Halal Food Authenticity: Does it matter to you?

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Halal Food
Authenticity
Does it matter to YOU?
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ABSTRACT

To the Muslims, the claim for Halal is not debatable. The current Halal food industry as required by 1.8 billion Muslims is estimated to be worth USD 1.6 trillion by 2018. In this respect, the Halal food industry is projected to be the fastest growing and most lucrative business segment in the very near future in view of the rapid increase in the Muslim population in the world. This enormous business potential will be further augmented by the preference, on the part of non-Muslims for the “safe, wholesome and clean” food concept referred to as the Halalan toyyiban. The Manufacture of Halal products, especially food requires an uncompromised understanding and knowledge of the Islamic laws and regulations along with the advancement in food processing and complex ingredients in the entire supply chain. However, unethical practices and fraud of the Halal logo is becoming a regular occurrence and poses a threat to the authenticity of halal products. Thus, authentication using specific, sensitive, easy-to-use and reliable scientific techniques based on DNA, proteins, lipids and metabolomics should be performed to complement the conventional audit-based certification process.
INTRODUCTION

Halal is an Arabic word which means lawful or permitted as dictated by Allah the Creator. Halal which transcends the entire facets of life of Muslims viz. the food and drinks they consume, social interactions, dress code and even financial transactions must be strictly adhered to as laid down in the Holy Quran and exemplified by the Sunnah (teachings and practices) of Prophet Muhammad (SAW). Halal food are food products which are prepared strictly following specific Islamic requirements referred to as \textit{Halalan Toyyiban} which ensures that such products are safe, wholesome and clean.

The demand for Halal food is slowly but surely exerting a major influence on the global economy as the world is projected to be populated by 2.8 billion Muslims by 2050; accounting for one third of the human population (Pew Research Center, 2016). To a certain extent, non-Muslims in the world, regardless of race are also attracted by the “safe, wholesome and clean” concept in foods which would further add on to the increase in the demand for \textit{Halalan toyyiban} food. Alserhan (2010) reported that Halal food constitute 16% of the global food industry which is worth USD632 billion. Report by Porter (2014) estimated the value of global Halal food market to be in the region of USD700 billion. A more recent report by Thompson Reuters (2015) predicted that the global halal food market to be worth more than USD1.6 trillions. However, although there exist such a huge potential in the development of the Halal food industry, there are still many challenging and pressing issues that need to be addressed. Food products could only be declared Halal when the entire supply chain (ingredients, processing, handling, packaging, storage and distribution) are certified Halal (Samsi et al., 2012). The players in the entire supply chain processes need to understand and be
fully committed to the Halal requirements and procedures. In this respect traceability from the point of view of the Halal industry is the ability to trace the Halalness of each product from its origin and nature of the ingredients through all the manufacturing processes until it reaches the consumer.

The failure to adhere to the Shariah requirements in the production of Halal foods has led to considerable doubts in the Halalness of the product itself and uncertainties pertaining to the integrity and authenticity of the Halal certification. These uncertainties are further aggravated by the absence of a unified Halal standard which has opened the floodgate for fake Halal certificate. In view of the above, there is a need for a comprehensive and effective authentication system to provide the much needed transparency on the production of Halal food. Thus, this lecture deliberates the scientific approaches developed over the years towards improving and refining Halal food authentication to facilitate the respective authorities to make a definitive decision on the Halalness of food and food products before the issuance of the Halal certificate.

TRACEABILITY SYSTEM IN HALAL FOOD PRODUCTION

To the Muslims, Halal is inseparable as it is their way of life. Halal food, being foremost in the minds of Muslims worldwide which make up one third of the human population has a major influence on the world’s economy (Power, 2009). Growth of world Halal market is expected to grow at 5.8% annually until 2020 while the market for certified Halal goods is expected to grow at 26%.

Malaysia was instrumental to the development of Halal standard MS 1500:2009 (Department of Standards Malaysia) which is now accepted as the benchmark of most Halal standards worldwide.
One of the major criteria in this standard is the traceability of the ingredients in Halal foods. The requirements for Halalness discreetly states that Halalness is not only fulfilled by evaluating the ingredients of the food but also encompasses aspects on safety and cleanliness which have to be strictly adhered to throughout the stages of production i.e. source of ingredients, processing, packaging, storage and transportation. Every stage along the supply chain must be ensured to have met all the requirements according to the Halal standards. It is a comprehensive concept of protection for Muslims which cover not only religious requirements such as exclusion of alcohol and pork but equally high on the priority list are the safety, wholesomeness and cleanliness of the product. This concept is known as the Halalan Toyyiban (which means Halal and good/preferred) which demonstrates the righteousness concept of Halal (Janis, 2004). However, for business purposes, a company may be required to comply with additional certifications such as the Hazard Analysis and Critical Control Point (HACCP) and Good Manufacturing Practices (GMP). The fulfillment of Halal together with other standards and requirements clearly demonstrates that the food product produced has achieved certain quality levels or standards that cannot be compromised particularly from the perspective of Muslim customers and to a lesser extent by non-Muslim consumers (Samsi et al., 2012).

**TRACKING AND TRACING OF HALAL FOOD**

As the awareness and education of Halal food consumers grow which is concurrent with the increase in demand for Halal food products, there is an accompanying increase in demand for more information with respect to the products purchased by consumers. Not only consumers are interested in a wider choices of Halal food
available but they also require information on procedures employed along the supply chain, assurance on food safety and quality, animal welfare practices and good environmental management (Schulze et al., 2006).

From the perspective of the Halal food industry, traceability can be used to trace the Halal status of a particular food product at every stage of the supply chain. It encompasses all information pertaining to activities that the Halal food products have gone through which include preproduction activities and in particular the origin of the ingredients/raw materials. By having a traceability system, critical Halal control points can be fully monitored and if the product is suspected to be contaminated or adulterated with non-Halal elements, detailed information can be retrieved to identify the cross contamination point and accordingly corrective measures instituted in view of the fact that Halal is all about credibility and quality control.

A comprehensive and reliable traceability system in the Halal food supply chain would therefore increase Halal transparency and strengthen Halal integrity. However reports by Norman et al. (2009), Mohd Albakir and Mohd Mokhtar (2011) and Yang and Bao (2011) pointed out that almost all existing tools to determine the Halal status of products are not only unreliable and lack security but there appeared to be an absence of real time product analysis and the test procedures require considerable time to be accomplished. Existing technologies such as those involving Radio Frequency Identification Device (RFID), barcoding and the Internet could be manipulated to make them appear as reliable tools for Halal traceability. The above observations do not auger well with the current flourishing worldwide Halal food industry which require an excellent and reliable traceability system to ensure that consumers
are persistently protected from unknowingly consuming non-Halal food which is against the Muslim faith.

Halal products inclusive of their ingredients, processing, handling, packaging, storage and distribution must strictly adhere to the Halal rules and regulations as stipulated in the Shariah law. In short, the entire supply chain processes and activities must be certified as Halal compliant. If a food product contains imported ingredients, then every single ingredient needs to be checked and verified and certified in terms of its Halalness, which inevitably involves investigations within its supply chain. The best terminology to illustrate this process is ‘traceability’. Traceability in the Halal industry is defined as the ability to trace the Halal status of each product from farm to the table, or from its raw material to the finished product. This process involves all players in the supply chain to have their utmost commitment and comprehensive knowledge on the Halal concept (Samsi et al., 2012). Hence, traceability is notably critical in the Halal supply chain.

