Chapter 7:

Strategic Approaches to Halal Lipid Authentication Using Instrumental, Chemometric, and Traceability Techniques

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Halal authentication techniques encompass cutting-edge tools to assess the halal status of various products across diverse sectors, including food, cosmetics, pharmaceuticals, and more. The adoption of these techniques plays a crucial role as confirmatory mechanisms within the global halal certification bodies (HCBs).. Despite their significance, analysts in halal-testing laboratories often encounter challenges, including difficulty in (1) selecting the most appropriate instrumental technique, approach type, and analytical method for lipid-based products, (2) comprehending the requirements for validating and verifying analytical methods (AMs), (3) navigating the integration of a series of chemometric techniques into instrumental methodologies, and (4) recognising the significance of traceability systems in supporting halal authentication. This chapter delves into these challenges, offering insights and recommendations to assist analysts in halal-testing laboratories. It emphasizes adopting suitable instrumental techniques to navigate the complexities inherent in halal authentication procedures, ensuring robust and reliable outcomes.

1 Introduction

Ensuring the integrity of halal products, particularly halal lipids, is paramount in the global market. Authenticating these products is not only adherence to religious dietary laws but also strengthens consumer confidence and safety. This chapter meticulously explores the diverse classes, functions, sources, and applications of lipids in everyday products. It sheds light on the pervasive issue of halal lipid adulteration arising from various lipid sources, including plants, animals, microbes, and synthetics (Devi et al., 2024). The halal status of lipid-based products sourced from each category is critically examined.

Central to this chapter is an in-depth examination of authentication techniques for lipid-based products, primarily through instrumental methods. Despite the availability of numerous authentication techniques, discussions on halal authentication of lipid-based products remain scarce. While instrumental methods such as Fourier transform infrared (FTIR) spectroscopy, gas chromatography (GC), liquid chromatography, and other instrumental techniques have been instrumental in identifying lipids, scant attention has been paid to crucial aspects such as analyte selection, sample preparation and analysis, and data analysis (Sani et al., 2023). Analysts face significant challenges in selecting the most suitable instrumental techniques for lipid-based halal authentication. Hence, this chapter addresses these challenges, offering practical guidance to enhance analytical precision and reliability. Beyond instrumental selection, analysts often overlook the imperative of validating and verifying the chosen analytical methods, hindering the establishment of methods tailored for halal authentication. This chapter underscores the necessity of validating and verifying analytical methods, outlining the ISO 17205 testing and calibration laboratory requirements (Hafis Yuswan et al., 2020)—an aspect often neglected by most authentication techniques.

With technological advancements, halal authentication techniques have evolved to incorporate chemometric techniques into established instrumental methods. While previous works predominantly focused on exploratory chemometric techniques like principal component analysis (Ismail et al., 2021b), many chemometric techniques have emerged for halal authentication of lipid-based products. However, due to limited discourse on selecting chemometric techniques, analysts often struggle to grasp the principles

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and workflow of these techniques (Sani et al., 2024). This chapter bridges this gap by elucidating the principles and seamless flow of a series of chemometric techniques complemented by results from both exploratory and predictive techniques.

A robust traceability system is indispensable to fortify the results of halal authentication. Regrettably, many halal certification bodies (HCBs) neglect to adopt traceability systems in their certifications, merely accepting halal declarations from manufacturers. Consequently, the synergistic integration of halal authentication techniques and traceability systems remains unexplored. This chapter underscores the significance of incorporating a traceability system as a complementary tool to halal authentication. It delves into the challenges and future directions of halal authentication and traceability systems in the halal industry. By incorporating these insights, it is hoped that halal certification bodies will develop comprehensive standards for halal authentication, ultimately enhancing the credibility and effectiveness of the global halal assurance system.

2 Classes, functions, sources and applications of lipids

Lipids are a diverse group of organic molecules that are insoluble in water but soluble in nonpolar solvents such as chloroform or benzene. They play crucial roles in living organisms, including serving as structural components of cell membranes, energy storage, and signalling molecules / agents. Lipids are categorised into five main classes: fatty acids, triglycerides, phospholipids (Haq et al., 2021), steroids, and waxes (Devi et al., 2024). Glycerol and fatty acids are the based structures for most lipids, except steroids. For instance:
- Triglycerides: consist of three fatty acid molecules attached to a glycerol backbone.
- Phospholipids: comprise two fatty acids and a phosphate group attached to glycerol.
- Waxes: are formed by long-chain fatty acids bonded to long-chain alcohols.

Although in the absence of glycerol and fatty acids, steroids are categorised as lipids due to their sharing fundamental characteristics and vital biological functions. This categorisation is based on their hydrophobic properties (insolubility), biological roles, and structural similarities with other lipid molecules.

Table **7.1** also shows the functions of each of the lipid classes, and their structures. For instance, fatty acids, phospholipids and steroids are important components of cell membrane structure. Fatty acids in phospholipids influence membrane fluidity and permeability. In contrast, phospholipids form a lipid bilayer that separates the cell's interior from its external environment, providing structural support and controlling the movement of substances in and out of the cell. Cholesterol, a type of steroid, is an essential component of cell membranes that helps maintain membrane fluidity and stability (Crowley et al., 2022). These lipids also interact in various cellular and physiological processes across different organisms and environments, including cell signalling, hormone regulation, energy storage and metabolism, membrane structure and function maintenance, water repellence, and protection against physical damage and predation.

2.1 Sources of lipids

Lipids are derived from plant, animal, microbial, and synthetic sources (as detailed in Table 7.1).

2.1.1 Plant sources

- Fatty Acids: Found in oils such as olive, coconut, and sunflower oil, which are rich in oleic, lauric, and linoleic acids.
- Triglycerides: Abundant in soybean, palm, and olive oils.
- Phospholipids: Present in soybeans and sunflower seeds.
- Phytosterols: Plant-derived steroid compounds like β-sitosterol, found in oils, nuts, and seeds.
- Waxes: Found on leaves, fruits, and stems, providing protection against water loss and pathogens.

2.1.2 Animal sources

- Fatty Acids: Found in fats and oils such as lard and fish oil, which contain palmitic and stearic acids.
- Triglycerides: Common in animal fats such as lard and tallow.

- Phospholipids: Predominantly found in cell membranes.
- Cholesterol: Concentrated in egg yolks, meat, and dairy.
- Waxes: Produced by animals, such as beeswax used in nest building and body protection.

2.1.3 Microbial sources

Microorganisms synthesize lipids, often via fermentation. For example, Dias et al. (2024) reported producing lipids from *Yarrowia lipolytica* with high unsaturation levels, primarily comprising 48% oleic acid, 20% palmitoleic acid, 17% linoleic acid, and 14% palmitic acid.

2.1.4 Synthetic lipids

Lipids can be synthesized chemically or enzymatically for various applications, including research, industrial processes, pharmaceuticals, and nutraceuticals. Synthetic lipids allow customization to meet specific requirements in biotechnology, healthcare, and nutrition (Ibarguren et al., 2014).

