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Life Cycle Analysis (LCA) of Halal Authentication Approaches on Industrial Food Waste for Gelatine Production: A Mini Review

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Abstract

Industrial food waste is a major issue that affects numerous countries. One of the techniques to reduce the generation of food waste is to recycle it for other purposes. Particularly, food waste from livestock industries can be utilised for gelatine preparation. However, the study on halal authentication of industrial food waste is still low. This review is conducted to identify the available protein analysis methods utilised in halal authentication of industrial food waste. It is also to compare and acknowledge the most reliable method. This review is performed in three steps: the planning phase, the conducting phase and the analysing phase. Reliable methods such as polymerase chain reaction (PCR), multiple reaction monitoring (MRM), fluorescent molecularly imprinted polymer nanogel (F-MIP-NG), lateral flow devices (LFDs) and loop-mediated isothermal amplification (LAMP) are identified and discussed. Their sensitivity limits are also recognised and compared.

1. Introduction

Waste is any material or substance that is no longer needed and has no further use or value in its current state. It can take many forms, including solid, liquid or gaseous. It can be divided into many categories according to its composition or origin (Jamal, 2020). According to their origins, waste can be sorted into commercial, institutional, municipal, and industrial categories. The industrial waste comes from various industrial factories; the livestock industry is one of them. Livestock industries generate a variety of food waste, consisting of meat, poultry and fish. According to Harvard (2023), food waste refers to edible food that is intentionally disposed of during the stages of retail or consumption, despite being suitable for eating. Food waste can be utilised and profited in numerous ways: biochemical processing, sewage treatment, and composting (US Environmental Protection Agency, 2018). As reported by Aksun Tümerkan (2021), food waste can also be processed into halal gelatine. This type of product life cycle applied in livestock industries is categorised as cradle-to-cradle (C2C); it is a sustainable framework which creates regenerative products that can be reused or recycled indefinitely. Nevertheless, every process in a product life cycle has its environmental impacts, which can be evaluated by the method of life cycle assessment (LCA) using an open-source software tool called OpenLCA.

Halal is an Arabic term that means 'permissible' or 'lawful'. *Halalan toyyiban* is an Islamic principle that emphasises the importance of consuming halal (lawful) and *toyyib* (good) food (Islamic Religious Council of Singapore, 2023). The term is derived from the *Qur'anic* verse that states,

> "O mankind, eat from whatever is on Earth
> [that is] lawful and good" (*Al-Baqarah* 2:168)

The principle encompasses the method of slaughtering animals and the measures in handling, storing, preparing and processing the ingredients used in food production (Abd Rahman & Abu Dardak, 2021). In Malaysia, a standard called MS 1500:2009 was specifically developed by the Technical Committee on Halal Food and Islamic Consumer Goods for halal food production. The importance of *halalan toyyiban* is recognised by Muslims worldwide, and it has become a significant part of their lifestyle as it highlights the holistic nature of Islam, which stresses the importance of physical, mental, and spiritual well-being.

As previously mentioned, halal gelatine can be produced from food waste. Gelatine is typically derived from animal collagen, which can be extracted from various collagen-rich sources, including bones, skin, and cartilage (Richter, 2021). In many cases, these sources of collagen are considered waste or by-products of the meat, poultry, and seafood industries. Depending on the collagen source, gelatine is either porcine, bovine or piscine. Halal gelatine is a type of gelatine that is permissible according to Islamic law. It is derived from sources that comply with Islamic dietary laws, prohibiting pork and un-slaughtered meat consumption. The use of halal gelatine has become increasingly important in the food and pharmaceutical industries as the Muslim population continues to grow

worldwide. According to Desilver & Masci (2017), the Muslim population is expected to account for 29.7% of the world's population by 2050. This population presents a significant market opportunity for halal products, including halal gelatine. In order to produce halal gelatine from food waste, animal origin needs to be identified. This production requires careful sourcing, in which several techniques can be utilised.

This mini-review paper is conducted to identify the available protein analysis methods utilised in halal authentication of industrial food waste. It is also to compare these methods to recognise the most reliable and efficient technique to be used.