Traceability as an enabler of data exchange is the key to Halal assurance. It augments Halal integrity by providing clear information with regards to origin and processes, link food safety to the process flow, ensure all planned safety measures have been carried out and all labeling are correct. Traceability also helps to decrease minimum stock levels, reduce wastage through shelf-life, reduce dependent picking, reduce dependency on experienced workers, remote control of sites and reduce the risk of breach of specification/desired practices. Figure 1 shows the 5 levels of Halal traceability system (Lehr, 2012).
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Figure 1  Level of Halal traceability system
ISSUES ON HALAL FOOD ADULTERATION

Harmonization of modern science and Islamic law is very important especially with regard to Halal authentication to protect from fraud and deception. In the food industry, pork and its derivatives are among the most widely used materials such as gelatin, sodium stearoylactylate, shortening, collagen, whey, calcium stearate, capric acid, myristic acid, oleic acid, pancreatic extract, bone ash and lard. In this context, Muslim researchers tend to stick with the majority view of Muslim jurists who forbid any sources of pig either flesh or lard and their derivatives. Most issues arising in Halal food production include porcine-based products in food and beverages, usage of gelatin from animal source which is classified as non-Halal, consumption of non-Halal food additives, contamination of food and beverages with alcohol, meat that is not slaughtered according to the ritual Islamic manner and last but not least the use of non-halal genetic materials in GM (genetically modified) foods (Ermis, 2017). Table 1 shows some cases of cross contamination of food products with porcine derivatives and mislabeling that concern Muslim consumers all over the world.
Table 1  Examples of food potentially cross-contaminated with porcine products and mislabelling cases

<table>
<thead>
<tr>
<th>Item</th>
<th>Location</th>
<th>Contaminant</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sausages</td>
<td>Mexico</td>
<td>pork</td>
<td>Flores-Munguia et al. (2000)</td>
</tr>
<tr>
<td>Meat pies and pastries</td>
<td>UK</td>
<td>Porcine DNA</td>
<td>Whitworth (2013)</td>
</tr>
<tr>
<td>Homemade food products</td>
<td>China</td>
<td>Mislabeled as Halal</td>
<td>Lipes (2013)</td>
</tr>
<tr>
<td>Cat Fish</td>
<td>Malaysia</td>
<td>Fed with porcine products</td>
<td>Harian Metro, 2014</td>
</tr>
<tr>
<td>Chicken sausages</td>
<td>Italy</td>
<td>Porcine DNA</td>
<td>Di Pinto et al. (2015)</td>
</tr>
<tr>
<td>Marshmallows, gummies and candies</td>
<td>Spain</td>
<td>Porcine DNA</td>
<td>Munoz-Colmenero et al. (2016)</td>
</tr>
<tr>
<td>Imported goat meat</td>
<td>Malaysia</td>
<td>Mix with pork</td>
<td>Utusan Malaysia, 2017</td>
</tr>
</tbody>
</table>
Shuhaimi Mustafa

THE NECESSITY OF LABORATORY TESTING

The growing concern in food authenticity and adulteration has resulted in increased awareness with reference to the composition of food products. The identity and source of the ingredients in processed or composite mixtures is not always readily discernable. Hidden ingredients from various sources present serious problem for Muslim consumers (Riaz and Chaudry, 2003). The high demand for transparency in the food industry has enhanced the development of methods for the analysis of food ingredients. Muslims are more attentive to the content of processed foods, for instance, since food chains are becoming longer and more complex. Hence all products produced for the needs of Islamic consumers must comply with Halal criteria. These criteria refer to the nature, origin, and the processing method of the food product (Bonne and Verbeke, 2008) which are as follows:

1. does not contain elements not permissible according to Islamic law,
2. has not been in contact with non-Halal substances during production, transportation and storage, and
3. is not stored in facilities or premises or transported using transportation shared with non-Halal food or ingredients.

Laboratory analysis can be used as proof of correct labeling when food products are delivered to consumers or buyers or to ensure that suppliers only deliver products devoid of any Halal substances. To ensure that the food industry has met the requirements in producing Halal foods, its production has to be verified. Certificates and labeling are required to convince consumers and buyers alike that the food products are manufactured according to Halal production procedures.
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Generally, the Halal certification process work flow includes 1) online registration of product(s), 2) delivery of supporting documents, 3) reviewing of supporting document, 4) auditing process, and 5) issuing of Halal certificate upon approval by certification panel (Figure 2).

Figure 2 General Halal Certification Process Work Flow (Manual Procedure for Malaysia Halal Certification, Third Revision, 2014)

Halal laboratory testing is never indicated in the certification process even though some critical ingredients are subjected to laboratory testing upon receiving complaint or reported to be doubtful. Laidan (2011) stated that a particular Halal-certified product cannot be genuinely guaranteed Halal without being tested in a certified Halal laboratory. Hence, verification on the
authenticity of the ingredients and sources acceptable to Muslim consumers are indeed essential. Sensitive and reliable methods for detection of Halal product adulteration are of paramount importance for implementation of Halal food labeling, regulations and products quality control. Various techniques including DNA-based methods, gas chromatography, liquid chromatography, differential scanning calorimetric (DSC) and Fourier transform infrared (FTIR) spectroscopy have been specifically proposed for the analyses of pork, lard, khamr and gelatin. It should be noted that there is no laboratory test in the market that could possibly determine the way in which an animal is slaughtered. Therefore the scientific testing within the Halal industry is strictly concentrated on adulteration either intentionally or unintentionally.

VARIOUS HALAL AUTHENTICATION TECHNIQUES FOR FOOD PRODUCTS ANALYSIS

Selection of the most suitable technique on Halal certification is dependent upon the type of suspected non-Halal compound(s), composition of the food product, the expected levels of non-Halal constituents, whether screening or confirmatory analysis is required and the measurement of uncertainty allowed. The specificity and sensitivity of detection methods determine the extent to which the absence of non-Halal ingredients can be demonstrated. More sensitive, cheap and simple detection methods for non-Halal ingredients are needed to support the verification of the Halal status of food products (Fels-Klerx et al., 2016).

There are various approaches to detect and quantify the level of adulterants in food products. The first approach is by determining the ratios between some chemical constituents and assuming that these ratios are constant in particular food products. This approach appears to be more convincing as addition in any food products
will modify or change this ratio or will highlight an anomaly in its chemical composition. This approach is usually associated with a set number of analyses and the use of chemometrics. Another approach is by identification a specific marker in food products, either chemical constituents or morphological characteristics, which proves the presence of adulterants in food products. The third approach is using analytical methods derived from physical analysis by taking into account the whole samples to show the adulteration effects on the physico-chemical properties (Cordella et al., 2002). Some of the analytical techniques are described in the following sections.

**DNA-based Halal Authentication**

Advances in gene technology have led to rapid development of detection methods based on genetic variations for Halal authentication. DNA offers advantages over proteins, including stability at high temperature, presence in all tissue types and greater variation with genetic code (Mackie, 1996). DNA is a comparatively stable molecule, provides universal and identical information from all tissues of an organism and capable of withstanding extensive heat, pressure and chemical stresses of food processing (Ali et al., 2011; 2012). In meat speciation, DNA-based techniques have been preferred over protein and lipid-based molecular identification protocols since most DNA biomarkers are extremely stable even under harsh processing conditions such as heat, pressure and chemical additives. This technique is equally stable in compromised states such as natural decomposition or degraded specimens where protein-based markers are denatured or degraded (Fajardo et al., 2010).
Polymerase chain reaction (PCR) has occupied a central position in all DNA-based investigations by virtue of its simplicity, cost-effectiveness, robust and extraordinary power of increasing target DNA copy numbers even from a single or a few copies to easily detectable quantities (Mohd Yusop et al., 2012). Conventional PCR is simple and useful but rely on end-point analysis and under such condition could not provide any quantitative information on the targets originally present in the sample. Real-time PCR has effectively overcome this limitation by direct and independent monitoring of cycle-to-cycle amplification, using a fluorescent-labeled signaling probe. The intensity of the fluorescent signal directly correlates to the accumulated PCR products in each cycle, providing detection in a real-time format at an early stage. It is inherently more accurate and more promising than the conventional PCR due to self-automation and devoid of the laborious agarose gel or polyacrylamide electrophoresis.

