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Comparative Study of DNA Extraction Methods for Halal Food Testing: Advantages, Disadvantages, and Recommended Methods

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ABSTRACT

Identification of halal status of food is a crucial aspect of the food industry, particularly in countries with a majority Muslim population. DNA extraction methods play a critical role in ensuring the halal status of food products by accurately detecting non-halal substances. This study aimed to conduct a comparative evaluation of various DNA extraction methods used in halal food testing. Several DNA extraction methods listed in RSNI3 ISO 21571:2005 were discussed: phenol-chloroform method (1), silica method (2), Cethyl Trimethyl Ammonium Bromida (CTAB) method (3), polyvinylpyrrolidone (PVP) method (4), and guanidine chloroform method (5). An economic and process effectiveness were also reviewed to identify the best method. Based on this analysis, the most suitable method for testing the halal status of various types of food sample was recommended. The results of this study are expected to serve as a guideline for testing laboratories and the food industry in selecting the optimal DNA extraction method to ensure efficient and accurate halal status of products.

Keywords: *Consumption, Comparative Study, DNA Extraction, Halal Food, Food*

1 Introduction

The food supply is the most essential aspect for human survival, therefore, it is important for us to pay serious attention to its quantity and quality [1]. With the majority of Indonesia's population being Muslims, reaching 207.2 million people or approximately 85% of the total population of 237 million people, it is important for us to consider the concept of halal and haram foods in Islamic beliefs, including beverages. The halal industry serves a vast global market, which includes more than 1.9 billion Muslims worldwide and non-Muslim consumers

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who are increasingly concerned about product quality and halal. In addition to food safety concerns, it is also important for the Muslim community to consider halal aspects when selecting food products. Therefore, it should be the government's responsibility to protect consumers from the risks of haram food that may be circulated in the market.

Generally, the testing of product halal is carried out through laboratory procedures. Some scientific procedures that are often used to detect contamination of non-halal ingredients in food products are HPLC (High-Performance Liquid Chromatography), ELISA (Enzyme-Linked Immunosorbent Assay), and IEF (Isoelectric Focusing). These methods use specific proteins as markers to reveal contamination. However, protein detection methods tend to be less effective when applied to processed foodstuffs. This is attributed to the nature of proteins that can denature during processing, resulting in inaccurate test results.

The use of DNA-based testing is considered a solution to this problem. Unlike proteins, DNA has a more stable structure and tends to be preserved despite exposure to high temperatures. One of the commonly used DNA testing methods to detect contamination in processed foods is Polymerase Chain Reaction (PCR) [2]. PCR is an efficient, fast, specific, uncomplicated, and sensitive molecular analysis technique for identifying the DNA of an organism [3]. The superiority of this technique has been demonstrated in detecting the presence of specific DNA in various processed meat food products, even at very low concentrations [4].

However, there are several limitations of DNA extraction methods in the identification of halal foods. In addition to the reagent limitations, DNA extraction methods may not be specific for identifying DNA. Among the many new DNA extraction methods currently being developed, The advantages and disadvantages of these methods and identifies the best method for general halal food identification applications were evaluated.

2 Methods

2.1 Literature Review

The first step in this methodology was to conduct a literature search. The literature review was performed using academic databases such as PubMed, Google Scholar, and related scientific journals.

2.2 Obtaining Data

The primary data were obtained from the draft of the Indonesian National Standard (RSNI) number RSNI: RSNI3 ISO 21571:2005. [5]

2.3 Data Analysis

Analyses were carried out with the support of data and information from the literature to find the best extraction method for certain food ingredients to be tested for halal.

3 Results and discussion

3.1 Identification of Halal Food Using DNA Extraction

DNA extraction is a crucial step in verifying halal food because, this process allows DNA to be isolated from food samples for molecular analysis. Through this technique, the presence of

DNA from non-halal sources, such as pork or other substances not compliant with halal regulations, can be accurately detected. This enables precise verification of the halal status of a food product, ensuring that Muslim consumers can consume such products with full confidence in their adherence to halal standards. The following are some common steps involved in testing food for halal compliance using a DNA-based approach (Figure 1).

