Gene Targets: • 16s rRNA (Universal). • Cyt-b (porcine specific)	1 pg/µL	(22)
Gelatin Capsule Shell	Detection Method: Duplex PCR DNA Extraction Method: DNeasy Mericon Food Kit (Qiagen, Germany)	Primer: Porcine-specific primers used were designed by other researchers. Gene Targets: Cyt-b gene (Porcine specific)	0.1% DNA or 0.1 gram/100 ml DNA	(10)
Health Supplement Gelatin Capsule Shell	Detection Method: PCR with WGA (Whole Genome Amplification) DNA Extraction Method: DNeasy® Blood & Tissue or DNeasy® Plant kit (Qiagen)	Primer: SFI11-Pig-F and SFI11-Pig-R Gene Targets: 16s rRNA gene (Porcine specific)	0.01ng/µL	(9)

Gelatin Capsule Shell	Detection Method: PCR RFLP DNA Extraction Method: Genomic DNA Mini Kit (Geneaid, Taiwan)	Primer: Porcine-specific primers used were designed by other researchers. Gene Targets: Cyt-b1 and Cyt-b2 genes (porcine specific) Restriction Enzymes: *BsaJ*I	3000 µg/mL	(23)

Table 3. Advantage and Disadvantage of the Method from Scientific Journal Data

Method Analysis	Advantage	Disadvantage
PCR MyTaq DNA Polymerase (16)	MyTaq DNA Polymerase enables accurate and sensitive detection of porcine DNA in collagen cream cosmetic products through efficient and specific DNA amplification at higher temperatures.	The heat resistance of the enzyme can result in non-specific amplification that may affect the method's specificity.
Real-time PCR (7,12,17,19)	A specific and sensitive method for detecting porcine materials in gelatin, gelatin capsules, and cosmetics with quantitative and real-time monitoring capabilities.	Complex modifications are necessary to enhance the interaction between gelatin and DNA during extraction, and selecting the right method is crucial for accurate detection.

PCR-RFLP (20,23)	It can detect products containing pork, beef, and fish in one test without any cross-reaction between the main targets (cow, pork and fish DNA).	It requires complexity in primer design and high technical expertise for interpreting restriction pattern, as this can affect the specificity and success of the amplification.
Duplex PCR (10,18)	It enables simultaneous detection of multiple target DNA sequences in a single reaction, thus saving time and improving efficiency.	There is a risk of false positives due to non-specific amplification caused by cross-reactivity or sample contamination.
TaqMan Qualitative PCR Multiplex Probe (21)	Specific, sensitive, and potentially cost-effective test for detecting 3 species at once (cow, pig and fish).	It is difficult to apply for samples containing genetically closely related DNA.
Conventional PCR (15)	Sensitive enough for detecting low percentage of bovine and porcine gelatin	It cannot be applied for quantitative analysis.
PCR with Loop-Mediated Isothermal Amplification (LAMP) (22)	LAMP is a cost-effective and accessible method for porcine detection as it does not require thermal cycling like PCR, operating at a constant temperature.	LAMP requires careful primer design to ensure specificity and accuracy, as improper design can lead to non-specific amplification and false-positive results.
PCR with Whole Genome Amplification (WGA) (9)	WGA can increase the quantity of amplifiable DNA, thereby enhancing the sensitivity and accuracy of detection.	WGA in PCR can increase the risk of cross-contamination from other positive samples.

4. Discussion

4.1 DNA Extraction/Isolation Methods

DNA isolation in cosmetics, particularly oil-based preparations, poses challenges due to the manufacturing process. Creams and similar cosmetics undergo extensive processing, resulting in minimal DNA content, making isolation more difficult (24). In the study by Zabidi *et al.* (16), the Genomic Wizard DNA purification kit yielded DNA concentrations ranging from 3.252-4.222 ng/µL, with DNA purity ranging from 1.0-1.4 (optimal DNA purity value: 1.8-2.0). The DNA concentration was relatively low compared to DNA isolated from processed food products (positive control), and the low DNA purity could be attributed to contamination with other proteins. Cream-based cosmetic products undergo extensive processing and contain additives, resulting in a small amount of DNA (24).

