

# Recent advances in halal food authentication: Challenges and strategies

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**Abstract:** Increasing public awareness of food quality and safety has prompted a rapid increase in food authentication of halal food, which covers the production method, technical processing, identification of undeclared components, and species substitution in halal food products. This urges for extensive research into analytical methods to obtain accurate and reliable results for monitoring and controlling the authenticity of halal food. Nonetheless, authentication of halal food is often challenging because of the complex nature of food and the increasing number of food adulterants that cause detection difficulties. This review provides a comprehensive and impartial overview of recent studies on the analytical techniques used in the analysis of halal food authenticity (from 1980 to the present, but there has been no significant trend in the choice of techniques for authentication of halal food during this period). Additionally, this review highlights the classification of different methodologies based on validity measures that provide valuable information for future developments in advanced technology. In addition, methodological developments, and novel emerging techniques as well as their implementations have been explored in the evaluation of halal food authentication. This includes food categories that require halal authentication, illustrating the advantages and disadvantages as well as shortcomings during the use of all approaches in the halal food industry.

## KEYWORDS

biosensor, electronic nose, food adulteration, gelatin, halal food authentication, haram, polymerase chain reaction

## 1 | INTRODUCTION

Consumption of halal food among consumers, specifically Muslims, has recently increased globally. The global halal food market has a great potential, and it is growing rapidly across every continent from Asia to the Middle East Africa, Europe, and America. The world has nearly 2 billion Muslims with halal food spending forecast at US\$ 1400 billion by 2020; this amount is predicted to grow more than 10 times to US\$ 15,000 billion by 2050. This automatically cre-

ates a potential and lucrative halal market in more than 50 countries globally. According to the Pew Research Center, the global Muslim population will reach 2.2 billion by 2030, indicating the importance of the halal market, as evidenced by the fact that the GDP of Muslim countries is increasing rapidly than that of the global West (Adekunle & Filson, 2020). Currently, halal industry is officially listed as a worldwide market worth US\$ 580 billion a year (Olya & Al-ansi, 2018). Alam and Sayuti (2011) established that the halal food industry developed at a rate of 7%

annually. The advancement of science and technology in the 21st century, specifically in the food processing industry, has prompted the assortment of ingredients in food products such as gelatin, genetically modified (GM) foods, and pharmaceutical products by Muslims (Abd Mutalib et al., 2015; Nikolić et al., 2009; Rohman & Windarsih, 2020;). Halal covers all aspects of food production from raw materials, food processing, and food handling services. They must comply with the supply chain standard of halal food and the country-based requirement or certification by the halal bodies, such as Jabatan Kemajuan Islam Malaysia (JAKIM), Majlis Ugama Islam Singapura (MUIS), Majelis Ulama Indonesia (MUI), and Central Islamic Council of Thailand (CICOT) (Kwag & Ko, 2019).

Muslim and non-Muslim consumers who purchase halal food products because of health, environmental, and quality concerns have diversified the market (Olya & Al-ansi, 2018). Muslims can only use halal products that meet specific requirements. Haram products, which are totally prohibited in Islam, such as alcohol and pork are the most common commodities mixed with the most popular halal foods, such as beef and chicken. According to the global consumption rate, the consumption of halal chicken and beef were 15.8% and 15.0%, respectively, in 2017 (Kwag & Ko, 2019). Nevertheless, factors such as feeding and slaughtering affect the availability of halal meats. Moreover, non-essential halal foods such as sushi or meatballs may necessitate the attention of Muslim consumers because sushi may contain mirin (rice wine) and meatballs may contain pork (Cahyadi et al., 2020;). As the food supply chain becomes wider and more complex, consumers, particularly Muslims, must be cautious of their food content (Bonne & Verbeke, 2008). Consumers must know what is in the food they eat; however, some manufacturers intentionally mislead consumers about the source or quality of food products for their financial gains. Furthermore, there is a significant risk that food adulteration and substitution may occur when demand for food and trade increases (Ortea et al., 2016). Owing to the importance of the halal market, producers and consumers must have a holistic understanding of how the dynamic of halal derived from Islamic Syariah law functions and is applied, which defines the rules for Muslims to follow on the basis of the Holy Quran and the Hadith. Halal is not easy to verify because odor, texture, or taste cannot determine whether the food is halal (Fischer, 2016). Therefore, most Muslim consumers ultimately depend on certification and labeling to assess whether food products are produced using halal practices (van der Spiegel et al., 2012). Because consumers find it difficult to determine the authenticity of food products as they may be tainted with haram products and improper slaughter could lead to falsification of food, novel advancements, or breakthroughs for performing rapid food screening on

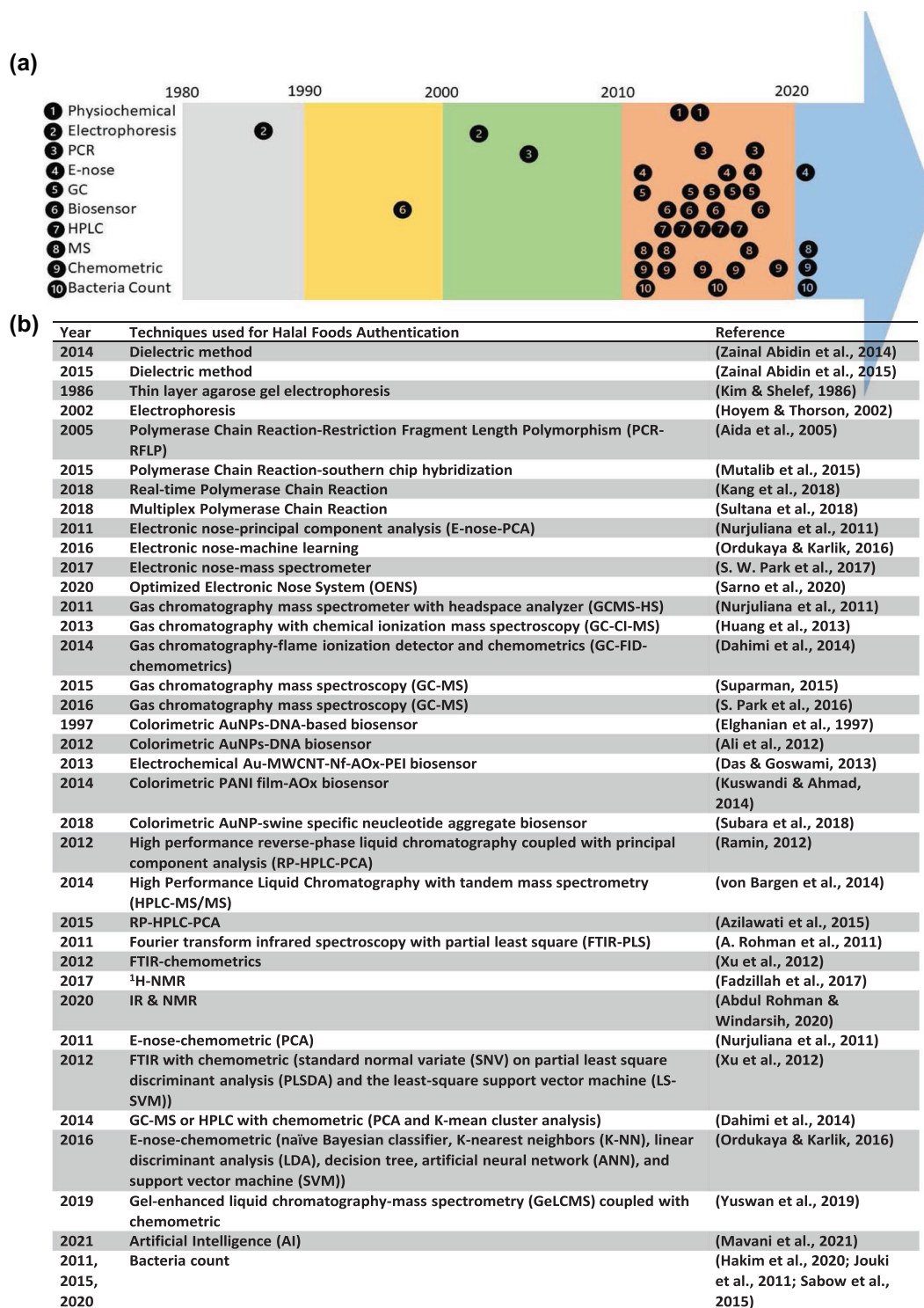
halal food products without compromising their sensitivity and accuracy are urgent. Additionally, they should be portable for real-time and on-site monitoring as well as affordable. Owing to the implementation of cutting-edge technologies and methods for food authentication, Muslim and non-Muslim consumers can confirm the safety of food for consumption. People began to be aware of the food they eat several decades ago, and they could distinguish and identify the composition of the food using traditional methods such as physicochemical approaches and electrophoresis. Although they are simple to use, these methods are ineffective for bulk/high-throughput screening and detection.

Currently, many efforts have been made to develop more effective halal-authentication detection systems. However, there is no discernible trend in the methodologies employed for the verification of halal food from 1980 to 2021, as shown in Figure 1. Over the decades, studies to improve the methods for halal identification have been conducted. Conventional techniques, such as dielectric and electrophoresis, were employed to detect halal components before the invention of polymerase chain reaction (PCR) in 1984. PCR has been commonly employed for electrophoresis since its introduction. Additionally, spectroscopy techniques have been used throughout the decades and they have recently become popular in combination with chemometrics for data processing and treatment.

Various review papers on authentication of halal food have been published (Mostafa, 2020; Rejeb et al., 2021; Zia et al., 2020). Compared to this review, the previous reports focused more on the halal market as a whole, as well as its future trends and growth. Furthermore, they only focused on halal authentication of a single food product, such as porcine meat and meat products. The significance of this review is to provide a comprehensive and critical overview of recent developments in the field of halal food authentication, review its efficiency, as well as provide insight into the advantages, disadvantages, challenges, and current limitations of the advanced techniques applied. This review aims to examine recent developments and novel emerging techniques as well as their applications in the field of halal food authentication. Moreover, the reliability measures of each methodology will be evaluated to provide useful information for future research development.

## 2 | TYPES OF FOOD THAT REQUIRE HALAL AUTHENTICATION

Because halal foods are more easily available in the market, the authenticity of halal food products has become a major concern for Muslims and non-Muslims (Feng et al.,



**FIGURE 1** (a) An overview of the timeline shows that there is no discernible trend in the technologies used for halal food authentication from 1980 to 2021. (b) The year in which the research was published, as well as the traditional/advanced halal food authentication methodologies utilized, along with their references

2017). Halal certification is required for all types of foods, particularly meat products. This is because meat products are a major component, and they are essential for human dietary. Meat and meat products are becoming more prone to illegal adulteration, replacement, and mislabeling as

their demand increases and prices rise (Chuah et al., 2016). Owing to the low-cost factor, counterfeiting meat products is a serious problem for consumers with religious reservations on the consumption of pork meat (Bonne & Verbeke, 2006). For instance, mixing and/or replacing beef

meat by pork meat is a problem that irritates Muslim consumers. This problem is extreme for Muslims living in non-Muslim countries where it is difficult to find halal-certified foods. There should be no traces of pork and its derivatives in halal foods. Moreover, Islam has dietary restrictions on the consumption of pork meat or other edible parts of pigs. In Islam, these foods are called “haram” and they are explicitly prohibited in religions. Because halal meat products are frequently tainted or adulterated with haram products such as frankfurters and meatballs, assessing food authenticity is the main challenge in the meat industry. Increased awareness of meat recognition would ensure product authenticity, protection of consumer safety, and compliance with international laws and religious practices. Although labeling allows consumers to monitor food product details and it is crucial in food safety, the authenticity and traceability of meat products cannot be guaranteed.