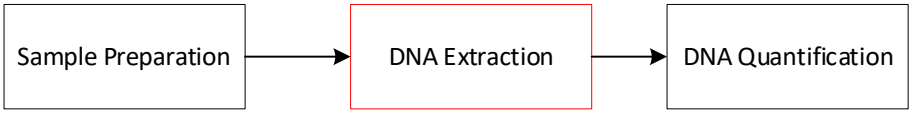

Fig. 1 Step for testing food for halal compliance using a DNA-based approach

Sample preparation is the initial step in DNA analysis, involving the collection and handling of biological samples to ensure their quality and integrity before DNA extraction. DNA extraction involves the separation and purification DNA from cells and cellular fluids, yielding pure DNA suitable for molecular analysis. Meanwhile, DNA quantification was performed to measure the concentration and purity of the extracted DNA to ensure an adequate amount and quality for further analysis. This paper focus on DNA extraction and several methods discussed in RSNI3 ISO 21571:2005.

DNA extraction is the procedure used to separate DNA from cells and fluids to obtain pure DNA suitable for molecular analysis. DNA extraction methods can be divided into two types: liquid-phase extraction and solid-phase extraction. The basic principle of DNA extraction involves releasing DNA from its matrix and subsequently purifying the DNA from PCR inhibitors, either simultaneously or sequentially. The process of DNA extraction involves several critical chemical reactions aimed at separating DNA from cell membranes, proteins, and other cellular components. the genetic information for the development and functioning of any organism is encoded in DNA or RNA sequences that are located inside the cell[6]. The cell has an outer boundary called the cell membrane, which encloses all the contents. The cell membrane act as a barrier and regulates the transport of materials between the cell inside and outside. The cell membrane must be disrupted or destroyed to access the DNA from inside the cell for molecular diagnosis as shown in Figure 2 [7].

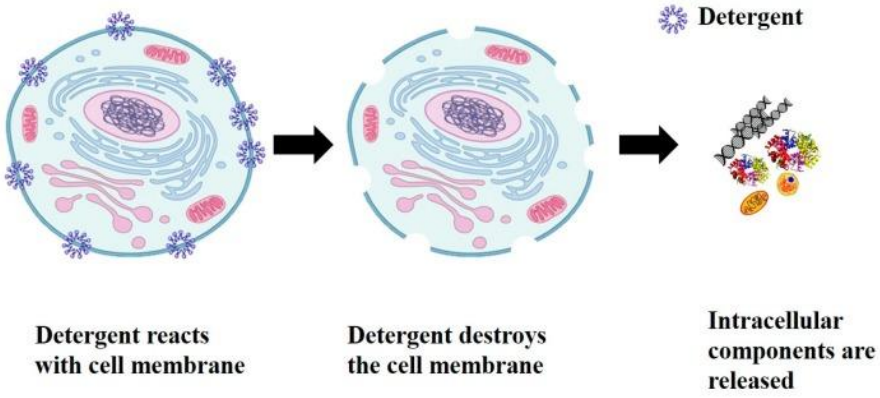

Fig. 2 Mechanism of Lysis Cell [7]

DNA extraction methods can be used to identify food halalities because they enable isolation and analysis of DNA from food materials. This method allows accurate detection of DNA from

non-halal sources such as pork or animals not slaughtered according to religious laws. Following DNA extraction, techniques like Polymerase Chain Reaction (PCR) can be employed to identify the species origin of the DNA, ensuring that food products are free from contaminants that do not comply with halal requirements. Therefore, this method provides robust assurance for manufacturers and consumers in scientifically and reliably verifying the halal status of food products. In RSNI3 ISO 21571:2005, five DNA extraction methods are discussed: the phenol chloroform method, Silica method, Cetyl Trimethyl Ammonium Bromide (CTAB) method, polyvinylpyrrolidone (PVP) method, and guanidine chloroform method.

3.2 Extraction of DNA Method

Figure 3 is a general flowchart of the five DNA extraction methods. All five methods generally follow the same process steps, which include sample preparation, cell lysis, contaminant removal using specific reagents according to each method, DNA precipitation, and finally washing.