From several studies, it is known that the most suitable kit for extracting porcine DNA from cosmetic products is the Power PrepTM DNA compared to other method (7). The Power PrepTM DNA extraction kit, which incorporates chloroform, is considered more efficient compared to other extraction kits. Chloroform or hexane can be added to fat-rich cosmetics like creams to alleviate PCR process inhibitions, enhancing detection capabilities (25–27).

The DNeasy Mericon Food Kit is the most used commercial kit for isolating pig DNA from gelatin samples or gelatin capsule shells, as indicated in **Table 2.** Additionally, some studies have developed specific DNA extraction methods to increase the yield of DNA for analysis. Yang *et al.* (11) compared DNA quality and PCR success rates of four commercial kits used for DNA extraction. The QuickGene DNA Tissue Kit L is the top-performing commercial kit for extracting porcine DNA from gelatin. It demonstrates the highest DNA concentration, optimal $A_{260/280}$ value indicating purity, superior amplification efficiency, and efficient handling of gelatin samples in terms of speed (11).

In addition, Mohamad *et al.* (12) optimized the DNA extraction from gelatin and gelatin capsules by modifying the DNA-gelatin interaction. They used the MasterPure DNA Purification Kit and found that adjusting the pH to 8.5 significantly increased the amount of extracted DNA compared to samples

4.2 Porcine DNA Detection Method

Polymerase Chain Reaction is an in vitro technique that amplifies target DNA fragments. It involves denaturation, annealing, and elongation stages using polymerase enzymes and deoxynucleotide triphosphates (dNTPs). Specially designed primers determine the target DNA fragment (14).

Identification of halal products based on mitochondrial DNA (mt-DNA) is estimated to be more accurate because it is more specific, reproducible, sensitive primers, affordable and has a faster processing time (28). In addition, the use of mt-DNA is also due to the high amount of mt-DNA found in mitochondria compared to DNA in nuclear cells or nuclear DNA (7). On the other hand, identification based on nuclear (chromosomal) DNA as the target gene is claimed to be less stable and subject to changes in the number of copies during the evolutionary process (29,30), but the use of nuclear DNA has the advantage of helping the PCR amplification process from degraded DNA extraction due to lengthy processing of samples (31,32). Mohamad *et al*. (19) conducted a study comparing the detection of porcine DNA in soft capsule samples using mitochondrial DNA and nuclear DNA gene targets. The findings showed that nuclear DNA detection provided a more sensitive limit of detection (1 pg gelatin DNA) compared to mitochondrial DNA (10 pg gelatin DNA).

In the PCR method, optimization is necessary at each stage, particularly in determining the annealing temperature and time. Annealing refers to the binding of primers to DNA fragments. Khayyira *et al*.'s (18) found that the optimal annealing temperature for duplex PCR with porcine DNA specific primers was 56°C. Temperatures of 55°C and 57°C resulted in non-specific bands, possibly due to primer non-specificity at low temperatures and denaturation at high temperatures.

Various PCR methods and their modifications, such as Real-Time PCR, PCR-RFLP, Multiplex-PCR, Duplex-PCR, and MyTaq DNA Polymerase PCR, have been utilized in the detection of porcine DNA in cosmetic products and health supplements. Comparison of various methods can be seen in **Table 3**, including their advantages and disadvantages. These modifications aim to enhance the detection sensitivity and accuracy of porcine DNA.