Besides pork, halal foods must be alcohol free. Alcohol, in particular ethyl alcohol or ethanol, is widely used in food production or as a byproduct in food processing. Furthermore, it is necessary for Muslims to consume the meat of animals slaughtered using proper halal methods and in accordance with Islamic rites. Porcine gelatin is widely used in industries, and this haram product is marketed not only to Muslims and Jews, but also to vegetarians and people allergic to swine ingredients. Candies, gummies, pastilles, and marshmallows are among the confectionery products that use gelatin. Moreover, gelatin is used in pharmaceutical products such as capsules because it protects medicinal drugs from harmful effects such as light and oxygen (Hom et al., 1975; Tonnesen & Karlsen, 1987). The pigment introduced into gelatin has a very high refractive index. Light's path can shift and be reflected out as it passes from a substance with a low refractive index to a pigment with a high refractive index, forming a protective barrier against harmful light radiation.

Furthermore, GM foods have attracted the attention of halal food consumers. GM food is a relatively novel food technology in which food is derived from GM organisms. A GM organism is one whose genome has been modified or replaced to express the desired physiological traits (Majid et al., 2015). GM foods are classified as non-halal under Malaysian standards. To comply with halal standards, halal food must be free of GM elements. Table 1 lists the types of foods required for halal authentication. As shown in Table 1, compared to PCR, electronic nose (E-nose) is often used in the analysis because sample preparation is more complicated, and more time is required for PCR.

### 3 | CONVENTIONAL AND ADVANCED TECHNIQUES FOR THE VERIFICATION OF HALAL FOODS

Conventional methods and research on novel advances for the evaluation of halal food authentication in various types of food products are discussed in the following sections. A decade ago, conventional methods such as physicochemical methods and electrophoresis were utilized for the analysis of halal food. Although the conventional methods are simple to use and inexpensive, sample preparation procedures are often time consuming and cumbersome, and the outcomes are difficult to interpret owing to the lack of concrete datasets (Zia et al., 2020). Therefore, R&D has been searching for novel breakthroughs for halal food authentication. To address needs such as low cost, portability, and real-time and on-site analysis, point-of-care devices for halal food authentication are useful because they enable rapid screening. For instance, 7FoodPillars launched a halal test kit, PorcineTrace Rapid Test Kit-Raw Meat, on the Malaysian market, which can detect approximately 0.05% to 0.1% (w/w) of adulterated porcine meat antigen in chicken and beef mixtures. Without the use of expensive tools, the test kit is easy to use, rapid, and cost-effective. Notably, France developed a portable “HalalTest” kit to detect the presence of pork in food in 2014. This test kit is inexpensive, with each device costing only 6.9 euros and giving 99% accuracy (Team, 2014). Nonetheless, advanced methodologies (including PCR, E-nose, gas chromatography mass spectrometer with headspace analyzer [GCMS-HS], high-performance liquid chromatography [HPLC], and molecular spectroscopy) that provide validated and reliable results are required for the confirmation and verification of the results. Moreover, artificial intelligence (AI), chemometrics, and Internet of Things (IoT) could be integrated with the aforementioned advanced techniques to enhance the accuracy, speed, and capability of multiplexed analyte detection further.

#### 3.1 | Physicochemical method (dielectric)

Dielectric techniques or electromagnetic radiation can be used to distinguish halal from non-halal meat as well as alcohol from beverages by measuring the interaction of components in food using electromagnetic energy. This technique does not require the use of heat or solvents. Exposing grounded meat and beverages to a potential to measure their dielectric permittivity is a simple technique.

TABLE 1 Types of food samples needed halal authentication

Types of food samples	Why need halal authentication?	Method used for sample preparation	Time required for sample preparation	Challenges/difficulties faced in sample preparation	Strategies used for preventing analyte lost during sample preparation	Analytical method used for authentication
Meats	To distinguish pork from other meats including beef, buffalo, quail, chicken, goat, and rabbit	DNA was extracted using DNeasy Tissue Kit (Qiagen Germany)	10–30 min	Aseptic conditions were required to avoid DNA contamination or degradation	The extracted DNA in solution is stored at 4°C before analysis	Polymerase chain reaction (PCR) (Murugaiah et al., 2009)
Sausages and meatballs	Usually substituted with pork meats for an economic reason	Precooking at 60°C in a heated water bath	10 min	To be analyzed, the sample must contain ambient vapors	The prepared sample was placed in septa-sealed vials and the sample vapor was pumped through the septa-sealed vial into the electronic nose with a side-ported sampling needle	Electronic nose with GCMS-HS (Nurjuliana et al., 2011)
Gelatin-based products (e.g., candy/marshmallows/pharmaceutical capsules)	Gelatin might be derived from pigskin, cattle bones, or cattle hides	Fine sample grinding and formation of KBr discs for FTIR DNA was extracted with QIAGEN DNeasy Blood and Tissue kit (Qiagen, USA) The gelatin solution was prepared by ultrasonically dissolving the gelatin samples in distilled water at 45°C for 20 min	5–10 min 10–30 min 10 min	Samples should be finely ground for FTIR analysis Gelatin capsules samples may increase the detection limit of an assay and trigger false-negative results For the calibration process, a large number of standard gelatin solutions, totaling 8l, must be prepared, ranging from 4% (w/v) to 20% (w/v)	Samples were taken from sausages and meatballs The prepared samples were stored at –20°C in sterile micro centrifuge tubes An ultrasonic bath was used to ensure complete dissolution of the gelatin sample	FTIR spectroscopy analysis (Rahayu et al., 2018; Xu et al., 2012) Polymerase chain reaction (PCR) (Abd Mutalib et al., 2015) Combining FTIR with chemometrics (Hassan et al., 2020; Hassan et al., 2018)
Confectionery products (e.g., candy/marshmallows/gummy/pastilles)	Gelatin is one of the ingredients that may be porcine gelatin	DNA was extracted from the samples using a column-based DNeasy <sup>®</sup> Mericon Food kit (Qiagen, Hilden, Germany)	10–30 min	Aseptic conditions were required to avoid DNA contamination or degradation	UV-treated apparatus (scissors) and sterile micro centrifuge tubes were used	Polymerase chain reaction (PCR) (Sultana et al., 2018)

(Continues)

TABLE 1 (Continued)

Types of food samples	Why need halal authentication?	Method used for sample preparation	Time required for sample preparation	Challenges/difficulties faced in sample preparation	Strategies used for preventing analyte lost during sample preparation	Analytical method used for authentication
Beverages (e.g., soft drinks/fruits juices)	To make the drinks taste better, ethanol was added in small quantities (permitted to be no more than 0.5% (v/v))	Ethanol was diluted to 0.10%, 1.0%, and 10% (v/v) for calibration purposes	Constant time of 10 s for reading	Optimization analysis must be performed to get the lowest detection of ethanol concentration by the portable E-nose with optimum bottle volume and headspace time	Optimal sample volume was analyzed upon obtaining optimal headspace generated time (HGT)	Portable electronic nose (E-nose) (Ab Mutalib et al., 2013)
Seasonings (e.g., soy sauce/vinegar)	During the fermentation process, various alcohols were produced	Red wines, orange, apple, and grape juices were prepared, followed by addition of 1% ethanol and a 0.1 M pH 7 phosphate buffer solution	10 min	During the fabrication of the biosensor, pH and temperature are critical, especially during the process of enzyme immobilization onto the film	When not in use, keep the developed biosensor and ethanol-based sample solutions at 4°C	Biosensor (Kuswandi et al., 2014)
GM food (soybean)	Muslim consumers have concern about the ethicality and halal status of genetically modified foods	The vial containing samples was agitated at 80°C at 350 rpm for 10 min	10 min	Seal condition was needed since the samples to be analyzed were volatile components	Sample-containing vials were hermetically sealed using PTPE/silicone septum caps	Mass spectrometry (MS) with electronic nose (E-nose) (S. W. Park et al., 2017)
		DNA was extracted and purified from samples using the CTAB protocol	The extraction of DNA from the sample takes about 4 to 5 h	CTAB detergent was added to lyse the plant cells and extract the DNA. The nucleic acids, on the other hand, are easily degradable at this stage. As a result, the time it takes to homogenize the sample after adding CTAB should be as short as possible	Before PCR analysis, polysaccharides and phenolic compounds should be removed from the sample since they can impede a variety of enzymatic reactions	Polymerase chain reaction (PCR) (Nikolić et al., 2009)

Heat lowers the solubility of proteins in solutions, whereas the exposure of meat protein to a solvent could disrupt its three-dimensional polypeptide chain.

Dielectric properties have been utilized and reported to distinguish different meats, specifically pork, chicken, and beef, in the frequency range of 0.5 to 50 GHz (Abidin et al., 2015). First, the meats were minced and sterilized using an Agilent N5245A PNA-X before dielectric measurement. Thereafter, an open-ended probe was slowly attached to the meat sample placed in a beaker to obtain the dielectric constant and dielectric loss factor. To distinguish pork from chicken and beef, a fluctuation was observed at low frequency below 20 GHz and two distinct peaks were observed at 7.43 and 31.19 GHz. The study did not specify any specific target analytes, but the two distinct peaks could be attributable to substances present solely in pigs, such as DNA, proteins, microorganisms, amino acids, enzymes, and others. Besides for the detection of meat products, dielectric properties have been explored for the detection of alcoholic content in beverages in the same frequency range as in a previous report (Abidin et al., 2014).

This method is rapid, simple, and low cost, with a meat discrimination accuracy of approximately 95% and an LOD of 0.5% alcohol concentration in a water mixture. However, the meat samples were sterilized (121°C) before being measured. This temperature could kill contaminating pathogenic microorganisms, which is an important parameter to consider when conducting a halal analysis. However, limited information can be obtained using this approach. Although there are distinct peaks for discriminating pork from chicken and beef, the biomarkers that contribute to the peaks are unknown.

### 3.2 | Electrophoresis

Electrophoresis is the movement of charged particles in a gel under the influence of an electric field. Electrophoresis on polysaccharide gels has been used to distinguish meat from various animal species. Gel electrophoresis of meat extracts was conducted on a polysaccharide gel in a glass tubing with a running time of approximately 40 min per sample. Thereafter, the gel was removed, and then followed by fixation of naturally colored myoglobin bands. The results indicated that the protein patterns or bands of each meat were specific and distinct (Figure 2). Pig species have an  $R_B$  (migration distance for myoglobin bands) value of 0.52 (Hoyem & Thorson, 2002). Currently, gel electrophoresis is used in conjunction with other equipment such as PCR to separate DNA fragments according to their size. Electrophoresis alone was used to identify different meat species before the PCR technology was fully developed and widely utilized. To acquire a supernatant for elec-

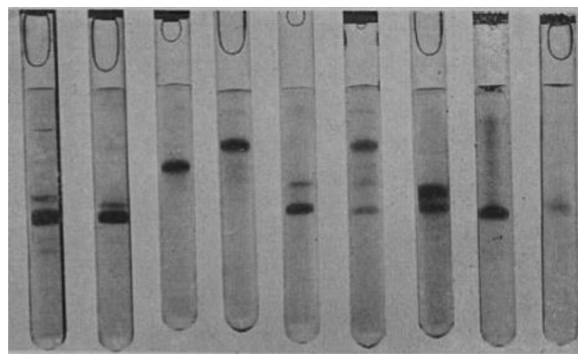


FIGURE 2 Myoglobin patterns in polyacrylamide gels from various meat sources. The bands for each type of meat are distinct and unique. Retrieved from (Hoyem & Thorson, 2002) COPYRIGHT © 1970, American Chemical Society

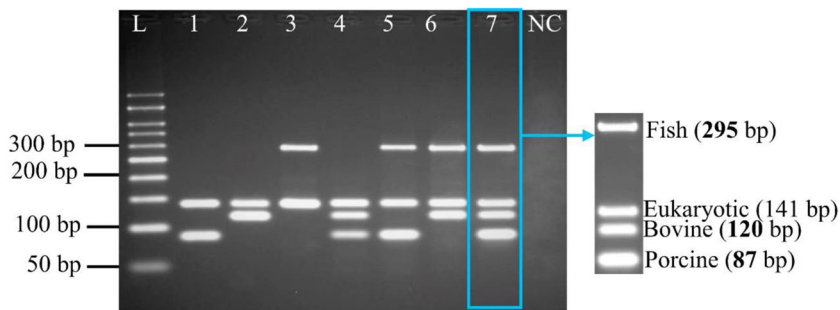
trophoresis, the meat samples were homogenized in a mortar or blended and then they were centrifuged. When the PCR technique was used, DNA from the meat samples was extracted and amplified for electrophoresis. Therefore, the electrophoretic distinct bands are based on the proteins present in each meat species, such as myoglobin.