2.2 Applications of lipids

Lipids have diverse applications across industries:
- Healthcare and Pharmaceuticals: Lipids serve as carriers in drug delivery systems and are integral to developing functional foods and nutraceuticals.
- Industrial Uses: They are employed in biofuels, lubricants, and cosmetics.
- Biotechnology: Synthesized lipids enable tailored functions for research and industrial innovation.

In conclusion, lipids are vital biomolecules with extensive structural, functional, and industrial significance. Understanding their classes, sources, and applications provides insight into their critical roles in biological and technological contexts.

Table 7.1: *Classes, functions, sources and applications of lipids*

Lipid class	Definition	Function	Sources	Application
Fatty Acids	These are the building blocks of many lipids. They consist of long hydrocarbon chains with a carboxyl group (-COOH) at one end. Fatty acids can be saturated (no double bonds between carbon atoms) or unsaturated (contain one or more double bonds)	Cell membrane structure: Fatty acids are essential components of phospholipids, which form the lipid bilayer of cell membranes. The composition of fatty acids in phospholipids influences membrane fluidity and permeability. Energy storage: Fatty acids are a primary energy source for many organisms. When broken down through cellular respiration, they yield large amounts of adenosine triphosphate (ATP), the energy currency of cells.	Plant sources: Fatty acids can be obtained from oils such as olive, coconut, and sunflower. These oils are rich in oleic, lauric, and linoleic acids . Animal sources: Fatty acids are also found in animal fats and oils, such as lard and fish oil. Animal fats contain fatty acids like palmitic acid and stearic acid. Microbial sources: Microorganisms can synthesize fatty acids; some fatty acids are produced commercially using microbial fermentation processes. Synthetic sources: Fatty acids can also be synthesized in laboratories through chemical processes for various industrial applications.	Food products: Cooking oils (e.g., olive oil, canola oil, soybean oil); Butter and margarine; Processed foods like cookies, pastries, and fried snacks. Food supplement: Dietary supplements, including omega-3 fatty acids.

Lipid class	Definition	Function	Sources	Application
Triglycerides	Triglycerides are the most common fat in the body and food. They consist of three fatty acid molecules bonded to a glycerol molecule. Triglycerides serve as a primary storage form of energy in adipose tissue.	Energy storage: Triglycerides are highly concentrated energy storage in adipose (fat) tissue. They store excess energy from food intake for later use during fasting or increased energy demand. Insulation: Adipose tissue acts as insulation, helping to maintain body temperature and cushioning vital organs. Protection: Triglycerides stored in adipose tissue protect organs and tissues mechanically.	Plant sources: Triglycerides are abundant in plant oils such as soybean oil, palm oil, and olive oil. Animal sources: Animal fats like those found in lard and tallow are rich sources of triglycerides. Microbial sources: Some microorganisms can accumulate triglycerides as storage compounds. Synthetic sources: Triglycerides can also be synthesized chemically or enzymatically for industrial purposes.	Food products: Cooking oils (e.g., olive oil, canola oil, soybean oil); Dairy products such as butter and cheese; Processed foods like cookies, pastries, and fried snacks. Cosmetic products: Lip balms, lotions, and moisturizers. Energy products: Biofuels.
Phospholipids	Phospholipids are a significant component of cell membranes. They comprise a glycerol molecule, two fatty acid molecules, a phosphate group, and a polar "head" group. Phospholipids have a hydrophilic (water-attracting) head and hydrophobic (water-repelling) tails, allowing them to form the lipid bilayer of cell membranes.	Cell membrane structure: Phospholipids are the primary components of cell membranes. They form a lipid bilayer that separates the cell's interior from its external environment, providing structural support and controlling the movement of substances in and out of the cell. Cell signalling: Some phospholipids act as signalling molecules within cells, playing roles in cell growth, differentiation, and apoptosis (programmed cell death).	Plant sources: Phospholipids are found in plant-based foods like soybeans and sunflower seeds. Animal sources: Animal tissues contain phospholipids, particularly in cell membranes. Microbial sources: Microorganisms like yeast can produce phospholipids. Synthetic sources: Synthetic phospholipids are produced in laboratories for various applications, including drug delivery systems and emulsifiers.	Food products: Dairy products such as butter and cheese, as well as soybeans and soy products (soy lecithin).
Steroids	Steroids are lipids characterised by a carbon skeleton consisting of four fused rings. Cholesterol is a	Cell membrane structure: Cholesterol, a type of steroid, is essential to cell membranes. It helps maintain membrane fluidity and stability.	Plant sources: Some plant species contain phytosterols and plant-derived steroid compounds. For example, β-sitosterol is found in plant oils, nuts, and seeds.	Food products: Vitamin D - Found in fortified dairy products and cereals.

Lipid class	Definition	Function	Sources	Application
	well-known steroid and is a crucial component of cell membranes. Steroid hormones such as estrogen, testosterone, and cortisol are essential signalling molecules.	Hormone regulation: Steroid hormones, derived from cholesterol, serve as chemical messengers that regulate various physiological processes, including metabolism, reproduction, immune response, and stress response. Regulation of metabolic processes: Steroids can modulate enzyme activity and gene expression, influencing metabolic pathways and cellular functions.	Animal sources: Cholesterol is primarily found in animal tissues, particularly egg yolks, meat, and dairy products. Microbial sources: Certain microorganisms can produce steroid compounds. Synthetic sources: Steroid compounds can be synthesised chemically for pharmaceutical purposes.	Pharmaceutical products: Estrogen hormone—Hormonal contraceptives, hormone replacement therapy; testosterone hormone—Anabolic steroids, hormone replacement therapy for males; cortisol hormone—Some anti-inflammatory medications and stress hormones.
Waxes	Waxes are esters of long-chain fatty acids and long-chain alcohols. They are water-repellent and are found in various protective coatings in plants and animals.	Water repellency: Waxes are hydrophobic and form protective coatings on the surfaces of plants and animals, helping to prevent water loss and protect against environmental stresses such as dehydration, UV radiation, and pathogens. Protection: In addition to their water-repellent properties, waxes provide mechanical protection against physical damage and predation.	Plant sources: Plant waxes are found on the surfaces of leaves, fruits, and stems, protecting against water loss and pathogens. Animal sources: Animal waxes, such as beeswax, are produced by certain animals for various purposes, including building nests and protecting their bodies. Microbial sources: Some microorganisms can produce waxes under certain conditions. Synthetic sources: Synthetic waxes are produced industrially for various applications, including cosmetics, coatings, and polishes.	Food products: Coatings on vegetables to retain moisture and protect from pathogens. Cosmetic products: Beeswax - Used in cosmetics, skincare products, and candles. Consumer speciality products: Carnauba wax is used in car waxes, shoe polishes, and dental floss, and animal waxes are used in wool and feathers to repel water and protect against environmental damage.

3 Halal lipids adulteration

Halal lipid adulteration refers to the deliberate or unintentional introduction of non-halal substances into lipid-based products. Types of adulteration include substitution, addition, and contamination, each posing unique risks to consumers and regulatory compliance. Adulterants such as animal fats, non-halal oils (Aziz et al., 2023), and synthetic compounds are frequently used to extend or mimic halal lipids, compromising the authenticity of the final product.