Species-specific PCR (Che Man et al., 2007; Haunshi et al., 2009; Mane et al., 2011; Karabasanavar et al., 2014), multiplex PCR (Dooley et al., 2004; Ali et al., 2015) randomly amplified polymorphic DNA (RAPD) (Arslan et al., 2006), PCR restriction fragment length polymorphism (RFLP) (Ali et al., 2012), real-time PCR (Ali et al., 2012a), DNA barcoding (Wong and Hanner, 2008), DNA microsatellites (Sonnante et al., 2009) and PCR product sequencing (McKenna et al., 2010) are some of the significant reports for fraud detection in food chain. Each method has its own advantages and drawbacks.

Species-specific PCR-RFLP is most commendable in meat speciation as the system exploit the genetic variations that exist within a defined region of target DNA and allowing differentiation of even closely related species by digestion of selected DNA
fragments with appropriate restriction enzymes (Fajardo et al., 2008). It was reported that PCR-RFLP assays with shorter DNA targets are advantageous due to higher availability and better stability of such biomarkers under extreme food processing conditions. However, hundred nanometers pore size of traditional agarose gel facilitates only separation of larger DNA targets (100 bp–10 kb) with good resolution (Salieb-Beugelaar et al., 2009). It cannot resolute restriction-digested smaller DNA targets, specially <50 bp. (Rohman et al., 2014b)

Real-time PCR assay combined with species-specific primers and TaqMan probe is particularly promising because, in addition to specific primers, it provides additional species screening through the specifically designed TaqMan probe, significantly enhancing the assay specificity and reliability (Tanabe et al., 2007; Rojas et al., 2010; Köppel et al., 2011; Sakai et al., 2011). Both the simplex (Rojas et al., 2010) and multiplex (Köppel et al., 2011) real-time PCR assays with TaqMan probes are available (Ali et al., 2012b).

Multiplex PCR is highly repeatable, time and cost saving since the system allows simultaneous identification of several species using a single PCR assay and affordable compared to above-mentioned methods (Dalmasso et al., 2004). It can simultaneously amplify primer mixtures in one-step PCR reaction thereby overcoming the weakness of single PCR detection which only amplifies a pair of primers. However, multiplex PCR has several disadvantages. Low amplification efficiency, variable efficiency on different templates and poor universality highlight the need for an advanced multiplex approach (Hanapi et al., 2014). Moreover, a comparatively longer and length-variable DNA template for different species is required. (Sakai et al., 2011). As longer DNA templates are not stable in the harsh conditions of food-processing and lengthy variable templates entail variable sensitivities for
different species (Rojas et al., 2010), multiplex PCR assays are not suitable for analysis of processed foods. Hanapi et al. (2014) developed a detection method through common primer multiple PCR (CP-M-PCR) system to simultaneously identify some of the common meats that are widely used in meat-based product industry. The authors claimed that the application of common primers (only one forward primer for all specific reverse primers) in multiplex PCR reduced the cost and increased the sensitivity of the analysis at a very low concentration of DNA and primer, thus reducing the limitations that generally occur in the conventional multiplex PCR (Ali et al., 2012a).

Simplex real-time PCR assays on other hand are comparatively easier to design and ensure more sensitive and robust quantitative detection of species in the raw (Mohd Yusop et al., 2012) and processed (Rojas et al., 2010) products. Thus, a real-time PCR assay targeting multicopy genes, such as mitochondrial (mt) genes, with shorter amplicon length is of great value as it ensures available targets even in degraded samples and increases assay sensitivity, making them more dependable and reliable (Bielikova et al., 2010).

It was reported that DNA-based assays with micro-fluidic technology coupled with a lab-on-a-chip bioanalyzer kit is partially automated and can effectively separate shorter nucleic acid fragments ≥10 bp differences in length with good resolution. This is an easy and user-friendly analysis approach integrating automated capillary electrophoresis (CE) in chip, giving higher resolution and speed with better reproducibility while using less reagents and samples over the traditional agarose gel electrophoresis (Funes-Huacca et al. 2004; Rahman et al., 2015; Ali et al., 2012b).

Some of the recent work related to exploiting porcine DNA analysis focusing on the use of PCR as biomolecular techniques to amplify the specific fragments of gene of interest are listed in Table
2. Both genomic and mitochondrial genes have been targeted for detection of porcine DNA in various products. PCR identification of species using mt-DNA has a series of advantages: Mt-DNA genes are present in thousands of copies per cell; the large variability of mt-DNA allows reliable identification of precise species in mixtures and intraspecific variability of mt-DNA overs the possibility of discriminating breeds currently used in industrial swine production (Montiel-Sosa et al., 2000). The use of specifically designed primers under restrictive conditions of PCR amplification could serve the same purpose by direct and specific identification of PCR-amplified mt-DNA fragments, avoiding subsequent sequencing or RFLP identification (Che Man et al., 2007).
Table 2: DNA-based PCR methods for Halal authentication

<table>
<thead>
<tr>
<th>Issues in food sample</th>
<th>Method of detection</th>
<th>Limit of detection</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork and lard in food products</td>
<td>cyt b PCR-RFLP</td>
<td>NR</td>
<td>Aida et al., (2005)</td>
</tr>
<tr>
<td>Pork and lard in food products (sausages and casings, bread and biscuits)</td>
<td>cyt b PCR-RFLP</td>
<td>NR</td>
<td>Aida et al., (2007)</td>
</tr>
<tr>
<td>Pork derivatives in food products</td>
<td>Species-specific PCR</td>
<td>NR</td>
<td>Che Man et al., (2007)</td>
</tr>
<tr>
<td>Pork adulteration in meat</td>
<td>Species-specific PCR assay utilizing mtDNA D-loop gene sequences</td>
<td>0.001 ng</td>
<td>Che Man et al. (2010)</td>
</tr>
<tr>
<td>Dog Meat Adulteration in Commercial Frankfurters</td>
<td>Cytochrome b (cytb)-based polymerase chain reaction (PCR)</td>
<td>0.02 ng</td>
<td>Ali et al., (2014b)</td>
</tr>
<tr>
<td>Canine DNA in chicken nugget</td>
<td>TaqMan probe RT-PCR</td>
<td>0.01%</td>
<td>Rahman et al., (2015a)</td>
</tr>
<tr>
<td>Canine DNA in Burger Formulations</td>
<td>Lab-on-a-Chip PCR-RFLP Assay</td>
<td>0.01 % (w/w)</td>
<td>Rahman et al., (2015b)</td>
</tr>
<tr>
<td>Event</td>
<td>Method</td>
<td>Sensitivity</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>-----------------------------</td>
<td>-------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Bovine, buffalo, and porcine materials in frankfurter formulation</td>
<td>mPCR- RFLP</td>
<td>as low as 0.1% adulteration</td>
<td>Motalib Hossain et al., (2016)</td>
</tr>
<tr>
<td>Detection of meat origin (pig, ruminant, avian and rabbit)</td>
<td>CP-M-PCR</td>
<td>0.1 ng</td>
<td>Hanapi et al., (2014)</td>
</tr>
<tr>
<td>Malayan box turtle (<em>Cuora amboinensis</em>) in the food chain and traditional Chinese medicines</td>
<td>Lab-on-a-Chip PCR-RFLP Assay</td>
<td>0.0001 ng</td>
<td>Ali et al., (2014b)</td>
</tr>
<tr>
<td>Pork in raw meat</td>
<td>Molecular Beacon probe RT-PCR</td>
<td>0.0001 ng</td>
<td>Mohd Yusop et al., (2012)</td>
</tr>
<tr>
<td>Lard detection in chocolate</td>
<td>porcine-specific real-time PCR</td>
<td>&lt;0.0001 ng</td>
<td>Rosman et al., (2016)</td>
</tr>
<tr>
<td>Canine species detection</td>
<td>Mitochondrial cytochrome b (cytb)-based polymerase chain reaction (PCR)</td>
<td>0.02 ng</td>
<td>Rahman et al., (2014a)</td>
</tr>
<tr>
<td>Dog meat adulteration in meatball formulation</td>
<td>cytb-based PCR</td>
<td>0.04 ng</td>
<td>Rahman et al., (2014b)</td>
</tr>
</tbody>
</table>