2. Methodology

For this review, three main steps are involved: the planning phase, the conducting phase and the analysing phase. The methodology flow can be seen in Figure 1 below.
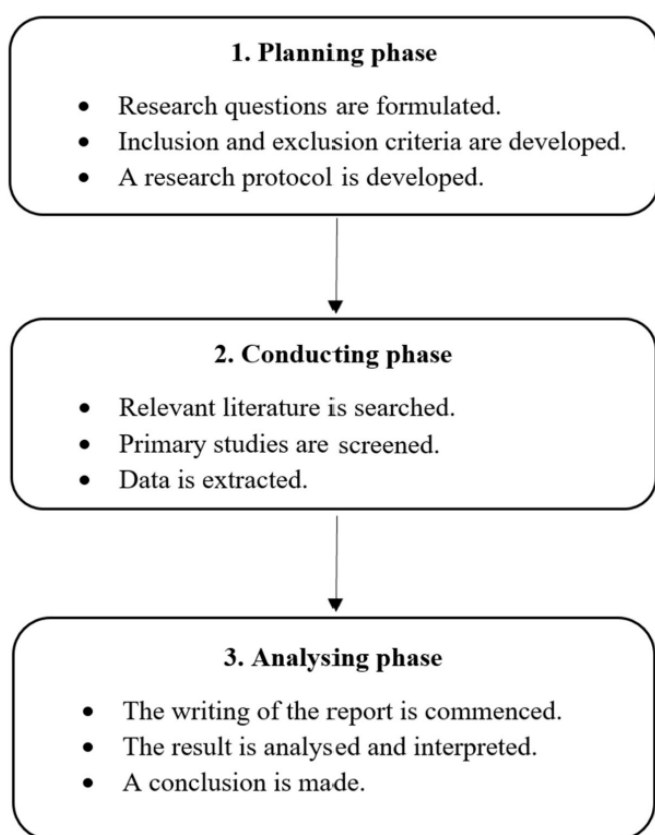

Figure 1: Methodology flow of the mini-review.

2.1 Planning phase

The first step, the planning phase, involves formulating a research question from a PICOS tool short for Population, Intervention, Comparison, Outcomes and Study.

Table 1: PICOS table for the review

Element	Keyword
Population	Industrial food waste
Intervention	Protein analysis methods
Comparison	None
Outcome	Halal authentication
Study	Scientific report papers

After the keywords are set as shown in Table 1, the research question can be put together: "What is known from the scientific report papers about protein analysis methods in halal authentication of industrial food waste?". The inclusion-exclusion criteria can then be determined, as shown in Table 2.

Table 2: Inclusion-exclusion criteria for the review

Factor	Inclusion	Exclusion
Timeline	Reports within 2013-2023	Reports before 2013
Subject area	Industrial food waste	Other types of waste
Publication type	Scientific report	Other types of publication
Language	English articles	Non-English articles

2.2 Conducting phase

The conducting phase involves searching for relevant articles using keywords on well-known search engines such as Google Scholar and PubMed and from backward referencing on obtained review papers. After title and abstract screening, a total of 10 articles investigating industrial food waste in the English language from the last decade (from 2013 until 2023) were acquired for this study. This mini-review targets key data in the articles, such as the objectives, analysis method, result and the industrial food waste investigated. The data is extracted and then synthesised in one table (Table 3) to be analysed. The articles are categorised based on the protein analysis methods utilised, in which the number of articles per method is shown in Figure 2.
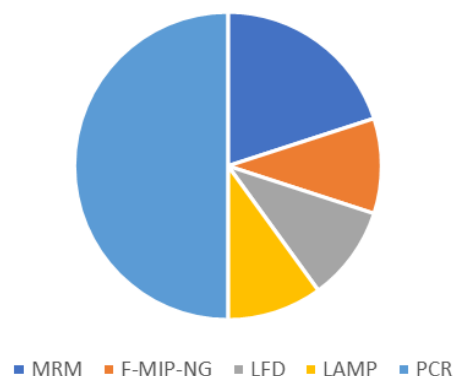

Figure 2: Percentage of studies based on methods utilised.