For Muslims, the halal status of lipids obtained from various sources can be a significant and critical concern, particularly when it comes to food and other consumable products regarding their source and processing.

3.1 Animal -based lipids

Lipids obtained from animals raise concerns regarding their halal status. In Islam, the consumption of certain animals is permissible (halal), while others are considered prohibited (*haram*). Additionally, the method of slaughter (*dhabiha*) must adhere to Islamic guidelines, including the recitation of the name of Allah at the time of slaughter and the use of a sharp instrument to sever the major blood vessels in the neck swiftly. Therefore, lipids derived from animals that are not slaughtered according to Islamic principles may be considered *haram (non-halal)*.

To summarize, the Islamic principles for slaughter(*dhabiha*) require:

The recitation of the name of Allah during slaughter.

The use of a sharp instrument to swiftly sever the major blood vessels in the neck.

Lipids obtained from animals not slaughtered according to these guidelines, or from prohibited animals, are considered *haram* (*non-halal*).

3.2 Pant-based lipids

Lipids obtained from plants are generally considered halal, as there are no specific restrictions on consuming plant-based products in Islamic dietary laws. However, the extraction and refinement processes may involve additives or processing agents that must also need to be checked for halal compliance (Idris et al., 2022).

3.3 Microbial lipids

Lipids produced through microbial fermentation, such as certain oils or fatty acids, are typically considered halal. As long as the microorganisms used in the fermentation process are halal and the production process does not involve haram ingredients or processes (Abdullah Sani et al., 2020), lipids obtained from microbial sources are generally acceptable for consumption.

3.4 Synthetic lipids

Synthetic lipids, chemically synthesized rather than extracted from natural sources, are subject to halal considerations based on the origin and nature of the raw materials and processes involved. Synthetic lipids may be considered halal if the raw materials and manufacturing processes comply with halal requirements and do not involve any haram substances.

3.5 Implications of halal lipid adulteration

Adulteration of halal lipids has significant consequences:

Health Risks: The use of non-halal or substandard adulterants can pose safety concerns.

Religious Violations: Such adulteration breaches Islamic dietary laws, directly impacting Muslim consumers.

Loss of Trust: Adulteration undermines consumer confidence in halal-certified products.

Certification Challenges: It complicates the integrity of halal certification systems and regulatory enforcement.

Global Supply Chain Complexities: Monitoring and ensuring compliance is increasingly difficult due to the intricate and international nature of supply chains.

In conclusion, addressing halal lipid adulteration requires robust regulatory frameworks, transparent certification systems, and rigorous compliance monitoring to ensure authenticity, maintain consumer trust, and uphold religious values.

4 Authentication techniques for lipid-based products

Authentication techniques enable the identification and quantification of critical components in lipid-based products. These techniques range from stand-alone instrumental techniques to the incorporation of instrumental and chemometric approaches. Since the demands for halal products have expanded beyond food items, developing analytical methods (AMs) through instrumental techniques has not solely focused on DNA testing. Techniques such as high-performance liquid chromatography (HPLC) and gas

chromatography-mass spectrometry (GC/MS) have demonstrated efficacy in halal testing (Azilawati et al., 2014; Azir et al., 2017; Ismail et al., 2021a). While other instrumental methods like Fourier Transform Infrared (FTIR) spectroscopy (Hashim et al., 2010) and Differential Scanning Calorimetry (DSC) (Naquiah et al., 2017) have also been explored for their potential in authentication. A standardized guideline for selecting appropriate approaches and AMs within instrumental techniques is lacking. This chapter proposes methodologies and approaches for lipid-based products that target and profile lipids for authentication. The critical points of this approach are summarized in
Table **7.2**. Sani et al. (2023) also discussed alternative approaches, such as protein-based and alcohol testing, in their previous study.

Table 7.2: *Approaches of instrumental techniques*

Approach type	Analytical method of instrumental techniques	Principle of sample analysis
Targetted	Fatty acid methyl esters	The sample undergoes extraction and derivatisation to produce fatty acid methyl esters (FAMEs), subject to the double separation of the FAMEs via GC x GC-TOF/MS (Indrasti et al., 2010).
Profiling	Functional and fingerprinting groups[1]	The analytical process commences with the extraction of samples using nonpolar solvent, followed by their examination using Fourier transform infrared (FTIR) spectroscopy. This analysis primarily targets identifying functional groups and generating spectral fingerprints (Zulkarnail et al., 2021), aiding in the characterization and differentiation of compounds within the samples.
Profiling	Thermal properties[1]	The sample analysis involves quantifying the thermal characteristics of a sample extract, whereby the resulting thermogram is juxtaposed with that of a porcine extract for comparison purposes (Mualim et al., 2018).
Profiling	Fatty acid methyl esters[1]	The sample is subjected to extraction and derivatization processes to FAMEs, which are then analyzed using GC/MS (Idris et al., 2021)
Profiling	Triacylglycerol	The sample undergoes the separation of triacylglycerols via high-performance liquid chromatography (HPLC) equipped with a refractive index detector (RID).

Note: [1]Result from this instrumental technique can be subjected to chemometric techniques to facilitate the halal authentication.

4.1 Instrumental techniques

4.1.1 Profiling of functional and fingerprinting groups via Fourier transform infrared (FTIR) spectroscopy

A widely utilized and highly sought-after instrumental technique for the authentication of oil-based samples is Fourier Transform Infrared (FTIR) Spectroscopy, which has been applied extensively across various industries. For instance, FTIR has been employed in the authentication of butter (Nurrulhidayah et al., 2013), plant-derived products (Yang & Irudayaraj, 2001), as well as cosmetics such as moisturizers and lotions (Rohman, Gupitasari, Purwanto, Triyana, Rosman, Shuhel Ahmad, et al., 2014). The extraction of these samples is typically carried out using standardized methodologies such as Soxhlet extraction, maceration, or other extraction techniques employing nonpolar solvents such as hexane, dichloromethane,

ethyl acetate, and diethyl ether. Following extraction, the nonpolar solvents are removed using a rotary evaporator at 40°C, and the resulting extracts are stored at -20°C before undergoing FTIR analysis.

FTIR operates by emitting infrared light at various wavelengths to interact with solid, liquid, or gaseous samples, thereby capturing the infrared spectrum corresponding to the absorption activities of sample molecules. These molecules absorb infrared radiation, leading to characteristic stretching and wagging vibrations, while the unabsorbed infrared is transmitted to the spectrometer for detection and recording. Numerous oil samples exhibit infrared-active properties, so they yield distinct spectra when subjected to FTIR analysis, facilitating their authentication as halal sources.

The measurement of absorbed infrared in oil samples typically involves first recording a blank spectrum using FTIR equipped with attenuated total reflection (ATR), followed by measuring the extracted oil sample. The actual spectrum is obtained by subtracting the recorded oil spectrum from the blank. While infrared radiation can be categorized into near (12500 - 4000 cm^{-1}), mid (4000 - 650 cm^{-1}), and far-infrared (650 - 200 cm^{-1}) ranges, mid-infrared spectra are of particular interest for halal authentication purposes.