Different binary mixtures of each meat were studied in another investigation of meat identification (beef, pork, chicken, and turkey) using thin-layer agarose gel electrophoresis. The relative ratios of the bands could be useful for predicting the fraction of certain meat because they correspond to the proportion of binary mixtures (Kim & Shelef, 1986). Another study employed the SDS-PAGE gel electrophoresis technique to distinguish between bovine and porcine gelatin capsules. The capsules were dissolved in water, and then ammonium sulfate was added to precipitate targeted proteins selectively for gelatin source discrimination. Thereafter, the supernatant was loaded into a polyacrylamide gel and electrophoresed at 200 V for 50 min. Porcine and bovine gelatins were successfully distinguished based on their sizes, which were 140 and 110 kDa, respectively. This approach was claimed to be 100% accurate. Since no expensive instrument is involved, the method is simple and inexpensive. However, it is time consuming and cumbersome. It takes between 1 and 2 h to complete. In addition, this approach can only provide limited quantification parameters.

### 3.3 | PCR

Currently, various methods have been developed to classify the species of origin in meat samples, and they are based on protein and DNA analyses. DNA-based identification is preferred for meat authentication because it is appropriate for both raw and processed meats, which contain high

**FIGURE 3** The electrophoresis image of the multiplex assay for distinguishing bovine, porcine, and fish DNA based on amplicon size (bp). Retrieved from (Sultana et al., 2018)  
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degraded materials such as macromolecular proteins and fats (Ali et al., 2012a; Juraj et al., 2017). Lard could be used as a substitute ingredient of oil in food manufacturing because of its low cost and ease of use. Aida et al. (2005) used the PCR-restriction fragment length polymorphism (PCR-RFLP) method to analyze the meat and fat contents of sheep, cow, chicken, and pig (Aida et al., 2005).

Compared to the conventional PCR, PCR-southern chip hybridization is a novel development and innovative approach for authenticating halal foods obtained from meat products such as porcine gelatin in highly processed foods (Abd Mutalib et al., 2015). This technique can detect a very small amount of swine DNA amplicons and it suggests that they are sensitive, reproducible, and undoubtedly useful for detecting swine DNA, which could validate the labeling of pharmaceutical products for consumers further. In addition, the use of real-time PCR is another breakthrough in the authentication of halal food. A study conducted by Kang et al. (2018) demonstrated that real-time PCR successfully revealed precise and sensitive detection of porcine gelatin in pure samples and complex mixtures of adulterated ramen stock powder (Kang, Lee, & Kim, 2018). This study shows that PCR provides a more reproducible and reliable identification of porcine gelatin in food samples using mitochondrial DNA (mtDNA). Although various simplex and duplex PCR assays are currently available to determine the origins of gelatin, they are lengthy, costly, and cannot reveal all information in a single study (Sultana et al., 2018). Therefore, the multiplex PCR assay is used to detect three major gelatins (bovine, porcine, and fish) in pure and halal confectionary products in a single assay (Sultana et al., 2018). To distinguish bovine, porcine, and fish DNA based on their amplicon size (bp), the electrophoresis image of the multiplex assay is shown in Figure 3. This technique provides better stability, sensitivity, as well as reliability in the screening of gelatin and it can be used in pharmaceutical and cosmetic products.

PCR is used to analyze meat-based products such as sausages and meatballs, gelatin capsules in pharmaceutical products, as well as confectionery products. A detection limit of 0.001 ng/ $\mu$ L porcine DNA and 5 pg/ $\mu$ L DNA from porcine gelatin have been reported for PCR. Moreover,

PCR offers a reliable, fast, sensitive, and specific approach for the identification of species because DNA can be discovered in all cell types of an organism with identical information. Furthermore, DNA is a stable molecule that can be used to analyze processed and heat-treated foods, and its lifetime is not limited by the lifetime of the organism. However, the real-time instruments and reagents for real-time PCR are costly. Furthermore, the sample cannot be reused for subsequent analyses, and the analysis is lengthy. This method also faces challenges, such as the requirement to select high efficiency and specific primers from genes for PCR amplification as well as the necessity to select appropriate DNA extraction methods and prepare sufficient template DNA for analysis. Table 2 lists the numerous types of PCR tests used for authenticating halal foods.

### 3.4 | E-nose

E-nose is a device or detection system designed to mimic the biological olfactory system of humans. It is based on semi-selective sensors interacting with volatile compounds (Di Rosa et al., 2017) that causes a physical change of the sensor during the adsorption of volatile compounds on its surface (Danezis et al., 2016). E-nose has been used in the food industry for the quality control of raw and refined goods with freshness and maturity testing (Chen et al., 2018; Ezhilan et al., 2019), shelf-life analysis (Gómez et al., 2008), authenticity tests (Wu et al., 2017), and identification of microbial pathogens (Sibila et al., 2014). According to Wang et al. (2019), E-nose was used with a gas chromatography-mass spectrometer (GC-MS) in the detection and quantification of meat adulterants (Wang et al., 2019). The GC-MS data has both qualitative and quantitative characteristics that are associated with the E-nose signals analyzed. In summary, the E-nose is suitable for the identification of adulterants in meat products, and its ability is significantly improved when it is integrated with an MS. E-nose is used to analyze meat products, edible oils, seasonings, and beverages. The advantages of E-nose include its low cost; rapid and accurate qualitative detection (this device has an advantage in analyzing food

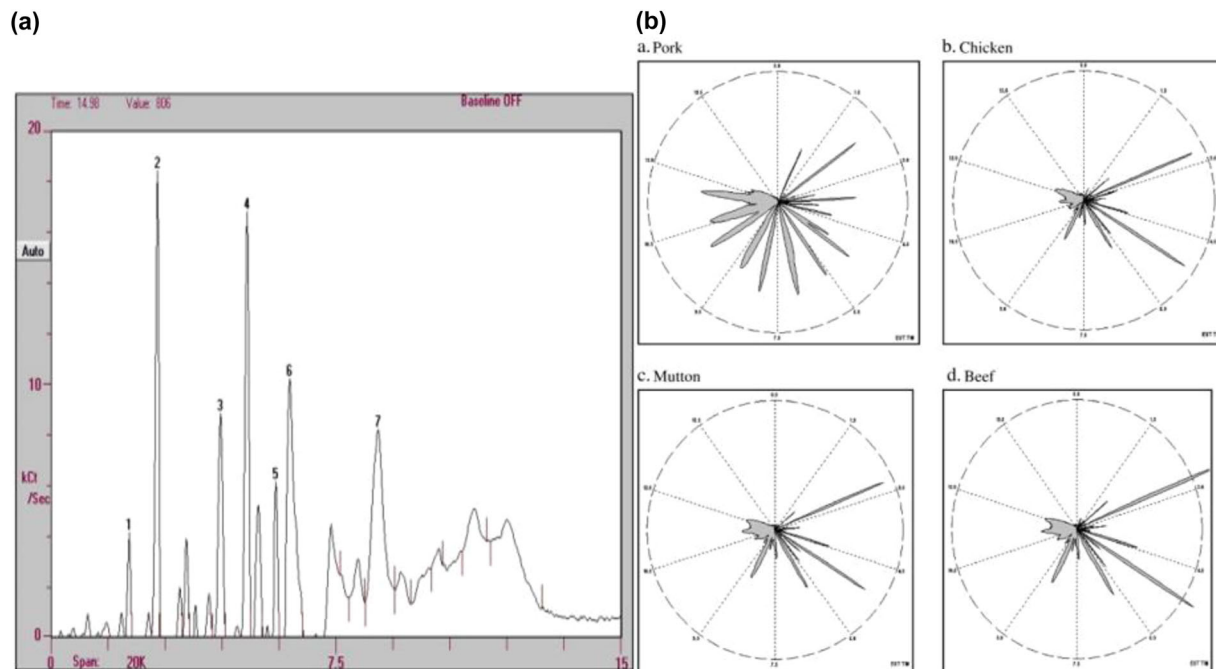
TABLE 2 Different types of PCR advanced techniques were used for different types of halal food authentication

Type of PCR	Type of food analyzed	Method validation involved	Reference
PCR-RFLP	Meat from sheep, cow, chicken, and pig	<ol style="list-style-type: none"> <li>1. DNA isolation</li> <li>2. Primer selection. In this study, cyt b1 (5'-CCA TCC AAC ATC TCA GCA TGA TGA AA-3') and CYTb2 (5'-GCC CCT CAG AAT GAT ATT TGT CCT CA-3') were used</li> <li>3. In a polymerase chain reaction, denaturation, annealing, and extension were utilized to amplify cyt b</li> <li>4. Using restriction enzyme (RE), a restriction fragment of the cyt b amplicon was produced (reveal the RE cutting pattern to figure out where the meat and fat come from)</li> <li>5. Electrophoresis</li> </ol>	Aida et al. (2005)
PCR-southern chip hybridization	Gelatin capsules and porcine canned meats	<ol style="list-style-type: none"> <li>1. DNA isolation</li> <li>2. Primer selection</li> <li>3. PCR amplification for southern hybridization on a DNA chip (biotin-labeled primer sets were used to amplify the DNA). The denatured biotinylated amplicons were hybridized with specific probes immobilized onto membrane. The biotin-labeled amplicons reacted with streptavidin-alkaline phosphatase and followed by colorimetric detection using the substrate nitro blue tetrazolium/5-bromo-4-chloro-3-indoyl-phosphate (NBT/BCIP). The positive spots were captured by Scanner System which allows the species-specific identification)</li> </ol>	Abd Mutalib et al. (2015)
Real-time PCR	Ramen stock powder containing porcine gelatin	<ol style="list-style-type: none"> <li>1. DNA isolation</li> <li>2. Primer selection</li> <li>3. SYBR Green I was used as a fluorescent dye in PCR amplification. This dye allows for the detection of four mitochondrial 99 markers (D-loop region, ATP8, ND5, and 12S rRNA) for porcine gelatin in ramen stock powder</li> <li>4. Analysis of the melting curve of primer with SYBR I Green dye for species-specific identification</li> </ol>	Kang et al. (2018)
Multiplex PCR	Porcine, bovine and fish gelatin, gummy, marshmallows, candy, pastilles, muscle tissue from buffalo, chicken, duck, lamb, goat, quail, frog, box turtle, pigeon, and rabbit, meat from dog, cat, rat, and monkey	<ol style="list-style-type: none"> <li>1. DNA isolation</li> <li>2. Primer selection</li> <li>3. A multiplex PCR assay using ABI 96 Well Verity Thermal Cyclers</li> </ol>	Sultana et al. (2018)

aromas because of its speed and high sensitivity, which requires only a small amount of sample); environmental friendliness; simple sample pre-treatment process; the ability to integrate it with other instruments, such as a mass spectrometer, to separate gases from samples into their corresponding peaks and thus making it easier to identify particular compounds (aroma profiling). Never-

theless, the E-nose loses sensitivity when analyzing high-concentration analytes and some sensors in the E-nose have relatively short lifetimes.