2.3 Analysing phase

The final step is the analysing phase. The extracted data from the selected articles are analysed, interpreted and summarised to answer the research question and accomplish the main objectives of this review. After the protein analysis methods are identified, the methods are compared to determine the most efficient technique to be utilised in halal authentication of industrial food waste.

3. Results and discussion

In the studies under review, different components were utilised to identify animal species, including meat extracts, processed meat and gelatine; these components are categorised as food waste, a type of waste purposely thrown out despite its edibility. Although different components were tested, all 10 studies investigated the protein level, allowing species differentiation.

Table 3: Extracted data from the reviewed articles

No	Food waste	Title	Method	Objective	Result	Author & year
1.	Processed meat	An SYBR Green real-time PCR assay to detect and quantify pork meat in processed poultry meat products	PCR (qPCR using SYBR Green Supermix)	To detect and quantify pork meat in processed poultry meat products.	The developed qPCR assay allowed the detection and quantification of pork meat in the linear dynamic range of 0.1 – 25% with high correlation and PCR efficiency. The proposed SYBR Green I qPCR method proved to be a powerful and simple technique, highly specific and sensitive for pork species identification without requiring any post-PCR treatment.	Soares *et al.* (2013)
2.	Gelatine capsule shell	Analysis of Porcine Gelatine DNA in a Commercial Capsule Shell Using Real-Time Polymerase Chain Reaction for Halal Authentication	PCR (qPCR using SYBR Green Supermix)	To develop a specific primer from mitochondrial D-loop capable of amplifying DNA from porcine gelatine in commercial capsule shells.	From two primers that have been designed specifically, only primer D-Loop108 had the capability to identify the presence of porcine DNA in fresh tissue and gelatine. The lowest concentration of porcine DNA in gelatine capsule shells is 5 pg.	Sudjadi *et al.* (2016)
3.	Skin crackers	Species-specific polymerase chain reaction (PCR) assay for identification of pig (Sus domesticus) skin in "Rambak" crackers	PCR (qPCR using DreamTaq Green Master Mix)	To develop the pig species-specific primer for identification of specific pig DNA in 'Rambak' cracker.	Analysis of experimental mixture meat demonstrated that 0.1% of pig tissues could be detected using a specific primer. The specificity of pig-specific PCR provides a valuable tool for the identification of pig skin and for avoiding its fraudulent substitution and adulteration.	Erwanto *et al.* (2016)
4.	Gelatine	Development of a new and sensitive method for the detection of pork adulteration in gelatine and other highly processed food products	PCR (qPCR using Tubigel)	To detect the presence of porcine DNA in commercial gelatine and processed foods.	The TübiGel method was found to have a detection limit of 0.01% porcine gelatine, whilst the Biotecon method had 0.1%, and the R-Biopharm method detected >5% porcine gelatine. qPCR of TübiGel method was also found to detect porcine DNA better than qPCR of commercial kits.	Yayla & Ekinci Doğan (2021)
5.	Processed meat	Detection of pork adulteration by highly-specific PCR assay of mitochondrial D-loop	PCR (standard PCR)	To perform authentic identification of pork in other species' meat.	The sensitivity of the detection of pork in other species of meat using unique pig-specific PCR was established to be at 0.1%. The technique is cheaper than qPCR. It can be used for the authentication of raw, processed and adulterated pork and products under the circumstances of food adulteration-related disputes or forensic detection of the origin of pig species.	Karabasanavar *et al.* (2014)
6.	Processed meat	New Sensitive High-Performance Liquid Chromatography–Tandem Mass Spectrometry Method for the Detection of Horse and Pork in Halal Beef	MRM (MRM and MRM3)	To detect trace contaminations of horse meat and pork in halal beef.	Able to detect down to 0.13% pork contamination in beef. Rapid and sensitive mass spectrometrical method for the detection of horse and pork by use of MRM and MRM3.	Von Bargen *et al.* (2013)