FTIR spectra encompass two main wavenumber ranges: the functional range (4000 – 1700 cm^{-1}) and the fingerprinting range (1700 – 650 cm^{-1}). Analysts typically subject the recorded spectra to Multivariate Data Analysis (MDA), which involves analyzing the entire wavenumber range (4000 - 650 cm^{-1}), the functional range (4000 – 1700 cm^{-1}), or the fingerprinting range (1700 – 650 cm^{-1}) to identify clusters of oil sources and significant wavenumbers associated with each source. Notably, the fingerprinting range (1700 – 650 cm^{-1}) is often utilized to discriminate between halal and non-halal sources. For instance, the detection of lard adulteration in vegetable oils has been achieved through partial least square and discriminant tests on spectra ranging from 1500 – 1000 cm^{-1} (Rohman et al., 2011), while the authentication of lard in cosmetic cream has been conducted using spectra ranging from 1200 – 1000 cm^{-1} (Rohman, Gupitasari, Purwanto, Triyana, Rosman, Ahmad, et al., 2014). Analysts may leverage built-in spectral libraries to identify the functional groups corresponding to significant wavenumbers, further enhancing the accuracy and reliability of FTIR analysis for halal authentication purposes. Based on the result of discriminant analysis (DA), Aziz et al. (2023) found that palm oil slightly differed from that of lard at the wavenumber range of 1453 cm^{-1} and 1415 cm^{-1} which corresponds to the bending vibrations of CH_2 and CH_3 aliphatic groups and OH carboxyl group, respectively.

4.1.2 Targeted fatty acid methyl esters via two-dimensional gas chromatography time-of-flight mass spectrometer

Oils are composed predominantly of fatty acids in both free and ester forms (Sani, Bakar, et al., 2020). Fatty acid methyl ester (FAME) profiling offers an alternative analytical approach for authenticating oil samples compared to targeted DNA methods. The presence of other constituents in oil samples often poses inhibitory effects when employing targeted DNA methods (Sani et al., 2021). Therefore, analysts identify targeted FAMEs using a two-dimensional gas chromatography time-of-flight mass spectrometer (GC x GC-TOF/MS). Unlike the spectral profiling of oils using FTIR, GC x GC-TOF/MS and gas chromatography-mass spectrometer (GC/MS) analyses of FAMEs are preferred due to their ability to identify specific FAMEs crucial for distinguishing between halal and non-halal sources.

Following a similar extraction method employed in FTIR analysis, the extracted oil undergoes esterification with 1 M sodium methoxide and hexane to yield FAMEs. The resulting nonpolar phase (upper layer) containing FAMEs is then injected into FAMEs separation by two different columns (SLB-5ms and DB-wax), allowing the discrimination of lard from other animal fats by three FAMEs. Indrasti et al. (2010) successfully identified targeted FAME biomarkers using a two-dimensional gas chromatography time-of-flight mass spectrometer (GC x GC-TOF/MS) without chemometric techniques. This study revealed a group of FAMEs, including constituents of methyl trans-9,12,15-octadecatrienoate (C18:3 n3t), methyl 11,14,17eicosatrienoate (C20:3 n3t) and methyl 11,14-eicosadienoate (C20:2 n6) that discriminated lard from goat, cattle, and chicken fats.

4.1.3 Profiling of fatty acid methyl esters via gas chromatography-mass spectrometer

The sample extraction, esterification and analysis for profiling the FAMEs via gas chromatography-mass spectrometer (GC/MS) follows a similar procedure for targeted FAMEs via GC x GC-TOF/MS, except that the separation of FAMEs via GC/MS occurs once prior to detection by the mass spectrometer. The FAMEs separation via GC/MS and an internal standard such as methyl tridecanoate (C13:0) or methyl heptadecanoate (C17:0) to mitigate sample matrix effects. The GC/MS analysis involves separating FAMEs using a polar-capillary (88%-cyanopropyl)aryl-polysiloxane (HP 88) column measuring 100 m in length, 0.25 mm in internal diameter, and 0.2 μm particle size. The retention time and mass spectra of separated FAMEs are validated against certified reference standards (Idris et al., 2021). Analysts quantify the concentration of each FAME by constructing a calibration curve with an R^2 value exceeding 0.98. Then, the analysts analyse various types of oils exceeding 30 replications for each oil type prior to subjecting the dataset to chemometric techniques.

Idris et al. (2021) employed principal component analysis (PCA), orthogonal partial least squares-discriminant analysis (OPLS-DA), and orthogonal partial least squares-regression (OPLS-R) on the FAME profile to identify discriminant FAMEs in palm oil and lard-containing fish feed. These discriminant FAMEs include methyl myristate (C14:0), methyl stearate (C18:0), methyl linoleate (C18:2), methyl linolenate (C18:3), methyl arachidate (C20:0), and second stereospecific (sn-2) of C16:0, C18:0, methyl oleate (C18:1), and methyl linoleate (C18:2).

4.1.4 Profiling of triacylglycerol via high-performance liquid chromatography (HPLC)

The lipid samples are ground and sieved at uniform size to ensure homogeneity and subjected to nonpolar solvent extraction at 60°C for 8 h in the Soxhlet apparatus. The extract is concentrated using a rotary vacuum evaporator at 40°C and frozen at -20°C in an amber glass container.

Analysts dissolved the extract in chloroform and separated the triacylglycerol via high-performance liquid chromatography (HPLC) equipped with a refractive index detector (RID). Prior to the separation of triacylglycerol, analysts inject 10 μL of triacylglycerol standards into the HPLC-RID and elute the extract with the flow of a pre-filtered acetone: acetonitrile eluent mixture at a 1.5 mL/min flow rate. The triacylglycerol standards separation occurs in the 12.5 cm × 4 mm i.d. Lichrosphere RP-18 column at 30°C, and the separated triacylglycerol elutes to RID. Yanty et al. (2017) separated and detected mixture of 19 triacylglycerol standards containing 1,3-distearoyl-2-oleoyl (SOS), 1-stearoyl-2-palmitoyl-3-oleoylrac-glycerol (SPO), dipalmitoyl-3-stearoyl glycerol (PPS), dilinoleoyl-3-linolenileoyl glycerol (LLLn), dilinoleoyl-1-oleoyl glycerol (OLL), dilinoleoyl-1-palmitoyl glycerol (PLL), dioleoyl-3-linoleoyl glycerol (OOL), dipalmitoyl-1-linoleoyl glycerol (PPL), dioleoyl-1-palmitoyl glycerol (POO), dipalmitoyl-3-oleoyl glycerol (PPO), dioleoyl-1-stearoyl glycerol (SOO), myristoyl-palmitoyl-linoleoyl glycerol (MPL), palmitoyl-oleoyl-linoleoyl glycerol (POL), palmitoyl-stearoyl-oleoyl glycerol (PSO), tristearoyl glycerol (SSS), trilinoleoyl glycerol (LLL), trimyristoyl glycerol (MMM), trioleoyl glycerol (OOO) and tripalmitoyl glycerol (PPP).