NR = not reported
Fourier-transform Infrared Spectroscopy

Infrared spectroscopy is one of the most powerful spectroscopic techniques for food analysis since it explains in detail the functional groups as well as chemical composition present in the infrared spectrum of specific substances (Merfort et al., 1997). Fourier-transform infrared spectroscopy (FTIR) has received considerable attention in quantitative analysis of fats and oils over the years due to the main advantage of easy sample preparation with reduced or no-sample pre-treatment steps (Sherazi et al., 2010). Its application in analysis of edible fats and oils can be considered as “green analytical chemistry” because this technique reduces or eliminates solvents and chemical reagents used in analysis which might be hazardous to human health and environment (Namiésnik, 2001; Nurrulhidayah et al., 2011). FTIR is a method of choice for the determination of major components present in foods as well as feeds and forage. It is widely preferred as its data can be processed and programmed to manipulate spectral information for development of multivariate analysis techniques, advanced construction of the spectrophotometers viz. better sources of radiation, detectors, and optical parts in general (Fadzlillah et al., 2013).

Combination of FTIR with multi-component analysis proves useful in quantitation of adulteration in Halal fats and oils products. This technique combined with partial least square (PLS) model is reported to have been used successfully in the detection of adulterants and quantitative analysis of lard in cake formulation (Syahariza et al., 2005) chocolate products (Che Man et al., 2005a) analyzing the mixture of lards in other animal fats (Che Man and Mirghani, 2001) in cod-liver oil (Rohman and Che Man, 2011a) in virgin coconut oil (Rohman and Che Man, 2011b) in palm kernel oil (Manaf et al., 2007) and many more.
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Che Man and Mirghani (2001) have developed a FTIR method for detecting lard in mixtures of other animal fats, such as chicken, lamb and cattle. The results demonstrated that the FTIR could qualitatively differentiate between the pure animal fats from their blends. A study by Syahariza et al. (2005), demonstrated that FTIR analytical approach could be adapted to detect and quantify the level of lard adulteration in cake formulation, particularly if the same type of shortening was used. However, in the case of lard adulteration in cakes, when variable lipid sources are used to formulate the products (e.g. other commercial shortening) the calibration model has to be designed to account for the variation. It is recommended that before the model can be applied a database comprising a collection of all shortening spectra have to be developed first to ensure the accuracy of the prediction. In cosmetics, the FTIR method has been developed for analysis of lard in a mixture with virgin coconut oil in cream (Rohman et al., 2011), in a mixture with palm oil in lotion (Lukitaningsih et al., 2012) and for analysis of lard extracted from lipstick formulation (Waskitho et al., 2016). Moreover, Rohman et al., (2011c) used FTIR to study pork adulteration in beefball formulations. They highlighted that there were some issues related to the adulteration of pork in beefball products. Their results showed that FTIR could be used for the detection and quantification of pork in beefball formulation for Halal authentication purposes. Guntarti et al. (2015) used this technique for analysis of wild boar meat in meatball formulation. Xu et al. (2012) has also developed a rapid discrimination method of pork in Halal and non-Halal Chinese ham sausages by FTIR and chemometric data analysis. Their study had resulted in the establishment of a reliable model for Halal/non-Halal discrimination of some mainstream and representative samples in China. Some of the recent works on the use of FTIR to discriminate lard from other fats and oils for
Halal purpose are listed in Table 3. Fadzillah et al. (2013) used FTIR-ATR and found that this technique could be used for the identification and quantification of mutton fat in butter formulation for source verification purposes. Detection and quantification of lard as adulterant in butter was also successfully carried out by Nurulhidayah et al., (2013) using FTIR-ATR approach.
<table>
<thead>
<tr>
<th>Issues in food sample</th>
<th>Software/analysis used</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lard mixed with other animal fats</td>
<td>PLS</td>
<td>Che Man and Mirghani, (2001)</td>
</tr>
<tr>
<td>Lard in cake formulation</td>
<td>PLS</td>
<td>Syahariza et al., (2005a)</td>
</tr>
<tr>
<td>Lard in chocolate and chocolate products</td>
<td>PLS</td>
<td>Che Man et al., (2005b)</td>
</tr>
<tr>
<td>Lard in biscuit</td>
<td>PLS</td>
<td>Syahariza (2006)</td>
</tr>
<tr>
<td>Lard mixed with lamb, cow and chicken body fats</td>
<td>PLS and DA</td>
<td>Rohman et al., (2010)</td>
</tr>
<tr>
<td>Lard mixed with cod liver oil</td>
<td>PLS and DA</td>
<td>Che Man and Rohman (2010)</td>
</tr>
<tr>
<td>Lard in other animal fats</td>
<td>PLS and DA</td>
<td>Rohman and Che Man, (2011)</td>
</tr>
<tr>
<td>Gelatin in raw materials</td>
<td>PLS and PCA</td>
<td>Hashim et al. (2010)</td>
</tr>
<tr>
<td>Lard in virgin coconut oil (VCO)</td>
<td>PLS and DA</td>
<td>Mansor et al., (2011)</td>
</tr>
<tr>
<td>Ingredient</td>
<td>Techniques Used</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>---------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Lard in vegetable oils</td>
<td>PLS, PCR and DA</td>
<td>Rohman and Che Man (2011b)</td>
</tr>
<tr>
<td>Lard in edible fats and oil</td>
<td>PCA and CA</td>
<td>Che Man et al., (2011)</td>
</tr>
<tr>
<td>Pig derivatives in meatball</td>
<td>PLS and DA</td>
<td>Rohman et al., (2011a)</td>
</tr>
<tr>
<td>Halal and non-Halal Chinese ham sausages</td>
<td>PLS-DA and LS-SVM</td>
<td>Xu et al., (2012)</td>
</tr>
<tr>
<td>Lard in cream cosmetics</td>
<td>PLS and PCR</td>
<td>Rohman et al., (2014)</td>
</tr>
<tr>
<td>Lard in frying oil</td>
<td>PLS and DA</td>
<td>Che Man et al., (2014)</td>
</tr>
<tr>
<td>Use of pork as beef in meatball</td>
<td>PLS and PCA</td>
<td>Kumiawati et al., (2014)</td>
</tr>
<tr>
<td>Beef jerky form pork</td>
<td>LDA, SIMCA and SVM</td>
<td>Kuswandi et al., (2015)</td>
</tr>
<tr>
<td>Pork in sausage</td>
<td>PCA and SVM</td>
<td>Schmutzlen et al., (2015)</td>
</tr>
<tr>
<td>Lard in chocolate</td>
<td>PLS, PCA</td>
<td>Suparman et al., (2015)</td>
</tr>
<tr>
<td>Lard in ink extracted from printed food packaging</td>
<td>PCA, SIMCA</td>
<td>Ramli et al., (2015)</td>
</tr>
</tbody>
</table>