7.	Processed meat	Meat authentication: a new HPLC-MS/MS-based method for the fast and sensitive detection of horse and pork in highly processed food	MRM (MRM and MRM³)	To develop an optimised method for the detection of horse and pork in different processed meat.	Identified marker peptides were sufficiently stable to resist the thermal processing of different meat products and thus allow the sensitive and specific detection of pork or horse in processed food down to 0.24% in a beef matrix system.\n\nSpecific and sensitive detection of horse and pork meat in different processed food matrices using MRM-based detection of marker peptides.	Von Bargen *et al.* (2014)
8.	Meat extract	Molecularly imprinted polymer nanogel-based fluorescence sensing of pork contamination in halal meat extracts	F-MIP-NG	To investigate pork contamination in halal meat extracts.	A detection limit for pork contamination was as low as 0.1% in the pork-contaminated beef extract samples was also achieved.\n\nUnder optimal conditions, the F-MIP-NG-based sensors exhibited high sensitivity, a detection limit of 40 pM, a linear range of 0.25 – 5 nM, and excellent affinity and selectivity towards PSA, compared to potentially interfering proteins. It was more efficient to detect beef contamination in 1 wt% pork contamination compared to the real-time polymerase chain reaction.	Cheubong *et al.* (2021)
9.	Processed meat	Development and validation of a rapid test system for the detection of pork meat and collagen residues	LFD	To develop a detection system capable of rapidly (~35min) identifying porcine residues.	A detection system was developed based on a lateral flow device (LFD) assay format capable of rapidly (~35 min) identifying porcine residues derived from raw meat, cooked meat, and gelatine down to 0.01%, 1.0%, and 2.5% contamination, respectively.\n\nThe LFD tests are suitable alternatives in that they do not require any machinery to operate, and report outcomes in ~35 min with the same or better level of sensitivity without any additional processing.	Masiri *et al.* (2016)
10.	Processed meat	Detection of porcine-derived ingredients from adulterated meat based on real-time loop-mediated isothermal amplification	LAMP (real-time LAMP)	To develop an efficient and rapid assay for detecting a porcine gene in meat products.	The amplification showed no cross-reactivity with 11 other meats. The established method required 20 min with an initial amplification curve of approximately 10 min and demonstrated a detection limit of 1.76 pg/µL porcine DNA, which equals to 0.0001%.\nThis method meets specificity, rapidness, robustness, and sensitivity criteria; its practical application will greatly aid in battling adulteration in the food industry.	Cai *et al.* (2020)

Available protein analysis methods utilised in halal authentication of industrial food waste are as listed below:

1. Polymerase chain reaction (PCR)
2. Multiple reaction monitoring (MRM)
3. Fluorescent molecularly imprinted polymer nanogel (F-MIP-NG)
4. Lateral flow devices (LFDs)
5. Loop-mediated isothermal amplification (LAMP)

3.1 Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a widely used molecular biology technique that amplifies specific DNA sequences in vitro. In accordance with the National Library of Medicine (2017), PCR is a method based on the ability of the DNA polymerase enzyme to extend primers annealed to a template DNA strand. It involves three basic steps: denaturation, annealing, and extension (Khan Academy, 2016). In the denaturation step, the DNA is heated to a high temperature to separate the double-stranded DNA into two single strands. In the annealing step, primers complementary to the target DNA sequences anneal to the single-stranded DNA template by the cooling process. DNA polymerase extends the primers in the extension step, producing a copy of the original DNA sequence. These three steps are repeated for multiple cycles, resulting in exponential amplification of the target DNA sequence. PCR is a molecular biology technique that can rapidly and efficiently amplify specific DNA sequences from small or degraded samples.