Following a similar setting, analysts injected the extract into the HPLC-RID. They confirmed the presence of triacylglycerols by comparing the retention time of triacylglycerol in the extract with the triacylglycerol standards. Idris et al. (2022) analysed lard and palm oil triacylglycerols in up to 45 replicates and subjected the dataset to chemometric techniques for authentication.

4.1.5 Profiling of thermal properties via differential scanning calorimetry

Analysts weigh 4–8 mg lipid extracts, hermetically seal them in the pan and analyze them at continual temperature settings to obtain thermal properties of heating and cooling activities. The cooling activity is carried out under the ~20 mL/min flow rate of 99.999% nitrogen purity. The DSC analysis produces a thermogram. Before the analysis, analysts measure heating and cooling activities for an empty hermetically sealed aluminium pan as the reference (Nina Naquiah et al., 2017). The thermogram is converted to comma-

separated values (CSV) format and subjected to chemometrics techniques, i.e. principal component analysis, discriminant analysis and partial least square regression (Idris et al., 2022).

4.2 Validating and verification of analytical method for authentication of lipid-based products

After selecting the most suitable approach among the available instrumental techniques, analysts must validate and verify the analytical method (AM) to ensure its fitness for purpose before issuing the testing report. This validation and verification process involves several steps: (1) understanding the rationale behind validating and verifying the AM, (2) identifying the specific requirements for validation and verification, and (3) preparing the procedure for conducting the validation and verification process (Sani et al., 2023).

Analysts are responsible for determining the performance characteristics (PC) of the AM, as outlined by the Department of Standards Malaysia (2018). These characteristics encompass specificity, calibration linearity, trueness, precision, sensitivity, ruggedness, accuracy, limit of detection (LOD), and limit of quantitation (LOQ), all of which must align with the nature and purpose of the AM. Typically, AMs are validated and verified following the rules and regulations stipulated by governmental bodies.

Within halal certification, adopting the halal authentication techniques is contingent upon the procedures established by the certification body (CB). For instance, the Central Islamic Committee of Thailand (CICOT), the halal CB in Thailand, mandates halal testing as a prerequisite for halal certification applications. These applications are then evaluation by Islamic scholars (Nawawi et al., 2017). The AMs intended for use in halal certification applications submitted to CICOT must undergo thorough comperhensive validation and verification to meet the CB's requirements (Sani, Jamaludin, et al., 2020). In contrast, halal testing for certification applications is not compulsory since the Department of Islamic Development Malaysia (JAKIM) accepts the halal declaration.

4.3 Incorporation of chemometric with instrumental techniques

The previous sub-topic discussed the approach types for authentication of lipid-based products, including targeted and profiling approaches. Unfortunately, the latter approach could not serve authentication without incorporating chemometric techniques. Most instrumental techniques predominantly utilize chemometric techniques for research purposes, often without investigating the sources of halal products using validated and verified analytical methods (AMs). Commonly employed software for carrying out chemometric analyses include XLSTAT (Sani et al., 2024), SIMCA (Idris et al., 2021), and Unscrambler X (Azilawati et al., 2015), among others. Instrumental techniques heavily rely on the DNA testing method due to several factors (advantages): the specificity of DNA testing method towards porcine, bovine, and fish DNAs (Sultana et al., 2018), the DNA stability as affected by pressure and heat treatments (Lubis et al., 2017), the ability of DNA testing to detect porcine DNA in both raw and processed meat (Abdullah Amqizal et al., 2017). Given these advantages, the DNA testing method does not necessarily require additional support from chemometric techniques for halal authentication, provided that the DNA methods meet various acceptability ranges for method validation performance criteria (U.S. Food and Drug Administration, 2020). Consequently, other analyte measurements and chemometric techniques tend to receive less attention.

As the demand for halal products extends beyond food, alternative AMs focusing on analytes, such as fatty acid methyl esters (FAMEs), triacylglycerols, thermal activities, etc., have demonstrated promising results. Analysts develop new AMs to offer alternative methods when the DNA testing method fails to detect porcine DNA in samples containing high oil content (Costa et al., 2012) or in the presence of inhibitors such as polyphenols, polysaccharides, metal ions, and detergents (Kim et al., 2018).

Instrumental techniques integrate chemometric techniques to simultaneously analyze analytes across various sources, including animals, plants, bacteria, etc., which exhibit distinct distributions of these analytes. Commonly measured analytes include infrared-active functional groups (Aziz et al., 2023) and FAMEs (Azir et al., 2017), among others, which are subjected to chemometric techniques such as principal component analysis, cluster analysis, and discriminant analysis. To mitigate the risk of false-negative or false-positive results, the application of chemometric techniques should adhere to specific requirements:

the AM must be validated and verified using actual samples, the dataset must be sufficiently diverse, the collected dataset should undergo preprocessing steps, including evaluation of missing data, outlier removal, and fulfilment of chemometric technique assumptions prior to their application (Sani et al., 2023).

4.3.1 Collecting adequate dataset for multivariate data analysis

Samples from various lipids origins, such as fish, bovine, and lard, undergo analysis with repeated measures. While a conventional guideline suggests collecting a minimum of 30 data points to ensure adequate representation of the population, it is advisable to increase the number of repetitions and subsequently conduct a sampling adequacy test, such as the Kaiser-Meyer-Olkin (KMO) test, at a significance level (α) of 0.01. The determined KMO value is categorized as follows: KMO < 0.5 = inadequate, 0.5 < KMO < 0.7 = mediocre, 0.7 < KMO < 0.8 = good, 0.8 < KMO < 0.9 = very good, and KMO > 0.9 = excellent, with only KMO > 0.5 considered adequate for the application of chemometric techniques (Sani, Yuswan, et al., 2020b). However, attaining a KMO value exceeding 0.9 ensures the adequacy of the dataset, particularly when outliers are identified and removed before applying chemometric techniques.

4.3.2 Data preprocessing

Before applying chemometric techniques, the dataset undergoes essential preprocessing steps to ensure the integrity of the data, prevent the loss or neglect of critical information, and discern between sample sources based on biomarker variability. These objectives are achieved by identifying missing data, detecting and eliminating outliers, and fulfilling assumptions underlying the chemometric techniques (Komsta et al., 2018). Analysts address missing data and outliers while ensuring adherence to chemometric technique assumptions.

Missing data typically arises from undetected variables or variables falling below the limit of detection (LOD). A common practice involves converting undetected values to zero and values below the LOD to 0.5LOD or 0.75LOD. However, converting LOD values to 0.65LOD is recommended to minimize distortions when applying chemometric techniques (Palarea-Albaladejo & Martín-Fernández, 2013).

Prior to employing chemometric techniques, the dataset undergoes outlier detection and removal. Outliers are defined as variable values significantly deviating from the majority of values. Multivariate outlier detection involves evaluating distances among data values using Mahalanobis Distance Measurement (MDM). MDM measures the distance of each sample source's values from the mean centre, identifying values further away as potential outliers. Additionally, the MDM/df value indicates potential outliers, with df representing the number of variables exceeding 2.5. Confirmation of outliers is achieved through box and whisker plots Grubbs or Dixon tests at a significance level (α) of 0.01 (Saiful et al., 2019).