Nurjuliana et al. (2011) established that the E-nose coupled with principal component analysis (PCA) was used to identify and detect the presence of pork in other meats and meat sausages (Nurjuliana et al., 2011). The E-nose



**FIGURE 4** (a) The chromatographic profile of raw meat aroma of pork from an electronic nose and (b) distinct and easily recognizable two-dimensional olfactory image of various meat sources generated by VaporPrint™. Retrieved from (Nurjuliana et al., 2011) COPYRIGHT © 2011, Elsevier Ltd

used in this study was based on a surface acoustic sensor (SAW). When volatiles are absorbed onto the surface of the sensor, the frequency of SAW is altered, which subsequently affects the detection signals. This allows the detection and identification of compounds using the E-nose. Compounds or odors, such as diacetyl and 3-hydroxy-2-butanone with a buttery odor, 2-methylpropanal with a pungent smell, heptanal with a fatty smell, trimethyl pyrazine with a roasted smell, as well as nonanal and decanal with a soapy smell, have been detected in raw pork. The peaks can be identified based on the Kovats index database. Figure 4 shows the chromatographic profile of raw meat aroma of pork from an E-nose, and distinct and easily recognizable two-dimensional olfactory image of various meat sources generated by VaporPrint™. This method provides a rapid, accurate, economical, and an environmentally friendly analysis (heat treatment is used to generate vapor and no or minimal solvent is used; thus, the principle of green chemistry is fulfilled) with a total analysis time of less than 60 s and a sample weight of 5 g. When this method is coupled with PCA, a total variance of 67% in terms of sample separation could be achieved using the first principal component. Therefore, this technique is useful for the identification of porcine-based ingredients for the authentication and verification of halal foods. Table 3 summarizes the various types of E-nose hyphenated techniques that have been reported for the authentication of various types of halal food.

### 3.5 | GC

GC is a separation method widely used to analyze volatile and semi-volatile substances, including aromatic compounds, in food science. It has various detectors for qualitative and quantitative analyses, including programmable temperature vaporization (PTV), pulsed flame photometric detection (PFPD), and halogen-specific detection (XSD), and the authenticity of food can be verified by obtaining a fingerprinting chromatography profile (Lehotay & Hajšlová, 2002). GC is coupled with mass spectrometry (MS) to identify and measure a wide range of substances. According to Cajka (2013), single quadrupole or ion trap mass analyzers with low resolution (unit mass) are typically utilized in food and environmental analysis, whereas triple quadrupole (tandem-in-space) or high-resolution magnetic double focusing sector instruments can be used for specific analyses (Cajka, 2013). Huang et al. (2013) reported that GC with chemical ionization-MS (GC-CI-MS) is a powerful technique for separating and identifying different components such as *N*-nitrosodimethylamine (NDMA), *N*-nitroso-*n*-methylethylamine (NMEA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosodi-*n*-propylamine (NDPA), *N*-nitrosopiperidine (NPIP), and *N*-nitrosodibutylamine (NDBA), and they used it to detect pork meat in halal food products owing to its ability to provide good precision, a wide linear range of

TABLE 3 E-nose hyphenated advanced techniques for different types of foods were used in halal authentication

E-nose hyphenated techniques	Type of food analyzed	Method validation involved	Reference
E-nose-PCA	Pork, chicken, mutton, beef, pork sausage, chicken sausage, and beef sausage	<ol style="list-style-type: none"> <li>1. Sample pre-treatment: Samples were stored at <math>-20^{\circ}\text{C}</math> to prevent deterioration of the samples' aroma components</li> <li>2. Sample preparation: Pre-cook in water bath at <math>60^{\circ}\text{C}</math> for 10 min. The vapor was injected into the electronic nose through a septa.</li> <li>3. Sample analysis is performed using a SAW sensor in the electronic nose apparatus</li> <li>4. Statistical analysis using PCA</li> </ol>	Nurjuliana et al. (2011)
E-nose-MS	Ethanol content in soy sauce	<ol style="list-style-type: none"> <li>1. Sample preparation: Soy sauce samples were thoroughly blended using a twister</li> <li>2. Sample analysis: The gas components were analyzed using an e-nose system and a mass spectrometer after the samples were introduced into an electronic system</li> </ol>	S. W. Park et al. (2017)
Optimized electronic nose system (OENS)	Minced pork and minced mutton	<ol style="list-style-type: none"> <li>1. Sample pre-treatment: Samples were grounded</li> <li>2. Sample preparation: Beef and mutton samples with various composition percentages were prepared.</li> <li>3. Sample analysis: Samples were placed in the sample chamber of the electronic nose for analysis</li> <li>4. Signal preprocessing with proper noise filtering</li> <li>5. Statistical parameters extraction such standard deviation, mean, kurtosis and skewness</li> <li>6. Dimensional reduction with PCA</li> <li>7. Classification with optimal classifier parameters using an optimized support vector machine (SVM)</li> </ol>	Sarno et al. (2020)
E-nose-machine learning	Fruit juice-alcohol mixtures and fruit juice-multiple alcohol mixtures (fruit juice: apple juice, orange juice, lemon juice, and cherry juice; alcohol: raki, vodka, tequila, whiskey, and gin)	<ol style="list-style-type: none"> <li>1. Sample preparation: Various fruit juice-to-alcohol/multi-alcohol ratios were mixed</li> <li>2. Sample analysis: The "Cyrano 320" which has 32 sensors was used to identify the aroma of each mixed drink</li> <li>3. Data classification using machine learning techniques such as the naïve Bayesian classifier, K-nearest neighbors (K-NN), linear discriminant analysis (LDA), decision tree, artificial neural network (ANN), and support vector machine (SVM)</li> </ol>	Ordukaya and Karlik (2016)

0.25–500 ng/mL, and an LOD of 0.03–0.36 ng/g (Huang et al., 2013).

In the authentication of halal food, after evaluating the qualitative detection and distinctions of pork from other types of meat using the E-nose, GCMS-HS was utilized for the aroma profiling and identification of components such as phenol, hexanal, 2-butanone, 1-methoxyl-2-methyl-2-pentanone, heptanal, benzaldehyde, heptyl ester 1-heptanol, 2-pentyl-furan, and octanal, 1-hexanal. Moreover, majority of the compounds were lipid oxidation products such as aldehydes, ketones, and

alcohols that provide flavors to pork and other meats (Nurjuliana et al., 2011). Based on the number of peaks and the corresponding retention time produced in the chromatograms, sausages containing pork could be identified. GCMS-HS has identified two compounds, heptanal and nonanal, which are oxidation products of oleic and linoleic acids with retention times of 11.821 and 21.288 min, respectively. Because these two compounds are the most abundant unsaturated fatty acids in pork, they can be used to identify pork in chromatograms. When GCMS-HS was used in combination with PCA,

a variance of 67% in terms of sample separation was achieved.

To separate lard from beef tallow and chicken fat, researchers employed a GC-flame ionization detector (GC-FID) and chemometrics such as principal components analysis and K-mean cluster analysis. The results indicated that lard samples have a low C16:0 fatty acid content, whereas beef tallow and chicken fat have a high C16:0 content. This approach could yield a limit of detection (LOD) of 0.5% of lard content in the sample (Dahimi et al., 2014). Another study used GC-MS to examine halal-certified chocolates. The presence of eikosadienoat 11.14 acidic compounds with a retention time of 36.6 min indicates the presence of lard in the sample. The LOD for this approach is a lard concentration of 10% in samples (Suparman, 2015). Additionally, GC-MS can be used to detect alcohol compounds (methanol, ethanol, *n*-propanol, *n*-butanol, and *n*-pentanol) for halal certification. This method gives an LOD of 0.25–1.16 mg/kg, and the recovery ranged from 9.079% to 101.50% (S. Park et al., 2016).

GC hyphenated methods such as GC-MS, GCMS-HS, GC-CI-MS, and GC-FID coupled with chemometrics are frequently used to examine meats- and lard-containing products. By separating the gases into peaks corresponding to individual components, this approach has the advantage of high accuracy and sensitivity. However, there is need for sample pre-treatment and it takes long to analyze a large number of samples. Depending on the complexity of the gases, testing a single sample can take between 1 and 2 days. Furthermore, the GC-MS is relatively expensive and requires skilled personnel to operate. The loss of volatile compounds during sample preparation and the selection of an appropriate column for analysis are the challenges that must be overcome when utilizing this advanced technique. Figure 5 shows the GC-MS chromatograms for determining alcohol compounds in fermented Korean food. Table 4 summarizes the various types of GC hyphenated techniques that have been reported for the authentication of various types of halal food.

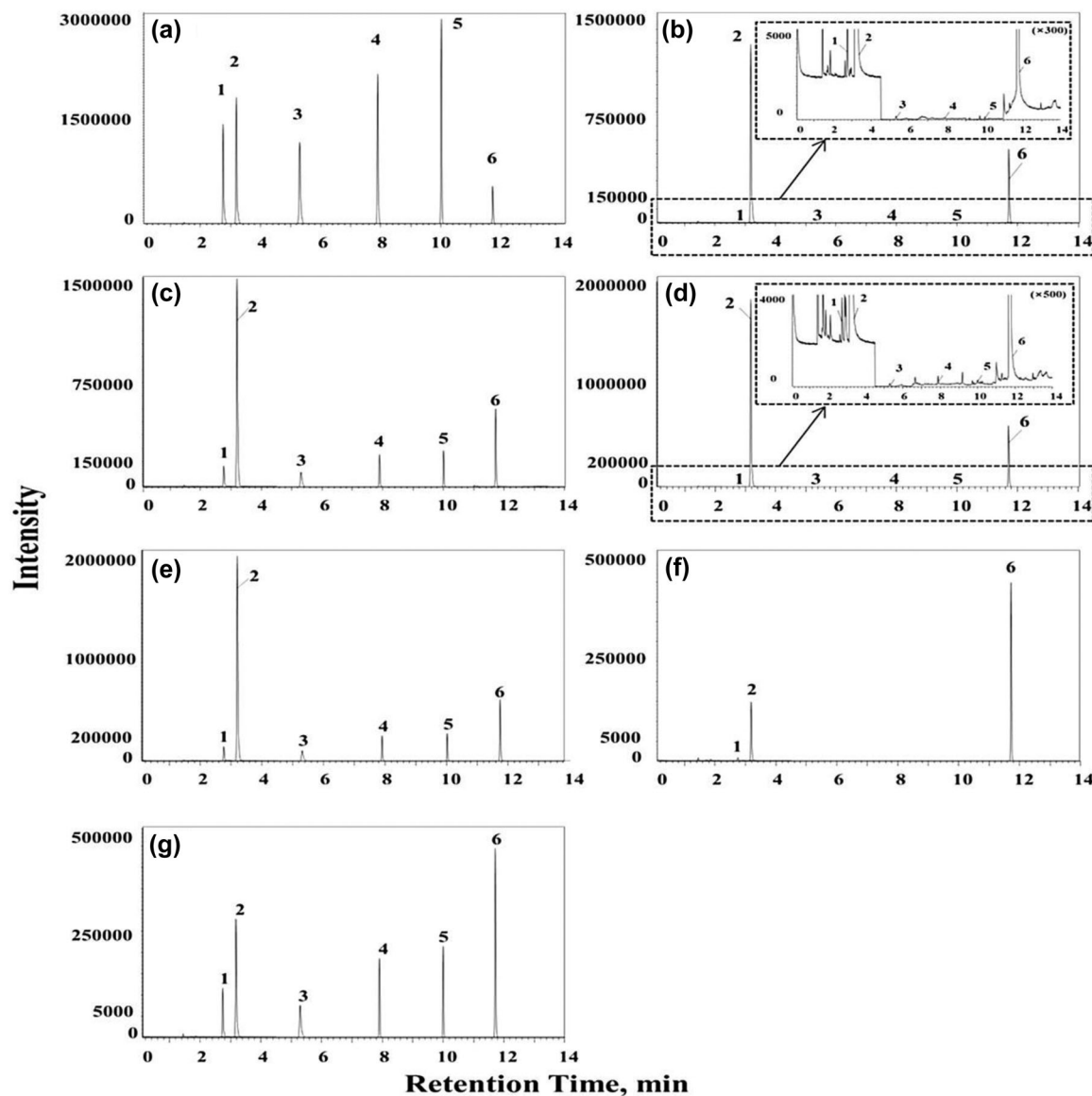
### 3.6 | Biosensors

The implementation of biosensor technology in the detection of halal food is a new era in food authentication platforms. It is a portable analytical tool that combines biological recognition elements such as enzymes, antibodies, DNA, and whole cells using a suitable transducer to transform biochemical recognition into electrical, optical, and any type of signal based on the transducer used (Neethirajan et al., 2018). The application of biosensors has attracted significant attention in the

food industry for the determination of markers such as food freshness, safety, and quality of different food products, specifically for the identification of non-halal components.