PCA: principal component analysis; DA: discriminant analysis; CA: cluster analysis; PCR: principle component regression; PLS: partial least square; SVM: support vector machines; LDA: linear discriminant analysis; SIMCA: soft independent modeling class analogy.
Electronic Nose Technology

Electronic nose (E-nose) is a rapid and easily operable, low-cost analytical tool in food authentication. Being a nondestructive analytical method it has a wide application such as quality control, discrimination of fake food and determination of origin of food. Basically e-nose is a bench-top portable machine designed to replicate the major functions of human nose for detection, recording, memory search and identification for profiling an aroma (Ali et al., 2012d). Although the applications of e-nose in food industry are well known in monitoring processes, shelf-life, freshness, authenticity, and other quality controls, the capability of e-nose in authentication of Halal status is rather limited.

The work by Che Man et al. (2005a) on surface acoustic wave (SAW)- based e-nose (zNose™) had successfully detected and quantitate the refined bleached deodorized (RBD) palm olein samples adulterated with lard (as low as 1 %) using the eNose™. A two-dimensional olfactory image called VaporPrint™, produced by the eNose™ could be used for immediate detection (qualitatively) of lard substances in sample admixtures.

Nurjuliana et al. (2011) combined eNose™ chromatograms with PCA for differentiation and discrimination of various animal fats such as lard, beef, mutton, and chicken fats. PCA provided a good grouping of samples: PC 1 and PC 2 accounted 61% and 29% variations of the data. All lard-containing samples produced a separate group from the samples that were free from lard. They also successfully applied eNose™ and PCA to differentiate and discriminate pork and pork sausages from beef, mutton, and chicken meats and their sausages. With a 67% total variance, PCA provides a good model to discriminate meat and their products into well defined clusters. However, the application of e-nose to discriminate
meat species in a complex matrix frequently encountered in commercial meat products has yet to be defined.

Ab Mutalib et al. (2013) has successfully developed a method for rapid detection of ethanol content in beverages using portable e-nose prototype. This portable e-nose is very reliable in the detection of ethanol in various beverages such as alcoholic beverages, isotonic drinks, soft drinks and fruit juices of different brands marketed in Malaysia. The device has high accuracy and reliability, where it could detect ethanol content as low as 0.1% (v/v). From routine screening processes products that carry the Halal label were found to contain a small amount of ethanol in beverages.

More recently, Park et al. (2017) has used e-nose coupled with MS for analysis of ethanol content in soy sauce. The e-nose MS data were analyzed by discriminant function analysis (DFA). Results obtained show more than 96.6% accuracy when the ethanol concentrations were greater than 0.5%. A high correlation between the first score of the DFA plot and the ethanol concentration was also observed. Results from their study show that mass spectrometry based on e-nose is an efficient method as a primary screening tool for Halal certification.

**Table 4: Halal product authentication using e-Nose**

<table>
<thead>
<tr>
<th>Issues in food sample</th>
<th>limit of detection</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lard in edible oil</td>
<td>1%</td>
<td>Che man et al. (2005a)</td>
</tr>
<tr>
<td>Pork in meat</td>
<td>1%</td>
<td>Nurjuliana et al. (2011)</td>
</tr>
<tr>
<td>Adulteration in edible oil</td>
<td>NR</td>
<td>Marina et al. (2009)</td>
</tr>
<tr>
<td>Ethanol in beverages</td>
<td>From &lt;0.1%</td>
<td>Ab mutalib et al. (2013)</td>
</tr>
<tr>
<td>Ethanol in soy sauce</td>
<td>&gt;0.5%</td>
<td>Park et al. (2017)</td>
</tr>
</tbody>
</table>

NR: not reported
Differential Scanning Calorimetry (DSC) for Halal Food Authentication

Thermal analysis (TA) is one of the analytical techniques used by food science researchers analysis of fats and oils. Differential scanning calorimetry (DSC) being the most versatile with a range of applications based on the principle of heat differences of a sample by thermo-physical transitions (exothermic and endothermic changes). The DSC provides the information on the melting and crystallization phenomena of oils that is directly influenced by their physicochemical properties such as fatty acid, triglyceride (TAG) composition and chemical structure. Each edible oil has a fingerprint profile in their thermal behaviors including melting and crystallization profile which is closely related to the chemical composition of the oil (Tan and Man, 2002).

The principle of DSC is to keep the sample and the reference at the same temperature in separate micro-ovens. The electrical power require for the compensation is equivalent to the calorimetric effect. DSC offers a direct method for studying the thermal properties of various materials and has a possibility to be developed as a quality control procedure for food adulteration (Mansor et al., 2011).

For Halal authentication, DSC has proven to be successful for the detection of adulterants such as lard and beef tallow in canola oils (Marikkar et al., 2002). Analysis of its heating thermogram enabled the detection of lard at 8% in canola oil. DSC was also used to determine the lard uptake in fried food products (Marikkar et al., 2003). When a food is fried with lard, it becomes a product enriched in lard. Based on DSC heating thermograms, lard contamination of tempeh and chicken resulted in a strong endothermic peak emerging in the range of 22-23 °C. Animal fats including lard have been used as adulterants in vegetable oils as well as being exploited to develop
new products in order to gain economical profits as lard is one of the cheapest fats available in the market (Foglia et al., 1993). One of the potential vegetable oils that could be adulterated with lard is virgin coconut oil (VCO). VCO is recognized for its nutraceutical properties which can act as primary prevention and treatment for many illnesses relating to atherosclerosis (Nevin and Rajamohan, 2004). Further, the physical characteristics of VCO are quite similar that of lard, being white to cream in color and are also solid at room temperature (Codex, Vol 8, 2001). Therefore, blending of VCO with lard would generate more profit as lard is comparatively cheaper than VCO. In view of this concern, the DSC technique was developed to study the thermal behavior of VCO adulterated with lard (Mansor et al., 2012). They showed that DSC was able to detect changes in the cooling and heating curves of VCO when adulterated with lard. Qualitative determination showed that the marker peak increased as the concentration of lard added increases. It can be concluded that, DSC provides unique thermal profiles and therefore suitable for Halal products analysis. Table 5 shows Halal product authentication using DSC.
### Table 5 Halal product authentication using DSC

<table>
<thead>
<tr>
<th>Issues in food sample</th>
<th>Limit of detection</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lard and randomized lard in RBD palm oil</td>
<td>10% (w/w)</td>
<td>Marikkar et al., (2001)</td>
</tr>
<tr>
<td>Adulteration of RBD palm oil with ERLD</td>
<td>10% (w/w)</td>
<td>Marikkar et al., (2002)</td>
</tr>
<tr>
<td>Monitoring lard, tallow and chicken fat</td>
<td>10% (w/w)</td>
<td>Marikkar et al., (2002)</td>
</tr>
<tr>
<td>Fat adulteration in Canola oil</td>
<td>10% (w/w)</td>
<td>Marikkar et al., (2002)</td>
</tr>
<tr>
<td>Lard adulteration</td>
<td>NR</td>
<td>Marikkar et al., (2002)</td>
</tr>
<tr>
<td>Detection of lard in selected food product</td>
<td>10% (w/w)</td>
<td>Marikkar et al., (2003)</td>
</tr>
<tr>
<td>deep fried in lard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lard in cooking oil</td>
<td>-</td>
<td>Mansor et al., (2012)</td>
</tr>
<tr>
<td>Lard in sunflower oil</td>
<td></td>
<td>Marikkar et al., (2012)</td>
</tr>
<tr>
<td>Lard in canola oil</td>
<td>5% (w/w)</td>
<td>Marikkar et al., (2014)</td>
</tr>
<tr>
<td>Lard in virgin coconut oil</td>
<td></td>
<td>Marikkar et al., (2011)</td>
</tr>
<tr>
<td>Lard in butter</td>
<td></td>
<td>Nurrulhidayah et al., (2015)</td>
</tr>
</tbody>
</table>

RBD: refined, bleached, deodorized; ERLD: enzymatically randomized lard; NR: not reported.