Fig. 3 Block Flow Diagram DNA Extraction

3.2.1 Phenol-Chloroform Method

This method includes material lysis (thermal lysis in presence of sodium dodecyl sulfate and a high EDTA content, nucleases from cells, and contaminants purification using organic solvents). DNA is recovered via precipitation using alcohol and salts, followed by rehydration. An alternate protocol uses a column-based purification method for the extracted DNA instead of alcohol precipitation. Cell lysis uses buffers containing enzymes and detergents. Organic extraction was conducted by adding equal amounts of water-saturated phenol dissolved in buffer to the aqueous DNA sample, agitating the mixture vigorously and then centrifuging to enable phase separation[8]. The aqueous top layer was then carefully transferred to a new tube, avoiding the phenol interface. Chloroform was then added to extract the remaining phenol. DNA concentration using ethanol precipitation. After washing with 70% ethanol, the DNA pellet was dried using a speed vacuum and dissolved in low-salt buffer. This extraction method is suitable for fresh meat, vegetables, grains, and cooked meat[5].

3.2.2 Polyvinyl-Pyrrolidone Method

In this method, cells are destroyed via thermal lysis with high concentrations of sodium dodecyl sulfate and EDTA reagents. After the cell lysis process, the DNA is removed from contaminants such as polyphenol, polysaccharides, metabolites, and soluble proteins. This method uses polyvinylpyrrolidone and ammonium acetate. The final step is alcohol precipitation, which is used to concentrate the DNA and remove residual salts. This stage not only concentrates the DNA but also helps eliminate residual impurities [5].

3.2.3 CTAB Method

This method consists of cell lysis, extraction, and isopropanol precipitation, followed by washing with ethanol. The lysis stage is useful for destroying parts of the cell via thermal lysis with the addition of CTAB. For some matrix, alpha amylase can be added to the lysis buffer to break down starch, if it contains amylum. In addition, proteinase K enzyme is needed to eliminate protein and r-nase A which remove RNA that can interfere with the next process. The next step is the extraction stage to remove contaminants. The final step is purification using precipitation and washing methods[5].

3.2.4 Silica Method

The Silica method involves a thermal lysis process with sodium dodecyl sulfate in the buffer. The next step is purification using silica resin with the addition of chaotropic guanidine-hydrochloride reagent. The essence of this method is the binding of nucleic acids into silica under conditions of low water activity. The final step was to remove contaminants from the resin using isopropanol. The final elution step used a low-salt buffer solution to recover the DNA[5].

3.2.5 Guanidium-Chloroform Method

The Guanidium-Chloroform method is widely used in DNA extraction at process at low pH. The principle of this method is to separate RNA from DNA after extraction. This method starts from the cel lysis stage using thermal lysis. Followed by cell extraction with the addition of guanidium thiocyanate solution for protein degradation.subsquently, chloroform was added, followed by centrifugation to separate the solution into an aqueous phase and the upper part containing RNA. Subsequently, precipitation and washing using alcohol were carried out[5].

3.3 Comparison and Economics

Table 1 presents the advantages and disadvantages of several DNA extraction methods used in RSNI3 ISO 21571:2005 for food halal testing[5]. The DNA extraction method using phenol-chloroform reagent is currently the most widely used method. According to the table 1, this method efficiently produces DNA copies. The phenol-chloroform method not only recovers nucleic acids in high quantities but also produces amplifiable copies. Additionally, the presence of phenol compounds can deactivate potential nucleases and infectious sources from the first step. Compared to the proteinase K method, the revised phenol-chloroform method yields the purest genomic DNA (gDNA). This protocol reduces working time by combining extraction with purification steps and allows operation at room temperature. The phenol-chloroform method can be used to extract samples with very low DNA concentrations (7.00-9.45 ng/µl) [9]. The extracted products are highly pure. The purity of DNA isolates is ideal for molecular analysis (1.80-2.10). The main drawback of this method is the use of chloroform, which is a hazardous chemical to both humans and the environment. This necessitates careful handling and proper waste disposal, leading to potential environmental and health risks. Moreover, this method requires a relatively long processing time of approximately 30 hours [10].