Ali et al. (2012) successfully developed a hybrid nanobio-probe (biosensor) to detect pig DNA, where the targeted DNA marker was a 15–30 nucleotide mitochondrial genome. A 27-nt A1UI-cut fragment of a swine cytb gene was employed for the study (Ali et al., 2012b). One percent of pork DNA was successfully detected and quantified using biosensors in extensively autoclaved binary admixtures of pork and beef. No inter-species detection was observed in the model experiments of the four most common types of meat (e.g., beef, chicken, pork, and fish). This indicates the promising use of biosensors to detect pig counterfeiting in halal and kosher meat as well as meat products because this method has an accuracy  $\geq 90\%$ . In addition to the identification of pork products, biosensors have been used to detect alcohol in food products. According to Das and Goswami (2013), alcohol biosensors have higher selectivity and specificity compared to spectrometric (short-wave near-infrared [SW-NIR] spectrometer) and chromatographic (GC/liquid chromatography) methods, which require expensive equipment and complex pre-treatment of samples. A biosensor was developed by immobilizing alcohol oxidase (AOx) on multiwalled carbon nanotubes-Nafion<sup>®</sup> (MWCNTs-Nf) encapsulated with polyethylenimine (PEI) on a gold electrode (AuE) to detect ethanol in solution (Figure 6). This method can provide a detection limit of 5  $\mu\text{M}$  of ethanol (Das & Goswami, 2013). In addition, Kuswandi and Ahmad (2014) used a biosensor to detect the content of alcohol in samples of fermented beverages. The biosensor was fabricated by immobilizing AOx on a polyaniline (PANI) film. When alcohol is detected, the biosensor changes color from green to blue owing to an enzymatic response in which ethanol is oxidized to acetaldehyde and hydrogen peroxide. Hydrogen peroxide further oxidizes the PANI film, resulting in the color change described above. This provides accurate results for detecting ethanol in fermented beverages as it has a detection limit of 0.001% and a lifetime of up to 7 weeks when stored at 4°C. Hence, it is useful for the halal verification in beverages and alcohol-containing foods (Kuswandi & Ahmad, 2014).

Biosensors are used to analyze beverages and meat products. This technique is low cost, requires less time, and can be miniaturized for real-time and on-site monitoring. Furthermore, this approach does not require user intervention and it requires minimal sample preparation (e.g., simple dilution). A challenge faced is the stability of the biosensors in real samples. Based on a study by Kuswandi et al. (2014), the stability of the ethanol biosensor was tested every week as the response of the biosensor remained 70%



**FIGURE 5** GC-MS chromatograms for determination of alcohol compounds in gochujang, soybean sauce, and kimchi. (a) Standard solution (1250.00 mg/kg), (b) blank gochujang, (c) spiked gochujang (spiked concentration 125.00 mg/kg), (d) blank soybean sauce, (e) spiked soybean sauce (spiked concentration 125.00 mg/kg), (f) blank kimchi, and (g) spiked kimchi (spiked concentration 125.00 mg/kg) using GC-MSD. Peaks: 1, methanol; 2, ethanol; 3, *n*-propanol; 4, *n*-butanol; 5, *n*-pentanol; 6, *n*-hexanol (IS). Retrieved from (S. Park et al., 2016) COPYRIGHT © 2016, Elsevier Ltd

after 8 weeks of the initial response (Kuswandi & Ahmad, 2014).

Elghanian et al. first reported the use of gold nanoparticles (AuNPs) for protein (single-stranded oligonucleotide) sensing in 1997 (Elghanian et al., 1997). The AuNPs were modified for polynucleotide identification via colorimetric detection. The reported AuNPs-DNA-based biosensor could replace the conventional methods (e.g., gel electrophoresis) because it can detect specific types of meat (e.g., pork meat) in the authentication of halal meat. AuNP-DNA-based sensing is rapid, requires a processing time of approximately 1 h throughout the analysis,

and it requires no additional equipment (e.g., PCR or liquid chromatography). The principle behind the colorimetric detection of AuNPs is based on specific aggregation of AuNP interacting with proteins (pork DNA). Proteins or DNA fragments from pigs could easily adsorb onto the surface, forming a protein “corona” surrounding the AuNPs. Particle aggregation affects the color intensity of the AuNPs and increases the absorption intensity. The aggregation is caused by the electrostatic properties of the DNA in pork samples, which results in distinct surface plasmon resonances (SPR). In addition to the concentration of pork DNA, the size of AuNP

**TABLE 4** GC hyphenated advanced techniques for different types of foods were used in halal authentication

GC hyphenated techniques	Type of food analyzed	Method validation involved	Reference
GCMS-HS	Pork, beef, mutton, and chicken	<ol style="list-style-type: none"> <li>1. Sample pre-treatment: Samples were stored at <math>-20^{\circ}\text{C}</math> to prevent deterioration of the aroma components of the samples</li> <li>2. Sample preparation: The samples were transferred to a headspace vial</li> <li>3. Sample analysis using a GCMS-HS with a non-polar column (J&amp;W Scientific DB-5). Mass spectra were obtained for compounds identification</li> <li>4. Statistical analysis using PCA</li> </ol>	Nurjuliana et al. (2011)
GC-CI-MS	Chinese pork sausage, bacon, hot dogs, ham, and Chinese cured pork	<ol style="list-style-type: none"> <li>1. Sample pre-treatment: Sodium nitrite was added to the meat samples as a preservative. The meat samples were cooked and blended in a Warring blender under liquid nitrogen</li> <li>2. Sample preparation: Microwave assisted extraction was used to obtain the supernatant</li> <li>3. Sample analysis by GC-CI-MS using a DB5-MS capillary column</li> </ol>	Huang et al. (2013)
GC-FID-chemometrics	Adipose tissue of lard, chicken, and beef	<ol style="list-style-type: none"> <li>1. Sample pre-treatment and preparation: Sample fats were extracted using the Bligh–Dyer method (1959), then lard, beef fat, and chicken fat were extracted using solvent extraction</li> <li>2. Sample analysis using GC-FID with a polar capillary column (HP88-Agilent Technologies, USA)</li> <li>3. Statistical analysis using PCA and K-mean cluster</li> </ol>	Dahimi et al. (2014)
GC-MS	Chocolate and lard (pig adipose tissue)	<ol style="list-style-type: none"> <li>1. Sample preparation: Soxhlet extraction of halal-certified chocolate</li> <li>2. Fatty acids analysis using GC-MS. The presence of the peak at retention time of 38.8 min, which is compound eikosadienoat 11.14 acidic compounds, indicates the presence of lard in chocolate</li> </ol>	Suparman (2015)
GC-MS	Korean fermented food (gochujang, kimchi, and soybean sauce)	<ol style="list-style-type: none"> <li>1. Sample preparation: Solvent extraction using DMSO</li> <li>2. Supernatants were subjected to GC-MS analysis</li> </ol>	S. Park et al. (2016)

affects the SPR absorption peak of AuNPs at a given wavelength.

Another similar study conducted by Subara and Jaswir (2018) showed that AuNPs modified with specific probes were used to detect 11 types of meat compounds, such as pork-shad, pork venison, and shad-venison. AuNPs ( $40 \pm 5$  nm) were utilized for colorimetric DNA biosensing, where the Graber Freeman method with monobasic anhydrous sodium citrate was used as a precursor for the synthesis of AuNPs. No additional equipment other than the naked eye, such as PCR, was used to measure the results. Because it contacts a swine-specific nucleotide, the AuNP detector switched color from pinkish red to grayish purple (owing to AuNP aggregation), indicating the detection of pork compounds. However, the drawback of this technique is that the detection limit of AuNPs ( $0.1 \mu\text{M}$  of DNA) was higher than that of the real-time PCR ( $0.1$ – $0.001$  ng of DNA). Although this method pro-

vides rapid detection and minimal equipment is needed, its detection limit is high. Additionally, specific probes are required for various animal meats as well as the production of stable nanoparticles suited for use in all media (Subara and Jaswir, 2018). Figure 6 shows the field emission scanning electron microscopy (FESEM) images of the modified surfaces of AuNPs-DNA-probe biosensors. Table 5 summarizes the various types of advanced biosensor techniques that have been reported for authenticating various types of halal food.

### 3.7 | HPLC

Amino acids are essential elements that form proteins. This occurs in foods in either free form or as peptides, polypeptides, or proteins. A high content of amino acids is found in food products such as meat, dairy products,

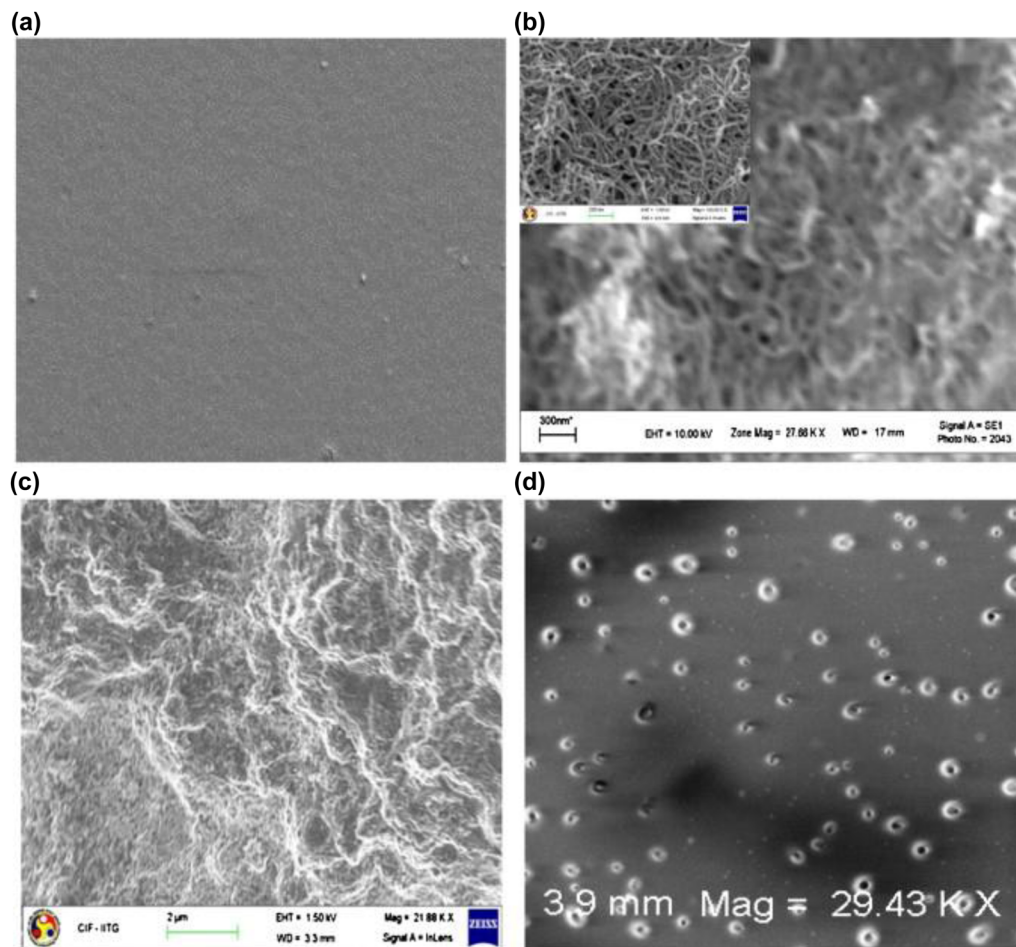


FIGURE 6 FESEM images of (a) AuE (30 KX), (b) Au-MWCNT-Nf (27.66 KX) with inset at 153 KX, (c) Au-MWCNT-Nf-AOx (21.88 KX), and (d) Au-MWCNT-Nf-AOx-PEI (29.43 KX). Retrieved from (Das & Goswami, 2013) COPYRIGHT © 2013, Elsevier B.V

and gelatin. Although amino acid analysis is a conventional method, few studies have been conducted to detect the composition of amino acid in meat products aimed at detecting halal verification. Recently, the composition of amino acids in gelatin has been identified using analytical techniques to determine the origin of various gelatin sources (Ishaq et al., 2020; Mariod & Fadul, 2013). HPLC is a popular analysis technique for identifying and quantifying amino acids when combined with a fluorescent or ultraviolet detector (Azilawati et al., 2016).