Following outlier identification and removal, the dataset undergoes scrutiny to ensure adherence to testing assumptions, including normalization, homoscedasticity, and linearity (Rani et al., 2019). Normality testing, such as the Shapiro-Wilks test at α of 0.01, is performed to identify non-normal biomarker values, which are transformed to achieve normal distribution. Homoscedasticity, ensuring equal variance dispersion within sample sources, is assessed using the Levene test at α of 0.01. In cases of non-compliance, appropriate transformations are applied. Linearity is evaluated by ensuring the model's R-value approximates 1 or transforming all biomarkers. Various transformation methods, including standardize n-1, standardize (n), centre, standard deviation-1 (n-1), standard deviation-1 (n), rescale from 0 to 1, rescale from 0 to 100, Pareto, and log transformation, are applicable to achieve compliance with chemometric techniques (Ismail et al., 2021b).

4.3.3 Exploring the correlations among variables and lipid-based samples via principal component analysis (PCA)

Integrating chemometric techniques for authenticating halal products has significantly advanced research in this domain. Techniques such as principal component analysis (PCA) have provided detailed insights into

significant variables relevant to halal authentication studies, established correlations, and identified key variables contributing significantly to sample sources (Abdullah Sani et al., 2021a).

To achieve these objectives, analysts conduct PCA, analysts select an optimal number of principal components (PC) with cumulative variability exceeding 75%, assess correlations among variables, delineate clusters of sample sources, and identify variables with high factor loadings (FL > 0.75) that significantly influence these clusters. A high cumulative variability indicates that the PCA explains a substantial data variability. After identifying significant variables, it is advisable to ensure that the new dataset comprising only these variables exhibits a Kaiser-Meyer-Olkin (KMO) > 0.5. Otherwise, additional data collection is warranted to meet this criterion before proceeding with further dataset analyses. The vector direction of the variables indicates their correlation in the variable plot. In this example, FAMEs are the variables analyzed using GC/MS.

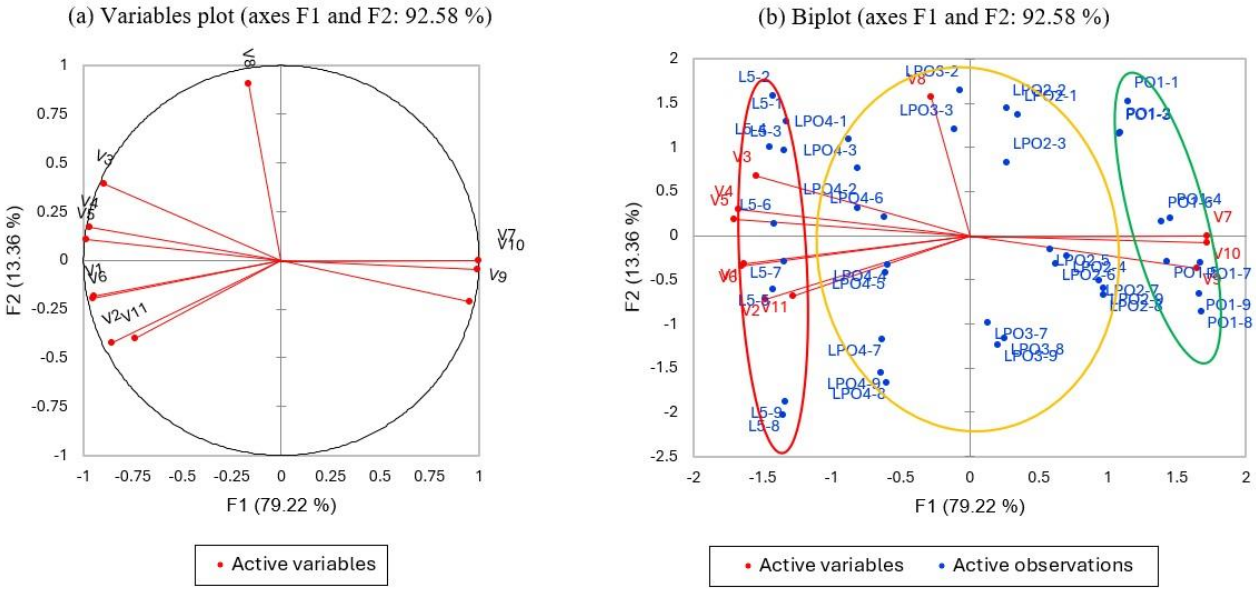

Figure 7.1: (a) Variable plot of fatty acid methyl esters (FAMEs: V1 – V12) and (b) biplot of FAMEs (V1 -V12) and lard (L), palm oil (PO) and lard-palm oil mixture (LPO) clusters.

Figure **7.1** (a) shows the correlation among the FAMEs (V1 – V12). For instance, V7, V9 and V10 have positive correlation due to their similar vector direction; V4 and V5 are negatively correlated against V9 due to their opposite vector directions; and V1 and V6 do not have any correlations with V8 since they make almost 90◦ vector directions. Of these FAMEs, all FAMEs except V11 have FL > 0.75, indicating that all FAMEs are significant in this study. Figure **7.1** (b) demonstrates three clusters, including lard only on the left, a mixture of lard and palm oil in the middle and palm oil only on the right. The FAMEs V7, V9, and V10; V1 – V6 and V11; and V8 dominate in lard only, lard-palm oil mixture and palm oil clusters, respectively. Also, the lard cluster has the lowest V7, V9 and V10, while palm oil has the lowest V1 – V6 and V11.

4.3.4 Validating principal component analysis (PCA) result via cluster analysis

Validation of the groupings developed through PCA is essential and can be achieved through cluster analysis (CA). The CA assesses the similarity or dissimilarity between sample sources by employing an agglomeration criterion, which clusters similar sample sources and constructs a dendrogram. The dendrogram visually represents the clusters by drawing a dotted horizontal line across the vertical axis, indicating several clusters through intercepts between the dotted line and the dendrogram's vertical axis (Sani, Yuswan, et al., 2020a). Figure depicts three intercepts that signify three distinct clusters of sample sources. Clusters 1, 2 and 3 are groupings of palm oil only, lard-palm oil and lard only clusters. For instance, in the context of halal authentication, the CA confirms the clusters identified by PCA.

The CA also shows the profile plot for the dendrogram to indicate which FAME variables are dominant in each cluster. Figure (b) shows that cluster 1 has the highest FAMEs - V7, V9 and V10 and the lowest V1 - V6; cluster 2 has a moderate level of all FAMEs except V8, which has the highest amount; and cluster 3 has the highest V1 – V6 and the lowest V7, V9 and V10. Analysts compare the dendrogram and profile plot from the CA with the variable plot and biplot from PCA and confirm the CA validation on the PCA result.