The second DNA extraction method involves Polyvinylpyrrolidone (PVP) reagent. The use of this reagent reduces the effects of phenolic compounds during DNA extraction. Phenolic compounds can potentially damage DNA if their levels are uncontrolled. Adding PVP in the

DNA extraction process enhances the resulting DNA concentration. Moreover, the materials used are non-toxic, unlike chloroform. One drawback of this method is its time-consuming process and the difficulty in obtaining the necessary raw materials or reagents.

The third DNA extraction method involves Cetyl TrimethylAmmonium Bromide (CTAB) reagent. Previous research indicates that DNA extracted using CTAB is highly stable and can be used for amplification even after approximately 2 years of extraction [11]. This stability is advantageous for long-term DNA extraction processes, such as those used in food halal testing. However, commercially available CTAB reagent kits are quite expensive. Additionally, it should be noted that polyphenolic compounds can inhibit amplification, not just mucopolysaccharides [11]. Furthermore, there is a possibility of partial DNA degradation during extraction with CTAB [12].

The next DNA extraction method involves the use of Silica. This method has been proven to produce DNA of the highest quality and requires a short duration for its DNA extraction process. However, this method yields very low amounts of gDNA (almost undetectable). This method can be considered the best choice for samples that require whole genome amplification [9]

The last method is DNA extraction using Guanidinium-Chloroform reagent. A notable feature of this method is its lack of reliance on any enzymes (except RNAse A) such as lysozyme to lyse gram-positive bacteria. Despite this, the method can still yield high-quality genomic DNA using a few simple steps. However, a major drawback is its continued use of hazardous chemicals like chloroform.

Table 1. Comparison of Advantages, Disadvantages, and Economical Aspects of DNA Extraction Methods

No	Methods	Advantages	Disadvantages	Estimated Extraction Cost
1	Phenol-Chloroform	- Produces high numbers of DNA copies - Easy to test - Produce amplifiable DNA copies - Can work at room temperature - Can extract samples with very low DNA concentrations - Phenol-chloroform extraction products are very clean.	- Hazardous chemicals - It requires a lengthy process	Rp. 425.000,00
2	Polivinil-Pirolidon (PVP)	- Reduced effects of phenolic compounds during DNA extraction - DNA concentration - Use of non-toxic materials	- Difficult to obtain reagents	Rp. 217.590,00
3	CTAB	- Extracted DNA is highly stable	- Potential degradation of partial DNA - Very costly	Rp. 402.831,00
4	Silica	- Produce high-quality DNA - Shortest processing time	- Very low gDNA yield (almost undetectable)	Rp. 83.960,00

5	Guanidinium-Chloroform	- Minimal use of enzymes - High yields of genomic DNA - Simple steps	- Hazardous chemicals such as chloroform	Rp. 219.042,00

An economic analysis of each type of DNA extraction method was also conducted. This estimation was obtained through a cost analysis of raw materials for each DNA extraction method according to the procedures in RSNI3 ISO 21571:2005. The cost of each material used was recorded based on prices from e-commerce sources and converted into the cost per unit of the material used in a single run of DNA extraction according to RSNI3 ISO 21571:2005. From the results of this analysis, it was found that the most expensive DNA extraction method is Phenol-chloroform, followed by CTAB, with costs exceeding Rp 400,000 per sample, as shown in Table 1. In contrast, the most economical DNA extraction method is using silica reagents.

3.4 Recommendations of Methods Based on Types of Test Samples

Based on Table 2, various products can be extracted using different methods, such as Phenol-Chloroform, Polyvinylpyrrolidone, CTAB, Silica, and Guanidium-Chloroform. The following is a detailed description of the products suitable for each extraction method:

1. Phenol-Chloroform Method:

The Phenol-Chloroform method is a classic method used for the extraction of DNA and RNA from cells. Phenol acts as a denaturing agent that disrupts cell membranes and proteins, while chloroform helps separate the aqueous phase and the organic phase, enabling the purification of nucleic acids. Products suitable for this method include various types of food and organic materials such as fermented soybeans, grains, dairy products, and meat. These products usually have a high cellular content, so they require strong denaturing agents to break down the cell membrane and release the nucleic acids.