Azilawati et al. (2015) used 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate as a derivative reagent for the identification of amino acid compositions in bovine, porcine, and fish gelatin (Azilawati et al., 2015). In this study, reverse-phase HPLC (RP-HPLC) coupled with PCA was used to distinguish all the gelatins tested. Hyp, Asp, Ser, Glu, Gly, Arg, Thr, Ala, Pro, Cys, Tyr, Val, Met, Lys, Ile, Leu, and Phe were among the 17 amino acids identified. For each study, RP-HPLC yielded a nearly identical amino acid composition. Therefore, PCA was used for further analyses. The results of this study indicated that 5 of the

12 samples contained porcine gelatin derived from commercial products of analytical grade gelatin and gelatin. This method provides a good peak resolution ( $R_s > 1.5$ ) for the RP-HPLC chromatogram, and PCA analysis could describe 96.5% of the total variance for the three principal components. Additionally, Jorfi et al. (2012) identified and distinguished pork from selected beef, mutton, chevon, and chicken meats using the RP-HPLC coupled with PCA based on their amino acid content Jorfi et al. (2012). The results indicated that valine, histidine, serine, alanine, and arginine were the most selective amino acids between pork and other meats. Based on this study, the amino acid content can be used as a marker for further analysis of halal authentication to differentiate pork meat. The PCA analysis of this study yielded 94.75% of the total variance for the three principal components.

In addition, a study reported the use of HPLC-MS/MS to discriminate between horses and pork in highly processed foods. Marker peptides have been used to distinguish pork from horses in which pork would have troponin T, myosin-4, and myosin-1, which are labeled as 1, 2, and 3

**TABLE 5** Biosensors advanced techniques for different types of foods were used in halal authentication

Biosensor techniques	Type of food analyzed	Method validation involved	Reference
AuNPs-DNA	Pork and beef burger meats	<ol style="list-style-type: none"> <li>1. A porcine-specific marker, a 27-nt A1UI-cut fragment of the swine cytb gene, was used for the swine-specific oligoprobe</li> <li>2. Colloidal AuNPs were synthesized</li> <li>3. A hybrid nanobioprobe was prepared by mixing the oligoprobe and AuNPs in a 3:1 ratio</li> <li>4. Samples preparation and calibration</li> <li>5. DNA isolation</li> <li>6. Fluorescence measurement</li> </ol>	Ali et al. (2012b)
Au-MWCNT-Nf-AOx-PEI	Beverages containing alcohol (ethanol)	<ol style="list-style-type: none"> <li>1. Preparation of a bioelectrode by immobilizing AOx on MWCNT-Nf encapsulated with PEI on AuE</li> <li>2. Cyclic voltammetry (CV) measurement with the bioelectrode as working electrode, platinum as counter electrode, and Ag/AgCl as reference electrode</li> </ol>	Das and Goswami (2013)
PANI film-AOx	Samples of fermented beverages	<ol style="list-style-type: none"> <li>1. Preparation of PANI film</li> <li>2. The AOx enzyme immobilization on the PANI film</li> <li>3. Detection of ethanol by color change from green to blue. ImageJ software can be used to calculate the RGB value</li> </ol>	Kuswandi et al. (2014)
AuNP-swine-specific nucleotide aggregate	11 types of meat mixtures such as pork-shad, pork venison, and shad-venison	<ol style="list-style-type: none"> <li>1. Synthesis of AuNPs</li> <li>2. Colorimetric detection of a swine-specific nucleotide by color change from pinkish red to greyish purple</li> </ol>	Subara et al. (2018)

in Figure 7. A horse would have troponin T, myosin-2, and myosin-1, which are labeled as 1, 4, and 5 in Figure 7. This method indicates a LOD of 0.24% (von Bargen et al., 2014). By splitting the samples into components based on polarity for analysis, this approach provides a simple, fast, and specific detection. However, because amino acids do not absorb radiation, a suitable chromophore or fluorophore is required for derivatization. Furthermore, this procedure destroys samples, and it takes a long time to perform a single analysis. The instrument is relatively expensive, costing approximately USD\$10,000, and it requires considerable competence to operate. Figure 7 shows the HPLC-MS/MS chromatograms of pure pork, beef, and horse meat. Table 6 summarizes the various types of HPLC hyphenated techniques that have been reported for authenticating various types of halal food.

### 3.8 | Molecular spectroscopy

Molecular spectroscopy is the study of the interaction of electromagnetic radiation with analytes at the molecular level. Non-halal compounds in food or pharmaceutical products such as proteins, lipids, and fatty acids can be easily detected at the molecular level, rendering molecular spectroscopy prominent in halal authentication. This approach has various advantages, including minimal or

no sample preparation, non-destructive operation, short analysis time, low cost, and less solvent, all of which are environmentally friendly. However, the complexity and overlap of some of the molecular spectra make it challenging to identify and analyze the compounds. To address the difficulties during analysis, this approach is combined with chemometrics. Infrared (IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy are two common types of molecular spectroscopy reported for halal analysis (Rohman & Salamah, 2018; Rohman & Windarsih, 2020).

It has been reported that IR spectroscopy can detect and analyze lard intentionally added to pharmaceutical and cosmetic products such as cream, lotion, and lipstick, as well as lard adulterated palm oil and porcine gelatin. For data treatment, the obtained molecular spectra were analyzed using chemometrics. The classification and quantification of IR spectra can be performed using a soft independent modeling class (SIMCA) or PCA and partial least square (PLS). IR spectroscopy coupled with PLS can predict lard content, whereas IR spectroscopy coupled with PCA can distinguish porcine gelatin from bovine and fish gelatins (Rohman & Salamah, 2018).

Fourier transform infrared (FTIR) spectroscopy combined with PLS calibration was used to investigate pork adulteration in beef meatballs. This model could give a root mean square of 0.742 using four principal compo-

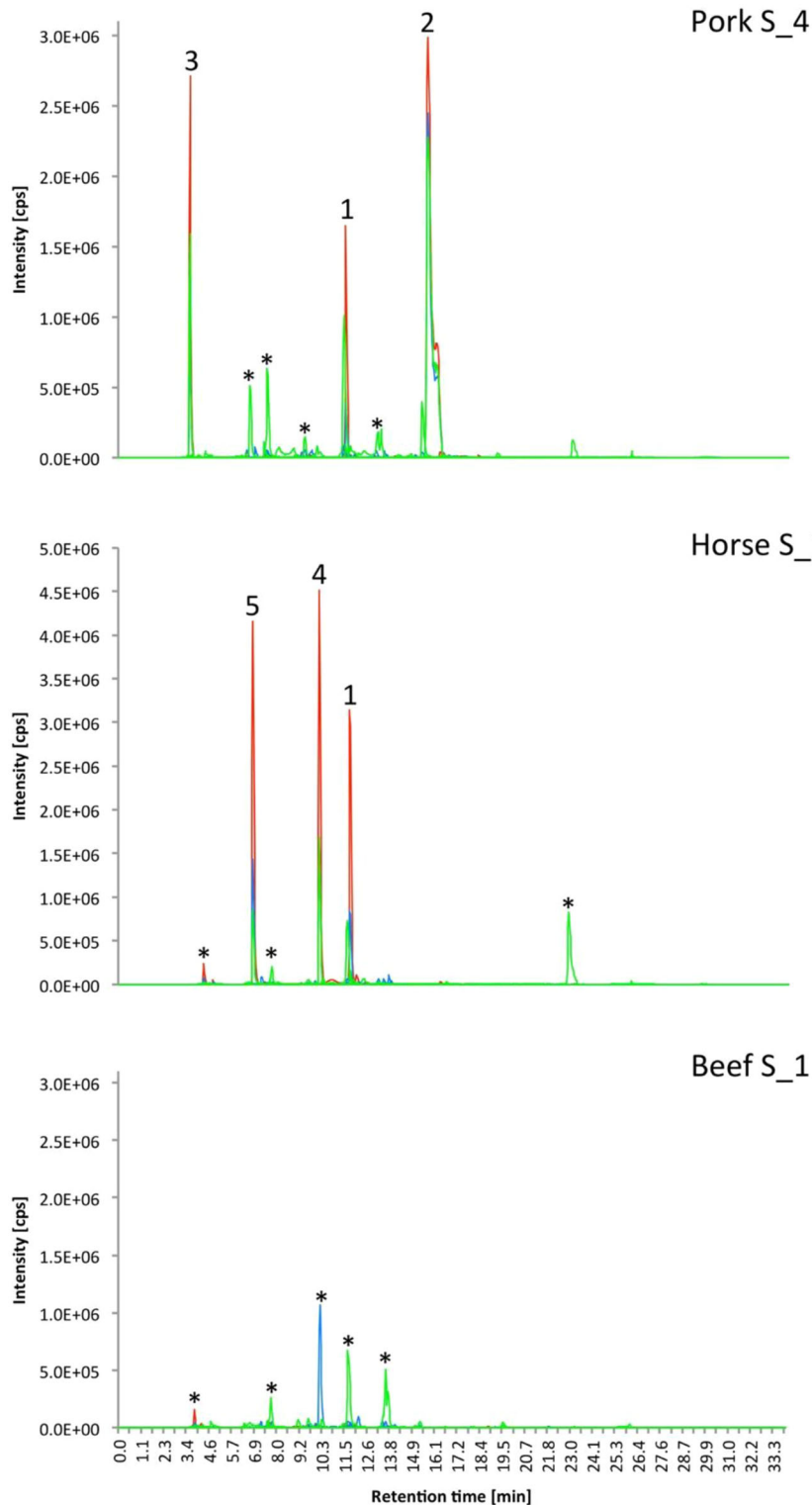


FIGURE 7 HPLC-MS/MS chromatograms of the pure pork, beef, and horse meat. Retrieved from (von Bargaen et al., 2014) COPYRIGHT © 2014, American Chemical Society

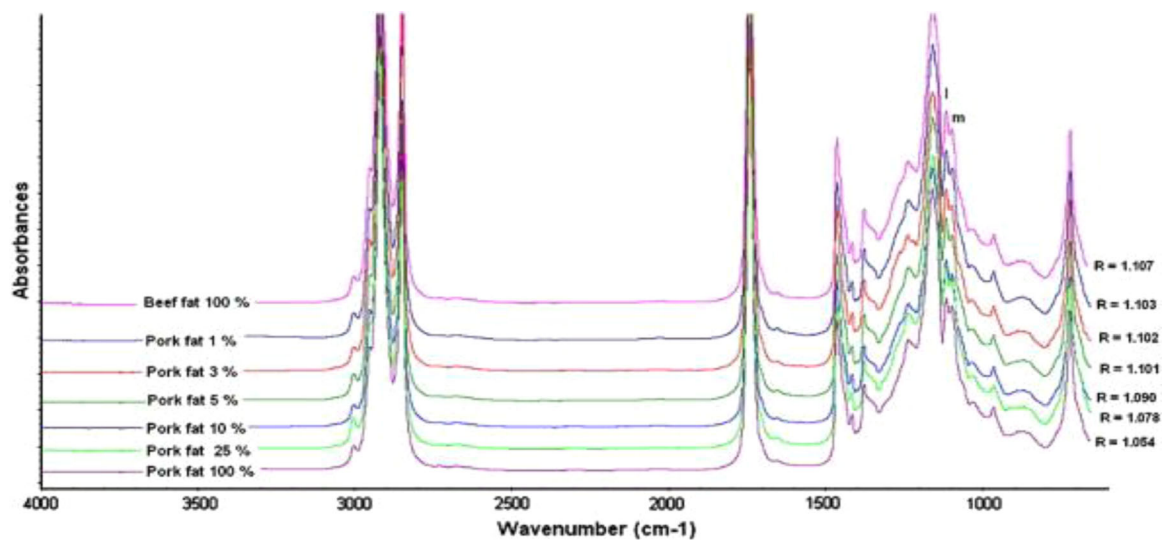
nents (A. Rohman et al., 2011). Additionally, a study used FTIR coupled with chemometrics such as standard normal variate (SNV) on partial least squares discrimination analysis (PLSDA) and least squares support machine vector (LS-SVM) to discriminate pork in halal and non-halal Chinese ham sausages. The sensitivity and specificity of PLSDA with SNV spectra were 0.913 and 0.929, respec-

tively, whereas those of the LS-SVM spectra were 0.957 and 0.0929, respectively (Xu et al., 2012).