### ELISA Technique for Halal Food Authentication

Enzyme-linked immunosorbent assay, also called ELISA, is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. ELISA has been applied as a diagnostic tool in medicine and plant pathology, as well as a quality control check in various industries. It is a sensitive,
Shuhaimi Mustafa

rapid, and specific for meat speciation. The ELISA uses polyclonal antibodies as a capture reagent and has been successfully applied to the species identification of fresh (Martin et al., 1991; Patterson et al., 1984) and cooked meat (Kang’ethe and Gathuma 1987; Berger et al., 1988; Andrews et al., 1992). In the Halal industries ELISA has been used for detection of pig derivatives qualitatively in the food samples, such as sausages from various types of meat (Jaswir et al., 2016). Although excellent results were obtained for the detection of pig derivatives in food, the technique lacks specificity which may lead to false negative or false positive findings.

Recently, ELISA was used for the detection of porcine in edible bird’s nests (EBNs) (Tukiran et al., 2015). In order to develop indirect ELISAs for porcine gelatin adulteration, anti-peptide polyclonal antibodies were raised against porcine species-specific amino acid sequences of the collagen (I) alpha2 chain (PAB1 and 2) and the collagen (I) alpha1 chain (PAB3). These antibodies showed acceptable affinity towards spiked samples with the ability to detect at least 0.05% porcine gelatin in EBNs.

In another work by Alina et al. (2012), sandwich ELISA method was successfully used to detect non-Halal plasma transglutaminase (TGase) in surimi-based products. Plasma TGase which is derived from blood of different species of mammalian animals use to maximize the gel strength. However, further study for optimization of the specificity of antibody used in the confirmation of TGase in surimi needs to be established.

Bio-sensors

A biosensor is a molecular sensor that combines a biological recognition mechanism with a physical chemical transduction technique (Cornell et.al., 1997). It can also be described as an analytical device incorporating a biological or biologically
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derived sensing element. It is aimed at generating a signal which is proportional to a specific analyte or group of analytes. The result is a simple, easy to use analytical devices that can be used by non-specialists to deliver sophisticated analytical results in decentralized locations in the field or even from the home. The biological component such as antibody, enzyme or nucleic acid may be extracted from nature subsequently, chemically modified or they may be synthesized \textit{de novo} (Turner, 2010). The general layout of a biosensor is as shown in Figure 3.

![Figure 3](image_url)

\textbf{Figure 3} Diagram showing the basic parts of a biosensor. The biological element produces a specific reaction with the substance that it is required to be measured, which in turn should be able to generate a signal that can be detected by the transducer (Kuswandi and Ahmad, 2014a)

Recently biosensors have been developed and applied in Halal authentication. Gold Nanoparticle Sensor for the visual detection of pork adulteration in meatball formulation was developed by Ali et al. (2012c). It was reported that the method is rapid (less than 10 min), reliable, and cheap for the selective detection of target DNA sequences in processed meat products. It does not need any instrument or surface modification chemistry and directly detects target DNA in non-amplified mixed genomic DNA. The procedure is very simple and relies on the color change of 20-nm GNPs following the addition of salt. The visual finding is solid and could be further confirmed by an inexpensive and reliable technique, absorption spectroscopy which incurs only the instrumental cost and reusable
cuvette. The use of absorption spectroscopy increases sensitivity and eliminates any sort of color-blindness error or ambiguity in visual detection by producing well-defined bands of aggregated and non-aggregated colloidal particles. The assay needs a shorter probe whose design is simpler than PCR primers. The method is applicable to analyze extensively degraded sample which may not be possible by with PCR which require longer targets.

In a the separate study, Ali et al. (2014a) used nanobiosensor to detect and quantify pork adulteration in meatball formulation. The sensor was found to be sensitive enough to detect 1% pork in raw and cooked meatballs. They claimed that this Species-specific hybrid biosensor based on gold nanoparticles (GNPs) was developed for the first time. The sensor was successfully applied to authenticate as low as 1% pork adulteration in ready-to-consume beef meatball preparations. The cross-testing results with various formulations of commercial meatballs also revealed the high specificity and sensitivity of the sensor for the pork DNA. The hybridization kinetics of the sensor was studied for the first time and hyperbolic relationship was observed between fluorescence intensity and target concentration. A linear curve was realized over the moderate concentration of the target and used to quantify potential targets in processed mixed meat products with more than 90% accuracy. The method eliminate the need of expensive RT-PCR, time-consuming electrophoresis and laborious blotting techniques for target DNA authentication. This approach has a very high potential for application in food analysis, genetic screening, bio-diagnostics and forensic investigations.

Kuswandi et al. (2014b) studied a simple visual ethanol biosensor based on alcohol oxidase (AOX) immobilized onto polyaniline (PANI) film for Halal verification of fermented beverage samples. This biosensor responded to ethanol via a color change
from green to blue due to the enzymatic reaction of ethanol which produces acetaldehyde and hydrogen peroxide when the latter oxidizes the PANI film. This biosensor consists of the immobilization of AOX onto PANI film by adsorption. The detection limit of the biosensor was 0.001\%, with 1.6\% reproducibility (RSD) and a shelf-life time up to seven weeks when stored at 4 °C. All these characteristics make the AOX/PANI biosensor a good alternative to other determination methods for Halal verification of ethanol in fermented beverage samples.

In another study by Kuswandi et al. (2017), a lateral flow immunoassay was developed and used to test pork adulteration in processed meats. The reliability of the assay was further investigated by comparing the results to those of commercially available ELISA kits. It was found that the correlation of two methods was excellent. Thus, the strip could provide a simple approach to detect pork adulteration in processed meats samples with high reliability. In their work, a high-affinity anti-Swine IgG polyclonal antibody was evaluated to develop a user-friendly, rapid, and sensitive immunoassay as a test strip assay for detecting low levels of pork adulteration in beef meatballs. In terms of gold nanoparticles (AuNPs) used for conjugation of IgG (swine antibody), very fine AuNPs within 20 nm were used since size is important as it could affect the nanosensor performance such as response time (Aveyard et al., 2008). A rapid immunoassay test to detect low levels of pork adulteration in beef meatball products was developed using anti-Swine IgG polyclonal antibody. The assay was completed in 5 min, after incubation time. Detection limit was 0.1\% (w/w), the reliability of the assay was good as the result in agreement when comparing to that of ELISA results. Thus, the immunoassay strip could provide a simple approach to detect low level pork adulteration in processed meat samples (i.e. beef meatballs) with high reliability.
Surface plasmon resonance based biosensor was used by Wardani et al. (2015) for quantitative differentiation between bovine and porcine gelatin. Their SPR-based biosensor could detect differences between both types of gelatin based on its sensitivity toward the gelatin concentration change, its reliability and limit of detection (LOD) and limit of quantification (LOQ) of the sensor. The LOD and LOQ of the sensor towards bovine gelatin concentration were 0.38% and 1.26% (w/w), while towards porcine gelatin concentration were 0.66% and 2.20% (w/w), respectively. The results show that SPR-based biosensor is a promising tool for detecting gelatin from different raw materials quantitatively.