There exist multiple varieties of PCR. Real-time PCR (qPCR) allows for real-time DNA quantification as the reaction occurs. This type of PCR uses fluorescent dyes or probes to detect DNA amplification as it happens. Over time, several types of qPCR-ready mixes have been developed. Soares et al. (2013) utilised an SYBR Green Supermix, a twice-concentrated mix with real-time fluorescence enhanced for dye-based qPCR. It consists of iTaq DNA polymerase, an antibody-mediated hot-start polymerase (Bio-Rad Laboratories, 2023b). In the study, PCR was conducted to detect and quantify pork meat in processed poultry. The assay detected pork meat in the range of 0.1 - 25%. Furthermore, Sudjadi et al. (2016) also utilised SYBR Green Supermix to develop a highly specific primer from the mitochondrial D-loop for DNA amplification of porcine gelatine in commercial capsule shells. Correspondingly, primer D-loop 108 detected the presence of porcine DNA in gelatine capsule shells at 5 pg. Both studies prove that SYBR Green for the method of qPCR is a powerful yet simple method of identifying pork meat.

Besides SYBR Green, DreamTaq Green PCR Master Mix (2X) is another ready-to-use mix utilised for qPCR. It contains DreamTaq DNA Polymerase, an optimised thermostable DNA polymerase with high sensitivity and yield for DNA synthesisation (Thermo Fisher Scientific, 2023). It was applied by Erwanto et al. (2016) to develop pig species-specific primers for identifying specific pig DNA in the 'Rambak' cracker. Their study proved that 0.1% of porcine DNA could be identified with the DreamTaq Green. It demonstrated that DreamTaq Green is as powerful as SYBR Green in pork DNA identification.

DNA detection kits were also developed for conducting qPCR. Yayla & Ekinci Doğan (2021) developed a method called TübiGel to detect the presence of porcine DNA in commercial gelatine and processed foods. It was proven that TübiGel has a porcine gelatine detection sensitivity of 0.01%, while commercial detection kits such as Biotecon and R-Biopharm only have a sensitivity of 0.1% and >5%, respectively. This showed that the newly designed kit, TübiGel, is more sensitive

to detecting porcine DNA in processed foods than commercial detection kits and ready-to-use mixes.

Apart from qPCR, another form of PCR commonly used in research is standard PCR. In contrast with qPCR, which allows real-time quantification, standard PCR detects the product only at the end-point (Bio-Rad Laboratories, 2023a). Karabasanavar et al. (2014) applied standard PCR in designing new highly specific primers to conduct genuine identification of pork in the meat of other species. As a result, a detection sensitivity of down to 0.1% was achieved. Although standard PCR is proven to be less sensitive than qPCR, it is also less costly.

3.2 Multiple reaction monitoring (MRM)

Multiple reaction monitoring (MRM) is an analytical technique used in mass spectrometry for the targeted quantification of proteins (You et al., 2013). According to Meng & Veenstra (2013), MRM is a type of mass spectrometry that involves selecting a specific precursor ion in the first stage, fragmenting it to produce multiple product ions in the second stage and monitoring the product ions within the third stage. The precursor and product ions selection is based on the specific mass-to-charge ratio (m/z) of the analyte and its fragments. The precursor and product ions are chosen based on their unique m/z values, which allows for specific detection of the analyte of interest. As stated by Rai & Satija (2021), MRM is a powerful method that provides higher selectivity than PCR for detecting low-abundance analytes in complex samples.

MRM can also be conducted with multistage fragmentation, known as MRM[3]. This technique enhances the sensitivity, specificity and selectivity of the method. Von Bargen et al. (2013) employed the method of MRM and MRM[3] to find trace contaminations of pork meat in halal beef. The results showed that MRM could detect 0.55% contamination, while MRM[3] detected down to 0.13% of porcine DNA. A year later, Von Bargen et al. (2014) established an optimised method for identifying pork in other species' processed meat. The MRM and MRM[3] detection were down to 0.24%, demonstrating a rapid 2-minute extraction process. This result proved that MRM and MRM[3] are quick yet sensitive techniques for DNA identification.