Similarly, proton NMR ( $^1\text{H-NMR}$ ) spectroscopy is suitable for determining the proton-bearing components of lard adulterated butter. The presence of lard is indicated by characteristic peaks in the region of 2.60–2.84 ppm. Moreover, a peak at 2.63 ppm that corresponds to the

**TABLE 6** HPLC hyphenated advanced techniques for different types of foods were used in halal authentication

HPLC hyphenated techniques	Type of food analyzed	Method validation involved	Reference
RP-HPLC-PCA	Bovine gelatin, porcine gelatin, fish gelatin	1. Gelatin samples were dissolved and subjected to RP-HPLC analysis 2. Raw data from RP-HPLC was projected to PCA for species discrimination	Azilawati et al. (2015)
RP-HPLC-PCA	Beef, mutton, chevon, and chicken	1. Sample preparation by hydrolysis in an oven 2. RP-HPLC analysis 3. Statistical analysis using PCA	Jorfi et al. (2012)
HPLC-MS/MS	Beef, pork, and horse meat	1. Sample pre-treatment by mincing and heating the meat samples 2. Extraction to obtain supernatant 3. SDS-PAGE gel analysis 4. HPLC analysis 5. MS/MS analysis	von Bargaen et al. (2014)

**FIGURE 8** FTIR spectra of beef and pork fats, as well as their mixtures. Retrieved from (A. Rohman et al., 2011) COPYRIGHT © 2010, Elsevier Ltd

chemical shifts of double-allylic methylene protons was observed in lard, but not in the  $^1\text{H-NMR}$  spectra for butter. For signal confirmation,  $^1\text{H-NMR}$  can be correlated with  $^{13}\text{C-NMR}$  (Fadzillah et al., 2017). Therefore, molecular spectroscopy is a useful technique for identifying non-halal components in halal foods. The challenges in interpreting complex spectra can be overcome by combining the approach with the chemometrics of multivariate analysis. This combination offers a rapid and reliable alternative to halal food authentication. Figure 8 shows the FTIR spectra of beef and pork fats, as well as their mixtures. Table 7 summarizes the various types of advanced molecular spectroscopic techniques that have been reported for authenticating various types of halal food.

### 3.9 | Chemometric methods/AI/IoT

Chemometric methods use multivariate statistics to extract data from the complex analytical data. Assessment of halal food authentication is principally based on regression and pattern recognition methods (Rohman & Windarsih, 2020). Furthermore, the analysis of halal food products can be conducted using multivariate methods of analysis that have allowed various quantitative and qualitative analyses. Yuswan et al. (2019) reported a gel-enhanced liquid chromatography-mass spectrometry (GeLCMS) approach coupled with chemometric methods for authenticating halal meat. However, the approach has certain challenges owing to the selection of potential protein markers (myofibrillar proteins) on one-dimensional

TABLE 7 Molecular spectroscopy advanced techniques for different types of foods were used in halal authentication

Molecular spectroscopy techniques	Type of food analyzed	Method validation involved	Reference
FTIR-PLS	Beef and pork	<ol style="list-style-type: none"> <li>1. Sample preparation by fine grinding the sample</li> <li>2. Soxhlet extraction of the sample</li> <li>3. Calibration by spiking pork into meatballs</li> <li>4. FTIR analysis</li> <li>5. PLS analysis</li> </ol>	A. Rohman et al. (2011)
FTIR-chemometrics	Halal and non-halal ham sausages	<ol style="list-style-type: none"> <li>1. Sample preparation by fine grinding the sample with KBr</li> <li>2. FTIR analysis</li> <li>3. Multivariate statistical analysis such as PLSDA on PLS and LS-SVM</li> </ol>	Xu et al. (2012)
<sup>1</sup> H-NMR	Lard and butter	<ol style="list-style-type: none"> <li>1. Lard sample was extracted from the adipose tissue of pig</li> <li>2. Butter sample preparation</li> <li>3. <sup>1</sup>H-NMR measurement and analysis</li> </ol>	Fadzillah et al. (2017)

gel electrophoresis (1DE). This is attributed to human error in the selection of protein bands from 1DE based on optical density in the conventional technique for identifying excising protein bands. Using multivariate PCA, the identification of potential protein markers such as myosin, actin, troponin, and tropomyosin for non-halal pork between halal beef and chicken was achieved using enhanced GeLCMS advanced techniques. The sequence of peptides identified was used to establish protein markers known as myofibrillar proteins in meat samples (pork, beef, and chicken) (Yuswan et al., 2019).

The chemometric approach is frequently utilized in the E-nose in terms of AI. It has been reported that PCA can be used to process raw data from the E-nose for halal authentication. The first principal component carries the most information, whereas the second principal component carries the maximum share of residual information. It is used to determine the similarities and differences between different datasets to structure a data matrix. After PCA analysis, pork could be discriminated from meats and sausages by determining the main volatile in the sample that has a major influence on the volatile profile generated by PCA, which is heptanal in this study (Nurjuliana et al., 2011). Additionally, it has been reported that machine learning techniques (AI) such as naïve Bayesian classifiers, K-nearest neighbors (K-NN), linear discriminant analysis (LDA), decision tree, artificial neural network (ANN), and SVM can be used to classify the raw data collected by the E-nose. For the naïve Bayesian classifier, a labeled training set is presented to the learning algorithm, and then the learner uses the training set to build a model that maps unlabeled instances to class labels. This technique can be used to predict the labels of instances that have not yet been labeled. For K-NN, the algorithm searches the training corpus for the K-NN of the unknown class and uti-

lizes the K-NN classes to weigh the possibilities. LDA is a signal-classification technique that maximizes class separability by maximizing the ratio of between-class variance to within-class variance, resulting in distinct clusters. A decision tree is an algorithm for automatically making decisions. ANN is an information-processing model capable of deriving and extracting patterns as well as detecting difficult-to-identify trends from complex datasets. SVM is used to solve the binary classification problem (a quadratic optimization problem) in which the error surface is free from local minima and has a global optimum (Ordukaya & Karlik, 2016).

For data treatment, chemometrics such as PCA and K-mean cluster analysis can be used with GC-MS or HPLC. The datasets subjected to PCA to reduce the number of descriptors while preserving the information of the original data. The PCA score plot and loading plots provide useful information and can distinguish very low concentrations of lard, such as 0.5% in beef tallow and chicken fat. When admixture samples were included in the study, cluster analysis was useful. Pure beef tallow, pure chicken fat, pure lard, and a mixture of lard in beef tallow and chicken fat were used in this study. Samples of lard, beef tallow, and chicken fat were grouped into three classes using K-mean cluster analysis, whereas the admixture samples were grouped according to their main fat component (Dahimi et al., 2014). FTIR is frequently used with chemometrics such as SNV on PLSDA and LS-SVM) in the discrimination of halal food. PLS is used to solve various regression problems, whereas SVM is a trade-off and generalization performance of a learned model by regression coefficient regularization. LS-SVM is a simplified version of SVM that obtains solutions by solving a set of linear equations rather than quadratic programming (Xu et al., 2012).

Currently, AI has been incorporated in the food sector and it has progressed to the halal food industry. As a control tool, AI can be useful in determining food quality, and it can be used to classify foods and make predictions. Additionally, it has been integrated with devices such as E-noses and near-infrared ray spectroscopy (NIRS) to enhance the accuracy and precision of halal food analysis. Fuzzy logic (FL), ANN, and machine learning (ML) are examples of AI. FL mimics the impeccable capability of human intelligence in decision-making and unraveling ambiguous data while solving problems. ANN mimics the human brain, and it can gain knowledge through learning and inter-neuro connections. ML is a computer algorithm that advances automatically with experience. For E-nose, AI techniques such as ANN, FL, and pattern recognition have been employed for recognizing odor in the authentication halal food. NIRS, ANN, FL, and ML allow classification and prediction of samples by extracting information from the complex physical and chemical information on the vibrational molecular bonds (Mavani et al., 2021).

Furthermore, IoT can be utilized to track and manage product information in the halal food industry. IoT refers to smart gadgets that connect to each other via the Internet and save data in the cloud. Radio frequency identification (RFID) can be used to track and trace the integrity of halal food throughout the supply chain. In addition, technologies such as transportation management system, warehouse management system, electronic data interchange, and global positioning system can be used to maintain the integrity of halal products in halal logistics services. However, one of the issues of IoT in the halal food supply chain is the accuracy and validity of shared status information of halal food, as there is a risk of misinformation and falsification. To address this shortcoming, a robust system of trust, decentralization, and collaboration has been established (Rejeb, 2018).

In conclusion, when combined with other analytical techniques such as spectroscopy, electrochemistry, and chromatography, as well as physical and biochemical techniques, chemometrics could be beneficial. Furthermore, in halal food authentication, AI can be combined with an analytical system to improve the accuracy, detection speed, and ability to detect multiplexed analytes. Moreover, integrating the IoT into the halal food supply chain can increase the authenticity of ingredients chosen for production.

Table 8 summarizes the comparison and highlights of the advantages, disadvantages, limitations, and challenges of the aforementioned advanced techniques for halal food authentication. The detailed comparison makes it simple to select the approach to use for non-halal component analysis. PCR is more suitable for detecting food components that are likely contaminated with pork because of its high

sensitivity. In addition, it can detect specific swine DNA down to 1–5 pg/ $\mu$ L. For alcohol-containing beverages or fermented foods, an E-nose coupled with chemometrics is preferred because it is suitable for detecting volatile compounds such as ethanol. Chemometrics has made the raw data of analysis from the E-nose presentable and easily distinguishable. Future research on genetically modified organism (GMO) food for halal authentication should also be conducted because the current information available in this field is insufficient.

#### 4 | BACTERIAL COUNT FOR HALAL SLAUGHTERED MEAT

Slaughtering is one of the main steps in halal meat production. Halal slaughter must be performed by a sane Muslim adult. The animal slaughtered must be in the category that permit for Muslim to eat, they must be alive and healthy at the time of slaughtering (Farouk et al., 2014). Blood must be drained out from the animal. Sabow et al. (2015) compared halal slaughter and slaughter with minimal anesthesia, through the bleeding efficiency, lipid oxidation, and microbiological quality (Sabow et al., 2015). Although the blood is drained off from the carcass, some blood is retained in the meat. The blood retained in the meat determines meat quality and microorganism contamination. Blood is a suitable medium for bacterial growth because many nutrients in the blood can support the growth of microorganisms. Contamination of meat with microorganisms can reduce meat quality, economic value, shorten its shelf life, and cause health problems (Jouki et al., 2011).