Real-Time PCR

Real-Time PCR is a technique used to quantify target DNA. RT-PCR or qPCR amplifies as well as calculates the number of target DNA molecules amplified. The principle of qPCR is the use of fluorescent dyes as probes or label dyes (14). Kim *et al*. (7) used RT-PCR to quantify pig DNA and obtained a threshold cycle (Ct) value. However, a high Ct value can potentially be a false positive due to amplification artifacts or residual fluorescence. To validate the Ct value, agarose gel analysis is recommended to confirm the presence of the correct target gene.

Multiplex and Duplex PCR

Multiplex and Duplex PCR are modified amplification methods that allow for simultaneous detection of multiple loci within a single PCR reaction. Multiplex PCR utilizes several specific primers (more than 2), while Duplex PCR utilizes 2 specific primers to amplify different target sequences (14). Khayyira *et al*. (18) carried out the detection of pig DNA using the Duplex PCR method. This study proves that the method can provide sensitivity and specificity in the detection of porcine DNA at low concentrations. In addition to its use for the analysis of halal products, the use of Multiplex and Duplex PCR has been applied in many areas of DNA testing such as gene deletion analysis, mutation and polymorphism detection, quantitative analysis, identification of viruses and parasitic bacteria (28,33,34). The advantage of using this method is that it can test 3 DNA contents at once in one test (20).

PCR-RFLP

PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) involves treating PCR amplicons with specific restriction enzymes to generate DNA fragments of varying sizes. These fragments are then separated by gel electrophoresis. The method is known for its simplicity, making it accessible to those with limited experience in molecular biology. However, PCR-RFLP requires specific restriction enzymes and faces challenges in accurately identifying variations when multiple SNPs (Single Nucleotide Polymorphisms) are targeted simultaneously (35). In the research journals used as data in this review, there are 3 studies using PCR-RFLP to detect porcine DNA in samples. Of the 3 studies,

2 studies used the restriction enzyme BsaJI and 1 study used the restriction enzyme BsaAI.

Modified Probe Real-Time PCR

Several studies have utilized commercial PCR kits, including MyTaq DNA Polymerase PCR, TaqMan Probe real-time PCR, and TaqMan Probe Multiplex Qualitative PCR. These kits employ similar PCR principles with the addition of DNA probes that specifically bind to DNA targets, exhibiting strong affinity (36). The type of probe used is the TaqMan probe, which is an oligonucleotide sequence which at the 5' end has a fluorescent label dye and the 3' end has a quencher label dye (37). The advantage of this TaqMan probe is that it can be easily synthesized and designed, but when the probe is not designed optimally, secondary chains such as hairpins, dimers, runs, and repeats can be formed during the PCR process (38).

Modified Amplification PCR

In addition to PCR methods, other amplification techniques such as Loop Mediated Isothermal Amplification (LAMP) and Whole Genome Amplification Kit (WGA) have been utilized to increase the amount of extracted DNA for detection purposes. These methods offer alternative approaches for DNA amplification in various studies. Tasrip *et al.* (22) utilized LAMP for DNA amplification. The LAMP method involves thermal amplification of DNA using specific primers, resulting in increased specificity of detection due to the recognition of multiple target DNA regions by four primers. Meanwhile, Lee *et al.* (9) used the WGA Kit to amplify the extracted DNA. WGA offers unbiased amplification of the entire genome, high yield, and scalability. It is suitable for various applications such as RFLP analysis, cloning, and DNA sequencing, and can amplify small starting material amounts (39–41). In the analysis conducted by Lee *et al.* (9), it was found that from samples treated with WGA, DNA fragments were produced in the range of 100 to 1000 bp.

5. Conclusion

Based on the collected research, there are many cosmetic and health supplement products that contain porcine DNA. Therefore, testing for porcine DNA is crucial to ensure the halal quality of the products. DNA-based methods, including the Polymerase Chain Reaction (PCR)

and its modifications, has been proven to detect the presence of porcine DNA in products like cosmetics and health supplements. The selection of DNA detection and isolation methods depends on the type of sample under examination, thus enhancing the accuracy and success of porcine DNA detection.

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