2. Polyvinylpirrolydon (PVP) method:

Polyvinylpirrolydon (PVP) is a polymer used to remove polyphenols during the nucleic acid extraction process. Polyphenols, which are often found in plants, can interfere with DNA extraction by binding to nucleic acids and inhibiting enzymatic reactions. PVP neutralises these polyphenols, making this method particularly effective for plant products and processed foods that contain polyphenolic components.

3. Cetyl TrimethylAmmonium Bromide (CTAB) method:

CTAB is a cationic detergent used to separate nucleic acids from polysaccharides and proteins. This method is particularly useful for plants that contain a lot of polysaccharides, such as grains and corn products. CTAB helps precipitate protein-polysaccharide complexes, allowing for cleaner nucleic acid purification.

4. Silica method:

The Silica method uses silica columns or membranes to bind nucleic acids under certain conditions, allowing purification by washing away other contaminants. This method is

particularly suitable for products that have a high nucleic acid content but require additional purification to separate from proteins and other compounds. Under specific conditions (such as in the presence of chaotropic salts), nucleic acids (DNA and RNA) have a strong affinity for silica surfaces. This is because the chaotropic agents disrupt the water structure, causing nucleic acids to bind to the silica while other macromolecules like proteins, lipids, and carbohydrates do not bind as effectively [13].

5. Guanidium-Chloroform Method:

The Guanidium-Chloroform method uses guanidium as a strong denaturing agent that lyses cells and inactivates ribonuclease (RNase) to protect RNA. Chloroform is then used to separate the aqueous phase containing nucleic acids from the organic phase. This method is very effective for purifying nucleic acids from samples that have many proteins and RNases. Guanidium salts (such as guanidium isothiocyanate) are powerful chaotropic agents. They disrupt the hydrogen bonding in proteins, causing them to denature. This denaturation inactivates enzymes like RNases and DNases that could otherwise degrade RNA and DNA, protecting the nucleic acids from enzymatic degradation [14].

Table 2. Effectiveness of Various DNA Extraction Methods on Different Food Products

Sample	Phenol-Chloroform	Polyvinyl pyrrolidone	CTAB	Silica	Guanidium-Chloroform
Fermented soybeans	Yes	No	No	No	Yes
Green materials	Yes	No	No	No	No
Baby biscuits	Yes	Yes	No	No	No
Baby milk	Yes	Yes	No	No	No
Bacteria and their spores	Yes	No	No	No	No
Barley seeds	Yes	No	No	No	No
Beef/pork pâté	Yes	No	No	No	No
Beer	Yes	No	No	No	No
Blue cheese	Yes	No	No	No	No
Brownies	Yes	Yes	No	No	No
Canned corn	Yes	Yes	Yes	No	Yes
Carrot seeds	Yes	No	No	No	No
Cereal bars	Yes	Yes	No	No	No
Cheese	Yes	Yes	No	No	No
Chicken nuggets	Yes	Yes	No	No	No
Chicory leaves	Yes	No	No	No	No
Chicory root	Yes	No	No	No	No
Chocolate cookies	Yes	Yes	Yes	No	No
Chocolate pasta	Yes	Yes	Yes	No	No
Cinnamon cookies	Yes	No	No	No	No
Compotes	Yes	No	No	No	No
Cornflakes	Yes	Yes	Yes	No	No