The SPR-based biosensor shows the quantitative ability to perform a simple and sensitive detection of the difference between bovine and porcine gelatin for a concentration range above 0.25% and under 9.9%, as cross-reactivity occurs at both concentrations. However, it still requires further investigation to compare these results with gelatin refractive indices at various concentrations used in this study. For advanced detection of gelatin contamination in food and pharmaceutical products, the development of immobilization method of gelatin molecules instead of the above simple deposition method is suggested.

Chromatography-based Techniques for Halal Food Authentication

The chromatography-based techniques offer rapid and reliable tools for the separation and quantitative analysis of major and minor components with highly similar chemical structures in complex foods. Due to their advantageous separation characteristics, numerous chromatographic techniques have been tested, accepted, and employed in the analysis of non-Halal derivatives in food products. However, the drawback of this technique is generally
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attributed to the multiple-step of sample preparation required before chromatographic analysis (Reid et al., 2006). Table 5 summarizes the application of chromatographic-based techniques for analysis of non-Halal derivatives in food systems.

HPLC is one of the most widely used analytical techniques for food authentication studies (Cordella et al., 2002). Its application for the detection of adulteration in foods has attracted much attention since the technique is applicable to almost all components present in food systems. Rashood et al. (1996) has carried out TG profiling of pork, beef, mutton, chicken, and turkey fats using a LiChrospher-100 RP-18 (5 μm) column. TG profiling of genuine and randomized lard has been investigated by these authors using a refractive index detector.

Marikkar et al. (2005) has identified lard contamination in vegetable oils such as palm oil (Pa-O), palm kernel oil (PKO), and canola oil (Ca-O) using HPLC in combination with the chemometrics of canonical discriminant analysis (CDA). Analyses were performed to monitor TG compositional changes in vegetable oil samples before and after adulteration with lard. The ability of the HPLC technique to monitor the triacylglycerol (TAG) compositional changes in the oil samples before and after adulteration lard was also studied by Marikkar et al. (2005). The results showed that qualitative determination of lard contamination in PKO was possible by a visual comparison of TAG profiles of PKO adulterated with different animal fats.

HPLC using a cation-exchange column and diode array detection was used by Wissiack et al. (2003) to differentiate pork from other animal meats to detect meat adulteration. Different peak patterns for extracted hemoglobins of pork, cow, or lamb were obtained to be further used for the qualitative assessment of meat adulteration. The identification of pork in meat products from 15 food animal
species (cattle, pigs, goats, deer, horses, chickens, ducks, ostriches, salmon, cod, shrimp, crabs, scallops, bull-frogs, and alligators) has been carried out using HPLC with electrochemical detection (Chou et al., 2007). Each species exhibited unique electrochemical profile.

Zhang et al. (2009) has successfully differentiated porcine and bovine gelatins based on marker peptides in tryptic-digested gelatins using HPLC-MS/MS. It was found that proline hydroxylation was a key factor affecting the peptide identification. They revealed that detection of marker peptides in the digested gelatin sample using HPLC-MS/MS is an effective method to differentiate between bovine and porcine gelatin. Norakasha et al. (2009) developed reverse-phase HPLC to differentiate gelatin coming from bovine and porcine by determining their amino acid compositions. The classification and characterization of gelatin based on the amino acid contents were carried out using PCA.

The use of liquid chromatography coupled with mass spectrometer (LC-MS) has grown exponentially in the last decade for authentication analysis by virtue of its sensitivity, extraordinary selectivity, and rapid rate of analysis. Dugo et al. (2006) have determined the triglyceride composition of lard, tallow, and their mixtures using HPLC in combination with atmospheric pressure chemical ionization mass spectrometry (APCI-MS). What did they show?

Analysis of lard adulteration in some food products depended upon the identification and determination of certain characteristic constituents. GC is a unique and versatile technique for analysis of volatile components. One of the main characteristics of GC is the minimization of band broadening. The formation of high and narrow peaks contributes to the high signal-to-noise ratio and improves the analyte detection (Mondello et al., 2004) Gas-liquid chromatography (GLC) was used for the detection of lard added in
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buffalo and cow ghee using an OV-17% column and flame ionization detector (FID). Analysis of fatty acids as methyl esters derivatives by GLC is an important method for authentication purposes. The compositional and positional distribution of fatty acids and TG has been performed using GLC. Individual and total saturated and unsaturated fatty acid compositions in total fats of both genuine and randomized lard were identical (Rohman and Che Man, 2012).

GLC combined with pancreatic lipolysis (which hydrolyzes fatty acids preferentially esterified at the 1,3-positions of TG) and chemometrics of canonical discriminant has been used for identification of lard contamination in some vegetable oils such as Pa-O and PKO. Jee (2002) has investigated the presence of lard in beef using GLC initially after treating with pancreatic lipase to produce 2-monoglycerides.

GC coupled with mass spectrometry (GC-MS) has been used for analysis of volatile flavor compositions in pork. The cooked pork contained a markedly low concentration of alcohols compared to that of the other muscles (cooked chicken and beef). The variations in alcohol and aldehyde concentrations might be responsible for the differences in the overall aroma characteristics of pork, chicken, and beef (Farag et al., 1983). Analysis of off-odor volatiles using GC-MS showed that the main volatile compounds present in irradiated pork were dimethyl disulfide, methyl sulfide, dimethyl trisulfide, S-methyl thiocianate, and methanethiol (Lin et al., 2007).

Comprehensive two-dimensional gas chromatography (GC×GC) has emerged as a powerful analytical technique in the compositional analysis of complex samples. Recently, attention was primarily devoted to the detection and analyte identification with time-of-flight mass spectrometry (TOF-MS) (Adahchour et al., 2008). GC×GC-coupled with an ion analyzer using time-of-flight mass spectrometry (GC×GC–TOF-MS) was applied to
differentiate lard, cattle fat, chicken fat, and goat fat by Indrasti et al. (2010). A similar study was carried out by Chin et al. (2009) for the differentiation of lard from other animal fats and cod liver oil. The results suggested that GC×GC–TOF-MS combined with chemometrics using PCA could be an effective means for such differentiation. Indrasti et al. (2010) also used GC×GC–TOF-MS to differentiate lard from several fats and oils, namely, butter, sunflower, corn, and palm oils by the determination of monoglyceride (MG) and diglyceride (DG) levels using DB17 and SLB-5ms as the primary and secondary columns, respectively. Indrasti et al. (2010) used a highly sophisticated chromatographic technique, GC × GC-TOF-MS, to study the fatty acid profiles of lard (LA) in comparison with other animal fats such as chicken (CF), cattle (CA) and goat fat (GF). Dias et al. (2010) demonstrated that gas chromatography hyphenated with time-of-flight mass spectrometry (GC-TOF-MS) was able to differentiate between lard and three other commonly animal-derived fats namely cattle fat, chicken fat and goat fat.