3.3 Fluorescent molecularly imprinted polymer nanogel (F-MIP-NG)

Fluorescent molecularly imprinted polymer nanogels (F-MIP-NG) are a relatively new material class that has gained increasing interest in nanotechnology. These nanogels are made up of a network of cross-linked polymers designed to selectively recognise and bind specific target molecules through molecular imprinting (Xu et al., 2021). F-MIP-NG are distinguished from other molecularly imprinted polymers by their ability to emit light when exposed to a specific wavelength of light. According to Huang et al. (2018), the fluorescent molecules serve as a signal transducer and allow for detecting the binding event between the nanogel and the target molecule. The signal produced by the nanogel can be easily detected and quantified using a fluorescence spectrometer, allowing for rapid and accurate analysis.

The method of F-MIP-NG was utilised by Cheubong et al. (2021) to investigate pork contamination in extracts of halal meat. In the study, a sensitive F-MIP-NG-based sensor was imprinted and established for rapid porcine serum albumin (PSA), lowering the porcine DNA detection sensitivity to 0.1%. The F-MIP-NG sensors revealed high sensitivity, affinity and

selectivity at optimal conditions, proving it more efficient than qPCR.

3.4 Lateral flow devices (LFDs)

Lateral flow devices (LFDs), also known as lateral flow assays, are paper-based diagnostic tests that use an immunoassay to detect the presence or absence of a specific analyte in a sample (Abingdon Health, 2022). LFDs detect and capture a target analyte in a sample using binding agents immobilised on a nitrocellulose membrane (Sadeghi *et al.*, 2021). The sample is typically applied to a sample pad at one end of the device, and the sample flows along the membrane due to capillary action. If the target analyte is present in the sample, it binds to the immobilised binding agent, resulting in a visible signal, such as a coloured line or dot, at the test line on the membrane. A control line containing a different immobilised binding agent that should always produce a signal if the device is working properly is also included to ensure the test is valid.

LFDs can be applied in numerous fields, including porcine DNA identification. Masiri *et al.* (2016) utilised LFDs to establish an identification system capable of rapidly detecting porcine residues. The system could detect down to 0.01%, 1.0%, and 2.5% contamination derived from raw pork, cooked pork and porcine gelatine, respectively. LFDs are simple alternatives for porcine detection; anyone can perform them as it does not involve complicated steps. Results are also processed within 35 minutes, proving LFDs to be rapid.

3.5 Loop-mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a highly sensitive and specific nucleic acid amplification technique that has become increasingly popular due to its ability to amplify DNA under isothermal conditions (Wong *et al.*, 2018). Unlike traditional PCR, LAMP does not require thermal cycling, which makes it particularly useful in resource-limited settings. LAMP amplifies target DNA using a strand-displacing DNA polymerase and a set of four to six primers recognising six or eight regions on the target DNA sequence. These primers are designed to recognise a target sequence and initiate a process of strand displacement and amplification in the presence of a constant temperature of 60 - 65°C (Marroki & Bousmaha-Marroki, 2022). According to Soroka *et al.* (2021), LAMP amplifies DNA faster and more efficiently than PCR; the former can amplify up to a billion copies in less than an hour, compared to a million copies by the latter.

Cai *et al.* (2020) established a rapid real-time LAMP method for porcine DNA detection in meat products. The method has a reaction time of 20 minutes and an initial amplification curve of 10 minutes with an identification limit of 1.76 pg/μL contamination, which equals 0.0001%. Compared with other DNA identification techniques, LAMP demonstrated itself to be a quick yet highly specific and sensitive method in porcine DNA detection.

4. Conclusion

There are various techniques to be used in DNA identification, including PCR, MRM, F-MIP-NG, LFDs and LAMP. Each technique was demonstrated to be highly specific, with a detection sensitivity of lower than 5%. Compared to other methods investigated, LAMP proved to be the most sensitive method in performing porcine DNA identification. With a rapid 20-minute reaction time and 10-minute amplification, LAMP detected down to 0.0001% porcine contamination in processed meat products of other species. As LAMP demonstrated to be the most sensitive method, MRM proved to be the least

sensitive. By the multistage fragmentation method known as MRM3, the lowest contamination percentage it could detect was down to 0.13%.

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