According to Hakim et al. (2020), the standard plate count (SPC) and coliform plate count (CPC) of microbial counts have been completed. SPC findings were sensitive and they showed that more residual blood in the meat could be found in non-halal slaughtered chickens, which can increase bacterial counts, thereby decreasing shelf life (Hakim et al., 2020). The degree of significance statistical analysis used in this study was  $p < 0.05$ . The non-halal approach had significantly prolonged period of bleeding ( $p < 0.05$ ), lower blood loss ( $p < 0.05$ ), longer death time ( $p < 0.05$ ), and higher SPC count ( $p < 0.05$ ) compared to the halal slaughter process. The CPC count was not significantly different. The high bacterial count in the sample was mostly because of the high volume of blood retained in the muscle. The bleeding rate during halal slaughtering was higher than that during non-halal slaughtering, leading to a greater amount of blood loss during halal slaughtering. The findings in this study are similar to those of previous studies that cutting the carotid arteries and jugular veins results in shorter bleeding time and death times; thus, halal slaughtering allows less blood to be retained in the meat.

**TABLE 8** Comparison and highlights of the advantages, disadvantages, limitations, and challenges of each of the aforementioned advanced halal food authentication techniques

Techniques	Validation parameters	Non-halal components	Advantages	Disadvantages/limitations	Challenges	Reference
Physio-chemical method (dielectric)	A LOD of 0.5% alcohol content in water was measured. An accuracy of about 95% for meat discrimination	No specific target analytes are mentioned but the peaks possibly related to DNA, proteins, microbes, amino acids, enzymes, and others in pork	This method is fast (less than 1 min per sample), low cost, and straightforward. Besides, it does not require heat or solvent treatment, which might disrupt the solubility and structure of the protein	This method can only provide limited information. Biomarkers, for example, are not known to correspond to distinct peaks	The sterilization process might kill bacteria in the sample, which is also a target analyte	(Abidin et al., 2015; Abidin et al., 2014)
Electrophoresis	An accuracy of 100% in distinguishing between porcine and bovine gelatin	Protein of meat species such as myoglobin	Simple and low cost because there is no complicated or expensive instrument involved	Limited information can be obtained from this method. The only parameter is the distance of the respective bands	Electrophoretic patterns are unstable at low pH	(Hoyem & Thorson, 2002; Kim & Shelef, 1986)
Polymerase chain reaction (PCR)	A detection limit of 0.001 ng/ $\mu$ L porcine DNA has been reported for PCR (2012). A detection limit of 5 pg/ $\mu$ L DNA from porcine gelatin has been reported for PCR (2016)	Swine protein and DNA	- This DNA-based analysis method is sensitive and specific because DNA can be found in all cell types of an organism with identical information - DNA is a stable molecule during the extraction process, unlike protein molecules which are heat labile and have a lifespan limited by the lifetime of the organism	- High cost of real-time instruments - High cost of reagents in real-time PCR - Sample destruction and time consuming to complete a full analysis - Contaminants may result in a false-negative test result - A short amplicon length could lead to low specificity	- Ought to select high efficiency and specific primers from genes for PCR amplification - Ought to choose suitable DNA extraction methods - Must prepare sufficient template DNA for analysis	(Aida et al., 2005; Che Man et al., 2012; Kang et al., 2018; Abd Mutalib et al., 2015; Sudjadi et al., 2016)

(Continues)

TABLE 8 (Continued)

Techniques	Validation parameters	Non-halal components	Advantages	Disadvantages/limitations	Challenges	Reference
Electronic nose (E-nose)	An accuracy of 98.10% for an optimized electronic nose system (2020). When this method is coupled with PCA, it gives a total variance of 67% in terms of separation of samples by first principal component	<p>Volatile compounds that give off an odor such as diacetyl, 3-hydroxy-2-butanone, 2-methylpropanal, heptanal, trimethylpyrazine, nonanal, and decanal</p>	<ul style="list-style-type: none"> <li>- The cost of analysis per sample is relatively low in comparison to HPLC, GCMS, and IR</li> <li>- Qualitative detection that is fast and accurate, with strong correlation between data from sensor analysis</li> <li>- It is environmentally friendly because it is a detection system that stimulates biological olfactory patterns</li> <li>- No complex pre-treatment processes</li> <li>- Can couple use with other instruments such as mass spectroscopy for other purposes such as quantitative analysis for various alcohols in the beverages</li> </ul>	<ul style="list-style-type: none"> <li>- Loss of sensitivity when analyzing high-concentration analyte</li> <li>- Some sensors in the E-nose have relatively short lifetimes (2 years)</li> </ul>	<ul style="list-style-type: none"> <li>- Less sensitive than human olfactory system</li> <li>- Poisoning of sensors may occur</li> <li>- All samples should be stored at -20°C and not pre-treated because the aroma would most likely be altered, affecting the results</li> </ul>	(Nurjuliana et al., 2011; Sarno et al., 2020; Wang et al., 2019)
Gas chromatography (GC)	<ul style="list-style-type: none"> <li>- GC-Cl-MS gives a wide linear range of 0.25–500 ng/mL and a LOD of 0.03–0.36 ng/g</li> <li>- GC-FID could give a LOD of 0.5% of lard content in the sample</li> <li>- GC-FID-PCA gives a total variance of 67% in terms of sample separation by first principal component</li> </ul>	<p>N-nitrosamines in meat products, fatty acid contents in meats</p>	<ul style="list-style-type: none"> <li>- Separating the gases from the sample into peaks corresponding to individual components provides high accuracy and sensitivity</li> <li>- It allows the analysis of a wide range of components in complex systems</li> </ul>	<ul style="list-style-type: none"> <li>- Need sample pre-treatment before analysis</li> <li>- Longer time to analyze a large number of samples than an electronic nose, as testing one sample might take anywhere from 1 to 2 days, depending on the complexity of the gas</li> <li>- The GCMS instrument is relatively expensive, costing around USD \$120,000</li> <li>- Operating the instrument necessitates a certain level of competence</li> <li>- The samples must be volatile</li> </ul>	<ul style="list-style-type: none"> <li>- Loss of volatile compounds during sample preparation</li> <li>- Selection of suitable column for analysis</li> </ul>	(Cajka, 2013; Huang et al., 2013; Lehotay & Hajslova, 2002; Nurjuliana et al., 2011; Sarno et al., 2020)

(Continues)

TABLE 8 (Continued)

Techniques	Validation parameters	Non-halal components	Advantages	Disadvantages/limitations	Challenges	Reference
Biosensors	An accuracy of $\geq 90\%$ (2012) A detection limit of $5 \mu\text{M}$ (2013) A detection limit of $0.001\%$ ethanol in beverages (2014)	Pork DNA and ethanol in beverages	- A low-cost and highly sensitive approach for detecting and quantifying targeted molecules by converting interactions into optical, electron, and magnetic signals - There is less time required because there is no requirement for the electrophoresis procedure that is required in conventional PC. - Miniaturization may be possible for real-time and on-site monitoring	- The stability of biosensor is checked every three working days as the sensitivity decreases with each interval - Calibration is required for each analyte	- Because of the inconsistency of the results, stability of biosensors toward real samples has been questioned	(Ali et al., 2012b; Das & Goswami, 2013; Kuswandi et al., 2014; Zia et al., 2020)
High-performance liquid chromatography	- HPLC-MS/MS gives a LOD of $0.24\%$ (2014)	Porcine amino acids	- Simple analysis by separating samples into components based on its polarity, therefore it is specific for targeted analytes - Fast detection ( $\sim 30$ min)	- High cost of reagent used - Sample destruction and time consuming to complete a full analysis - The instrument is relatively expensive, costing around USD \$10,000, and it requires expertise to operate	- Require suitable chromophore or fluorophore for derivatization	(Azilawati et al., 2016; Azilawati et al., 2015; Jorfi et al. (2012); von Barga et al., 2014)
Molecular spectroscopy	- This method favors qualification and identification over quantification for halal analysis	No specific halal components mentioned	- It is easy to operate, rapid, and requires little or no sample preparation - It is non-destructive at which the sample is reproducible - It gives characteristic fingerprint spectra for sample differentiation	- The instrument is relatively expensive, especially for NMR, costing between USD \$40,000 and 150,000 - The operation of the NMR instrument requires expertise - Because the spectra contain thousands of channels, some data may be redundant in IR	- Because fingerprint spectra can be difficult to analyze, they are often coupled with chemometrics for data analysis and peak interpretation	(Fadzillah et al., 2017; Rohman & Salamah, 2018; Rohman & Windarsih, 2020)

(Continues)

TABLE 8 (Continued)

Techniques	Validation parameters	Non-halal components	Advantages	Disadvantages/limitations	Challenges	Reference
Chemometric methods	- This method is normally coupled with biochemical, mass spectrometry, chromatography, and spectroscopic for data treatment	—	- It is non-destructive because it applies a mathematical and multivariate statistical analysis approach for data treatment	- This is an auxiliary method at which it cannot be stand-alone in halal analysis. It needs to be coupled with other instruments, and datasets for data analysis must be obtained from other sources	- In calibration datasets, overfitting of the model or overoptimistic multivariate calibration performance is acceptable, but in validation datasets, the performance is unacceptable	(Rohman & Windarsih, 2020; Yuswan et al., 2019)
Bacterial count	CPC gives a range of $8.9\text{--}28.1 \times 10^4$ cfu/g SPC gives a range of $12.6\text{--}91.2 \times 10^4$ cfu/g	—	- Because it involves counting bacteria colonies on SPC and CPC plates and does not involve an expensive instrument for analysis, it is simple and low cost	- Time consuming in sample preparation (1–7 days)	- Apply to a natural analyte with a low concentration (dilute to ppm level) - Both the halal and non-halal samples must be in the same environmental condition to compare their SPC and CPC	(Hakim et al., 2020; Sabow et al., 2015)

This bacterial count method is simple and inexpensive, as no complex instrument is required (by counting the bacterial colonies on the SPC and CPC plates); however, it is time consuming because SPC takes 48 h and CPC takes 24 h for incubation.

#### 4.1 | Conclusion and future perspectives

Over the recent years, various advanced analytical techniques have been evaluated for their suitability for food authentication, specifically for halal food products owing to food adulteration. Evaluation of halal food authentication has previously been conducted by panels of trained human experts, but this conventional approach has various disadvantages such as lengthy procedures, high cost, and it is subjective. Recent advances and technology offer various advantages over conventional approaches, including speed, accuracy, objectivity, reliability, environmental friendliness, and lack of sample pre-treatment. However, none of these methods can be extended to the same food source. Based on this review, biosensors have the greatest potential in the future, as a point-of-care device, to accurately and rapidly identify food products containing pork, alcohol, and other prohibited meats (e.g., horse and donkey) or even any animal that is not slaughtered using the halal slaughter method. Biosensors have relatively low cost and detection time compared to other reported advanced techniques. Additionally, they have the ability to miniaturize to enable portability, real-time, and on-site detection, such as for use in Olympic games or any major international events. The storage stability of the biosensors for real sample analysis should be enhanced further to ensure the accuracy and reliability of detection in halal food authentication analysis. Furthermore, we suggest that it is better to prevent cross-contamination with a disposable type of biosensor to avoid false-positive detection arising from leftover analyte residue from the previous analysis when a reusable biosensor is used. For future perspectives, the incorporation of AI, where ML can be integrated with the biosensing system in the analysis of halal food authentication to enhance the accuracy, speed, and its ability to perform multiplex analyte detection, should be considered.

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#### AUTHOR CONTRIBUTIONS

**Pei Chi Ng:** Writing—original draft. **Nur Amy Syahira Ahmad Ruslan:** Writing—original draft. **Ling**

**Xuan Chin:** Writing—original draft. **Musa Ahmad:** Writing—review and editing. **Sharina Abu Hanifah:** Writing—review and editing. **Zanariah Abdullah:** Writing—review and editing. **Sook Mei Khor:** Conceptualization, writing—review and editing, supervision, project administration, funding acquisition, resources.

## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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