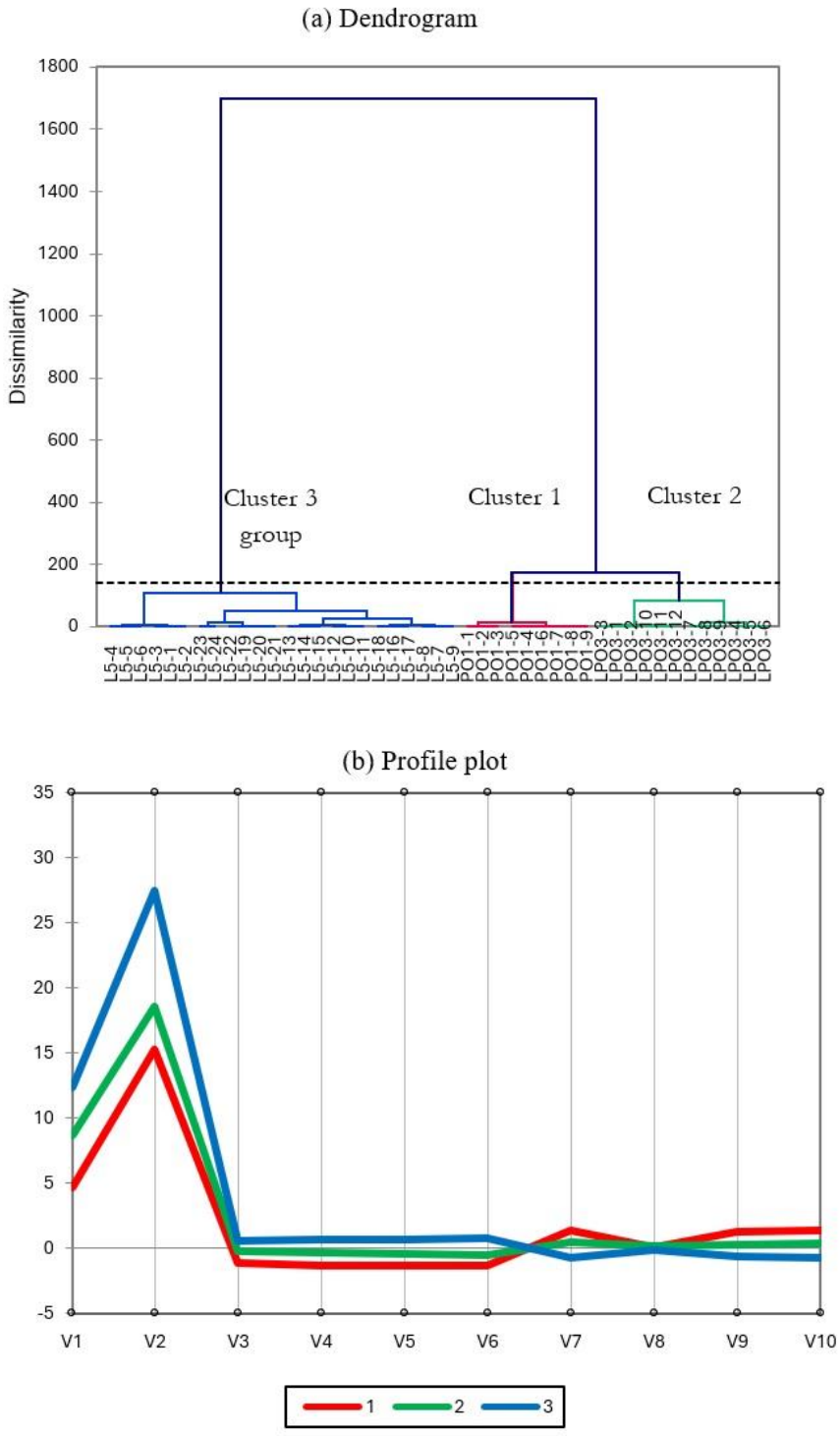

Figure 7.2: (a) Dendrogram of three clusters of sample source and (b) profile plot for each cluster

4.3.5 Authenticate lipid-based sources via discriminant analysis

Discriminant analysis (DA) is both an explanatory and predictive technique that verifies the validated PCA (Principal Component Analysis) clusters thriugh CA (Cluster Analysis), identifies and/or confirms the significant biomarkers contributing to these clusters, and authenticates unknown samples (Zulkarnail et al., 2020). The set-up of the dataset for DA requires structured approach, involving separation of data into training, validation, verification and testing datasets. These subsets should comprise the significant variables determined/identified in PCA and validated by CA (Table 7.3). To maximize classification accuracy, the dataset derived from PCA and CA is supplemented with validation and verification subsets. Using the newly established dataset from PCA and CA, along with validation and verification datasets comprising sample numbers of at least 20% of the training dataset, the analyst aims to achieve 100% correct classification of the sample source clusters. The verification dataset, in particular, is based on analysing actual samples containing the identified variables. For instance, to authenticate the halal status of cooking oil, the analyst shall analyze fatty acid methyl esters (FAMEs) in replicates of actual cooking oil using GC-MS and collect this dataset as a verification dataset. Nevertheless, achieving 100% correct classification in the verification dataset is challenging due to the matrix effect in actual (real-world) samples. For example, Abdullah Sani et al. (2021b) achieved 98% correct classification, while Sani et al. (2024) achieved 93% accuracy in classification when employing the discriminant analysis on their verification dataset.

Subsequently, analysts apply a Fisher distances test at a significance level ($\alpha = 0.01$) to assess the differences between the clusters in the training dataset. A p-value below 0.01 indicates statistically distinct clusters, thus confirming the significant variables contributing to these clusters. The results of the Fisher distances test enhance the discriminant model's reliability for authenticating unknown samples. Consequently, DA successfully develops a discriminant model to authenticate the unknown samples. Table 7.3 shows the results of the authentication of unknown samples by the discriminant model. Using this validated discriminant model, unknown samples can be authenticated and classified based on their dependent variables or clusters from the training dataset. For example, the results may indicate that an unknown sample is palm oil, lard, or a lard-palm oil mixture.

Table 7.3: *Classification matrix of training, validation, verification and testing datasets by discriminant analysis*

Dataset	Correct classification, %	Number of samples and p-values of Fisher distance[1,2]			Total samples
		Palm oil	Lard	Lard-palm oil	
Training dataset					
Palm oil	100.00	40 (1)	0 (< 0.0001)	0 (< 0.0001)	40
Lard	100.00	0 (< 0.0001)	40 (1)	0 (< 0.0001)	40
Lard-palm oil	100.00	0 (< 0.0001)	0 (< 0.0001)	40 (1)	40
Total	100.00				120
Validation dataset					
Palm oil	100.00	20 (1)	0 (< 0.0001)	0 (< 0.0001)	20
Lard	100.00	0 (< 0.0001)	20 (1)	0 (< 0.0001)	20
Lard-palm oil	100.00	0 (< 0.0001)	0 (< 0.0001)	20 (1)	20
Total	100.00				60
Verification dataset (e.g. actual cooking oil)					
Palm oil	100.00	20 (1)	0 (< 0.0001)	0 (< 0.0001)	20
Lard	100.00	0 (< 0.0001)	20 (1)	0 (< 0.0001)	20
Lard-palm oil	100.00	0 (< 0.0001)	0 (< 0.0001)	20 (1)	20
Total	100.00				60
Testing dataset					
Unknown 1		Palm oil			
Unknown 2			Lard		
Unknown 3				Lard-palm oil	

Note: [1]Ten (V1 – V10) out of 11 FAMEs are confirmed as the significant variables ($p < 0.01$).
[2]Calculated p-value of Fisher distance < 0.01 indicated that the three clusters are significantly different.