Recently, immuno-chromatographic assays using nano-sized colloidal gold particles were developed. These assays are capable of rapidly detecting pork in both raw and cooked samples at a low cost without using any special equipment or requiring skillful techniques by just observing the color change (Ali et al., 2012c). The method is best suited for conducting preliminary screening of large numbers of routine samples before using the traditional DNA or protein based methods for confirmation, which can enable an enhanced surveillance program of the Halal meat products supply.
Table 5 Chromatographic-based techniques for Halal authentication

<table>
<thead>
<tr>
<th>Method</th>
<th>Food products</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>Pork and lard in meat products</td>
<td>Saeed et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>Lard in meat products</td>
<td>Rashood et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>Pork in beef and sheep products</td>
<td>Jee et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>Pork in meat products</td>
<td>Wissiack et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>Lard in animal fats and vegetable oils</td>
<td>Marikkar et al. 2005</td>
</tr>
<tr>
<td></td>
<td>Pork in meat species</td>
<td>Chou et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Gelatin in raw materials</td>
<td>Norakasha et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>Differentiate bovine and porcine skin gelatin</td>
<td>Hafidz et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Lard in meat lipids</td>
<td>Saeed et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>Lard in vegetable oils</td>
<td>Marikkar et al. 2004</td>
</tr>
<tr>
<td></td>
<td>Lard in fried oils</td>
<td>Marikkar et al. 2003</td>
</tr>
<tr>
<td>Method</td>
<td>Sample Description</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>GLC (FID detector)</td>
<td>Lard in meat lipids</td>
<td>Saeed et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>Lard in milk lipids</td>
<td>Farag et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>Lard in milk lipids</td>
<td>Farag et al. (1982)</td>
</tr>
<tr>
<td></td>
<td>Lard in fried oils</td>
<td>Marikkar et al. 2003</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Lard in animal fats</td>
<td>Dugo et al. (2006)</td>
</tr>
<tr>
<td>GC-FID</td>
<td>Lard in animal fats</td>
<td>Farag et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>Lard in vegetable oils</td>
<td>Marikkar et al. 2005</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Pork in cooked meat</td>
<td>Wittasinghe et al. (2001)</td>
</tr>
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<td>GC</td>
<td>Lard in milk fat</td>
<td>Goudjil et al. (2003)</td>
</tr>
<tr>
<td>GC (FID detector)</td>
<td>Lard in vegetable oils</td>
<td>Marikkar et al. (2005)</td>
</tr>
<tr>
<td>GC×GC–TOF-MS</td>
<td>Lard in animal fats</td>
<td>Indrasti et al. (2010)</td>
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<td>Chin et al. (2009)</td>
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<td>HS-SPME-GC-MS</td>
<td>Alcoholic beverage</td>
<td>García-Martín et al. (2010)</td>
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<td>MALDI-TOF-MS, LC-MS</td>
<td>Porcine gelatin</td>
<td>Zhang et al. (2009)</td>
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CONCLUSION

The progression of science and technology has revolutionized the production of food and ingredients to fulfill the demand of consumers and industries in terms of improving quality as well as decreasing processing time and production costs. However, along the processes, alteration and adulteration could happen that change the characteristics of the final product from its origin making its identification complicated. Therefore, the development of specific and sensitive techniques for the authentication of food products is timely.

This presentation highlighted innovations in the authentication methods for Halal products that have been developed. These methods offer various levels of applications from screening to confirmation of contaminants at different concentrations. DNA-based assay developed by our team was able to detect as low as 0.1% (v/v) porcine DNA spiked into meat samples with a detection limit of 0.001 ng/μL porcine DNA. Meanwhile, protein-based assay using competitive indirect ELISA identified gelatin sources from confectionary products which exhibited low cross reactivity. Furthermore, the advancement of GC-MS/LC-MS technologies and other analytical techniques coupled with statistical analysis enable the detection of lard adulteration in fats and oils products. Moreover, the development of biosensors and halal test kits for rapid screening of adulterants in food products will assists the religious authorities in issuing halal certificate. These analyses become more and more sensitive, the trust and confidence in Halal products will increase which is important for the credibility of the Halal certification.
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BIOGRAPHY

Shuhaimi bin Mustafa was born in Hutan Melintang, Perak in 1972. He obtained his Bachelor of Science Degree in Food Science and Technology from the Faculty of Food Science and Biotechnology, Universiti Pertanian Malaysia in 1996. He did his final year project under the supervision of Prof. Dr. Jamilah Bakar. It was the experience in Prof. Dr. Jamilah Laboratory that inculcate his deep research interest. Following graduation, he continued his MSc. studies in the same Faculty with Prof. Dato' Dr. Mohd Yazid Abdul Manap as his supervisor. He completed his MSc in 1999 and published several papers in international journals. He was amongst the first NSF recipient to pursue a PhD studies in 1999 in UPM under the supervision of Prof. Dr. Mohd Yazid Abdul Manap and graduated with PhD in Food Biotechnology in 2003.

Upon graduation, he was appointed as a lecturer in the Department of Microbiology in 2003. He moved forward fulfilling his duty as a lecturer by giving lectures to students in Microbiology and Advance Food Microbiology as well as securing several millions of public and private research grants. He was promoted to Associate Professor in 2006 and full Professor in 2012.

In 2006, the late Prof. Dato’ Dr. Yaakob Che Man invited him to join Halal Products Research Institute as the Head of Laboratory of Halal Products Authenticity and in 2008, he was promoted to the position of the Deputy Director’s post at the same institute until February 2017. He is currently the Deputy Dean (Research and Post Graduate Studies) in the Faculty of Biotechnology and Biomolecular Sciences, UPM. The twelve years term at the Institute was really the productive years whence he managed to published more than 200 articles, graduated 10 PhD and 20 MS students, won several local and international prestigious awards and commercialized two research outputs. HaFYSTM-Porcine DNA
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Detector and Thohira™-Aquaformula (www.thohira.com) were his two research outputs produced at the Halal Products Research Institute that are currently being marketed globally.

Apart from teaching, research and networking, he is also active in Non-governmental Organization and sports. He was the Honorary Secretary and President of Malaysian Society for Microbiology and represent the University in cycling and golf events.
ACKNOWLEDGEMENTS

This compilation of research works would not have been possible without the financial support from UPM as well as Malaysian Government through the Ministry of Higher Education and Ministry of Science Technology and Innovation.

I am especially indebted to Prof. Dr. Jamilah Bakar, my final year project (FYP) supervisor, Prof. Dato’ Dr. Mohd Yazid Abdul Manap, my Ph.D and Master Degrees supervisor and the late Prof. Dato’ Dr. Yaakob Che Man. They have been supportive of my career goals and taught me about both scientific research in particular and life in general more than I could ever give them credit for here. I would like also convey my gratitude to Former Deans of Faculty of Biotechnology Dato’ Bakar, Prof. Ali, Datin Khatijah and the current Dean of Biotech Prof. Arbakariya for their endless support to enable me to carry out my research and use the biotech facility when I was at Halal Products Research Institute.

My sincere and heart felt appreciation also goes to all my post graduate students who tirelessly and with much enthusiasm conducted various research projects over the course of many several years. I would also like to express my appreciation to the staff of Halal Products Research Institute and staff of Faculty of Biotechnology without whom without which my research goals would not have been achieved. Every experiment conducted by my students was accomplished with the help and support of my fellow researchers (Nur Fadhilah, Syariena, Sahar and Raja Hafidz) and collaborators. Without their contribution my job as a lecturer would have undoubtedly been more difficult. I would not forget PSP team especially Dr. Samsilah Roslan, Dr. Wan Nurhayati and Mr. Zakir for their guidance and support to commercialize my research outputs.
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Nobody has been more important to me in the pursuit of this career goal than the members of my family. I would like to thank my parents, whose prayers, love and guidance are with me in whatever I pursue. They are my ultimate role models. Most importantly, I wish to thank my loving and supportive wife, Rossida Mamat, and my two wonderful children, Nadiah Syahirah and Muhammad Faris Irfan, who have always been a source of inspiration and encouragement.
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