Cracked rice	Yes	No	No	No	No
Dessert cream	Yes	Yes	Yes	No	Yes
Dried pea seeds	Yes	No	No	No	No
Corn biscuits	Yes	Yes	No	No	No
Maize-feeding oil cakes	Yes	No	Yes	No	Yes
Corn flour	Yes	Yes	Yes	Yes	Yes
Corn gluten feed	Yes	No	Yes	Yes	Yes
Corn seeds	Yes	No	Yes	Yes	Yes
Hard cassava pellet feed	Yes	No	No	No	No
Cassava meat	Yes	No	No	No	No
Fresh and cooked meat	Yes	Yes	Yes	No	No
Melon flesh	Yes	No	No	No	No
Melon seeds	Yes	No	No	No	No
Minced meat	Yes	Yes	Yes	No	No
Muesli ingredients	Yes	No	No	No	No
Muesli	Yes	Yes	Yes	No	No
Green bean sprouts	Yes	No	No	No	No
Oat seeds	Yes	No	Yes	No	No
Potato tubers	Yes	No	Yes	No	No
Rapeseed-feeding oil cake	Yes	No	Yes	No	No
Glupacolza	Yes	No	No	No	No
Rapeseed seeds	Yes	No	Yes	No	No
Sausage	Yes	Yes	Yes	No	Yes
Baking mix	No	No	Yes	No	No
Bouillon cubes	No	No	Yes	No	No
Sweet and sour candies	No	No	Yes	No	No
Caramel cream	No	No	Yes	No	No
Cattle cakes	No	No	Yes	No	Yes
Cereals	No	No	Yes	No	No
Chocolate bars	No	No	Yes	No	Yes
Chocolate cream	No	No	Yes	No	No
Chocolate	No	No	Yes	No	No
Cookies	No	No	Yes	No	No
Corn beer	No	No	Yes	No	No
Dextrose	No	No	Yes	No	No
Pralines filling	No	No	Yes	No	No
Pastries	No	No	Yes	No	No
Fish	No	No	Yes	No	No
Fish fingers	No	No	Yes	No	No
Flakes of whole soybean	No	No	Yes	No	Yes
French fries	No	No	Yes	No	No
Gravy	No	No	Yes	No	No
Boiled ham	No	No	Yes	No	No

Honey	No	No	Yes	No	No
Instant food	No	No	Yes	No	No
Corn stalks	No	No	Yes	No	No
Corn embryos	No	No	Yes	No	No
Corn leaves	No	No	Yes	Yes	No
Native corn starch	No	No	Yes	No	No
Corn oil	No	No	Yes	No	No
Corn protein	No	No	Yes	No	Yes
Corn seeds	No	No	Yes	No	No
Corn semolina	No	No	Yes	Yes	Yes
Margarine	No	No	Yes	No	No
Mixed animal feed	No	No	Yes	No	No
Green bean seeds	No	No	Yes	No	No
Mustard leaves	No	No	Yes	No	No
Popcorn	No	Yes	Yes	No	No
Potato chips	No	Yes	Yes	No	No
Potato starch	No	No	Yes	No	No
Rape leaves	No	No	Yes	No	No
Rape-press cake	No	No	Yes	No	No
Rapeseed oil	No	No	Yes	No	No
Raw soy lecithin	No	No	Yes	No	Yes
Ready-to-eat food	No	No	Yes	No	No
Salami	No	No	Yes	No	No
Salty snacks	No	No	Yes	No	No

4 Conclusion

DNA extraction is a crucial step in verifying halal food because, this process allows DNA to be isolated from food samples for molecular analysis. In RSNI3 ISO 21571:2005, five DNA extraction methods: (the phenol chloroform method, Silica method, CTAB method, polyvinylpyrrolidone (PVP) method, and guanidine chloroform method) were discussed. Based on the flow diagram blocks that have been outlined, all methods share the same general steps. However, each method uses different reagents.

The Phenol-Chloroform method is suitable for various types of food and organic materials such as fermented soybeans, grains, dairy products, and meat. These products usually have a high cellular content. Polyvinylpyrrolidone (PVP) is particularly suitable for products that have a high nucleic acid content but require additional purification to separate from proteins and other compounds. CTAB is particularly useful for plants that contain a lot of polysaccharides, such as grains and corn products. The Silica method is particularly suitable for products that have a high nucleic acid content but require additional purification to separate from proteins and other compounds. The Guanidium-Chloroform method is very effective for purifying nucleic acids from samples that have many proteins and Rnases.

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