4.3.6 Quantification of non-halal contaminant in the lipid-based sample via regression analysis.

Following the authentication result by the DA (Discriminant Analysis), samples suspected of lard adulteration undergo regression analysis (RA) for further scrutiny. The RA, including partial least squares regression (PLSR), principal component regression (PCR), orthogonal partial least squares-discriminant analysis (OPLS-DA), multiple linear regression (MLR), and others, are utilized to construct regression models for determining the level of adulteration in unknown samples. This stage necessitates the availability of separate training, validation, verification, and testing datasets comprising only the significant variables identified in PCA, validated by CA and verified by DA.

The training and validation datasets include sample source clusters with predetermined percentages of non-halal adulteration, e.g. lard. In contrast, the verification dataset comprises known non-halal adulterant concentration prepared in the sample. For instance, the verification dataset of lard-adulterated palm oil entails the preparation of a known percentage of lard in the palm oil. Abdullah Sani et al. (2021b) work, although not related to lipid-based products, prepared 0.01%, 0.03%, 0.05%, 0.07% and 0.1% of porcine gelatine (adulterant) in marshmallows and kept this dataset as verification dataset. The testing dataset, however, comprises unknown samples. Before proceeding with RA, these datasets must meet the KMO > 0.5 requirement. Once this criterion is satisfied, analysts conduct the RA at a significance level (α) of 0.01 (Sani et al., 2024).

In cases where multiple types of regression analyses are performed, such as establishing and comparing the effectiveness of PLSR, PCR, OPLS-DA, MLR, etc models, the determination coefficient (R^2), mean square error (MSE), and root mean square error (RMSE) of each RA model shall be compared. The RA model is selected, which exhibits the highest R^2 value closest to 1, along with the lowest MSE and RMSE. These criteria indicate that the chosen model has the lowest probability ($\leq$ 1%) of incorrectly determining the level of adulteration. Additionally, the independent sample t-test value of the predicted and actual adulteration levels is evaluated to aid in selecting the optimal regression model. A p-value > 0.01 for the independent sample t-test signifies acceptance of the null hypothesis, indicating no significant difference between the predicted and actual adulteration levels. This result validates the effectiveness of the selected regression model as a reliable tool for halal authentication. Table 7.4 shows the RA model's effectiveness in determining the lard adulteration in palm oil using FAMEs as variables. As the p-value of the independent sample t-test between the prepared lard adulterant and the prediction of lard adulterant is more than 0.01, the predicted lard adulterant is not significantly different from the prepared lard adulterant in palm oil. This result indicates that the RA model could be the quantification tool in determining the percentage of lard adulterant in palm oil.

Table 7.4: *Effectiveness of regression analysis model in determining the lard adulteration in palm oil in the validation dataset*

Sample	Prepared lard adulterant, %	Prediction of lard adulterant, %	p-value of independent sample t-test value
PO1 – PO5	0%	0.03 ± 1.54	0.9596
LP01 – LP05	25%	25.29 ± 1.39	0.6681
LP06 – LP10	50%	49.56 ± 0.72	0.2455
LP11 – LP15	75%	75.13 ± 0.82	0.7359
L01 – L05	100%	99.98 ± 1.55	0.9802

5 Importance of traceability system

Although this chapter outlines the approaches of instrumental technique incorporated with the chemometric techniques for halal authentication, the traceability systems that track the movement of ingredients and products throughout the supply chain, from production to consumption, are essential in supporting the laboratory testing report. The critical traceability components include documentation,

labelling, and data management systems. Ingredients or products identified as adulterated shall be examined by comparing them with the traceability system on the premises. International certification bodies (CBs) have adopted this practice to cross-check the possibility of deliberate adulteration or unavoidable cross-contamination while handling the ingredients or products. The CB will revoke the premise's halal certification if the former incident occurs. Hence, it is imperative to establish a traceability system.

Keeping documents related to procurement, production, storage and delivery within the supply chain holds a traceability chain that enhances transparency and accountability, enabling stakeholders to promptly identify and address instances of adulteration (Jabatan Kemajuan Islam Malaysia, 2020). The traceability system is part and parcel of the procedure for product recall in the occurrence of adulteration. Traceability systems bolster consumer confidence and facilitate regulatory compliance by providing a comprehensive audit trail. Despite the benefits, implementing effective traceability systems faces challenges such as data standardization, interoperability, and cost. Moreover, the complexity of global supply chains necessitates collaboration across industry sectors and regulatory agencies, which has become a challenge in the halal industries.

6 Challenges and future directions

The progression of advanced instrumental techniques encounters persistent challenges in developing new and effective and reliable analytical methods (AMs). While the validation of AMs has often met performance characteristic (PC) requirements, many struggle to ascertain the halal status of actual samples or products due to the complexity of sample matrices and analyte denaturation resulting from sample heat treatment. Analyzing diverse actual samples is recommended to ensure the applicability of validated AMs and to enhance the limit of quantification (LOQ) to differentiate between the blank baseline and actual samples. Another significant challenge is the potential risk for cross-contamination within the supply chain of actual samples can contribute to false-positive results. Halal testing should be conducted at the manufacturing site rather than retail stores to address this concern. However, the voluntary nature of halal testing, as determined by halal certification bodies (CBs), can impede the development, validation, and verification of new AMs for instrumental techniques. For example, in Malaysia, JAKIM accepts/allows halal certification applications without requiring halal testing due to its built-in halal assurance system, leading to a decline in the development of new AMs using instrumental techniques. Conversely, the Central Islamic Committee of Thailand (CICOT) in Thailand has made halal testing and traceability evidence mandatory for halal certification, resulting in an increase in new AMs at the Thailand Halal Science Centre to support manufacturers' halal declarations (Sani et al., 2023).

Despite these challenges, advancements in spectroscopy, chromatography, and sensor technologies offer promising avenues for improving the speed and accuracy of authenticity testing. Integrating blockchain-based systems and Internet of Things (IoT) devices enables real-time tracking and verification of halal lipids, thereby reducing the risk of adulteration throughout the supply chain. It is anticipated that these state-of-the-art technologies are anticipated to play a pivotal role in addressing adulteration issues in lipids and lipid-based products, paving the way for more reliable and efficient halal authentication systems.

7 Conclusion

The integrity of halal lipids is critical for ensuring compliance with religious dietary laws, protecting consumer health, and maintaining market trust. The challenge of adulteration of lipid-based products could be addressed by establishing accurate authentication techniques, including validated and verified analytical methods of instrumental techniques combined with chemometric techniques. Also, by implementing robust traceability systems and leveraging advanced authentication techniques, stakeholders can significantly mitigate the risks of adulteration and uphold the authenticity and reliability of halal products.

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9 Conflict of interest statement

We declare no conflict of interest.

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