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Review

Analytical approaches for the determination of adulterated animal fats and vegetable oils in food and non-food samples

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ABSTRACT

Edible oils and fats are crucial components of everyday cooking and the production of food products, but their purity has been a major issue for a long time. High-quality edible oils are contaminated with low- and cheap-quality edible oils to increase profits. The adulteration of edible oils and fats also produces many health risks. Detection of main and minor components can identify adulterations using various techniques, such as GC, HPLC, TLC, FTIR, NIR, NMR, direct mass spectrometry, PCR, *E*-Nose, and DSC. Each detection technique has its advantages and disadvantages. For example, chromatography offers high precision but requires extensive sample preparation, while spectroscopy is rapid and non-destructive but may lack resolution. Direct mass spectrometry is faster and simpler than chromatography-based MS, eliminating complex preparation steps. DNA-based oil authentication is effective but hindered by laborious extraction processes. *E*-Nose only distinguishes odours, and DSC directly studies lipid thermal properties without derivatization or solvents. Mass spectrometry-based techniques, particularly GC–MS is found to be highly effective for detecting adulteration of oils and fats in food and non-food samples. This review summarizes the benefits and drawbacks of these analytical approaches and their use in conjunction with chemometric tools to detect the adulteration of animal fats and vegetable oils. This combination provides a powerful technique with enormous chemotaxonomic potential that includes the detection of adulterations, quality assurance, assessment of geographical origin, assessment of the process, and classification of the product in complex matrices from food and non-food samples.

Abbreviations: GC, Gas Chromatography; GC–MS, Gas Chromatography-Mass Spectrometry; GC-FID, Gas Chromatography-Flame Ionization Detector; HPLC, High-Performance Liquid Chromatography; HPLC-MS, High-Performance Liquid Chromatography-Mass Spectrometry; TLC, Thin Layer Chromatography; HPTLC, High-Performance Thin Layer Chromatography; FTIR, Fourier Transform Infrared Spectroscopy; RS, Raman Spectroscopy; SERS, Surface-Enhanced Raman spectroscopy; NIRS, Near-Infrared Spectroscopy; ATR, Attenuated Total Reflection; FS, Fluorescence spectroscopy; SFS, Synchronous Fluorescence Spectroscopy; LIFS, Laser-Induced Fluorescence Spectroscopy; LED-IFS, LED-Induced Fluorescence Spectroscopy; MIR, Mid-Infrared; NMR, Nuclear Magnetic Resonance; LF-NMR, Low Field Nuclear Magnetic Resonance; MALDI-MS, Matrix-assisted Laser Desorption/ Ionization Mass Spectrometry; ESI-MS, Electrospray Ionization-Mass Spectrometry; TOF, Time-of-Flight; *E*-Nose, Electronic Nose; GC-SAW, Gas Chromatography-Surface Acoustic Wave; DSC, Differential Scanning Calorimetry; PCR, Polymerase Chain Reaction; PCR-RFLP, PCR coupled with Restriction Fragment Length Polymorphism; CE-SSCP, Capillary Electrophoresis Single-Strand Conformation Polymorphism; DA, Discriminant Analysis; LDA, Linear Discriminant Analysis; PCA, Principal Component Analysis; PLS, Partial Least Squares; PLS-DA, Partial Least Squares-Discriminant Analysis; OPLS-DA, Orthogonal Partial Least Square-Discriminant Analysis; OCPLS, One-class Partial Least Squares; SIMCA, Soft Independent Modelling of Class Analogy; HCA, Hierarchical Cluster Analysis; UHC, Unsupervised Hierarchical Clustering; SVM, Support Vector Machine; LS-SVM, Least squares support vector machines; MCR-ALS, Multivariate Curve Resolution-Alternating Least Squares Analysis; ANOVA, One-way Analysis of Variance; PLS-R, Partial Least Squares Regression; CARS, Competitive Adaptive Reweighted Sampling; ECR, Elastic Component Regression; IPLS, Interval Partial Least Squares; SIPLS, Synergy Interval Partial Least Square; SLLE, Supervised Locally Linear Embedding; DFA, Discriminant Factor Analysis; CDA, Canonical Discriminant Analysis; CBTs, Classification Binary Trees; SVM-C, Support Vector Machine Classification; GILS, Genetic Inverse Least Squares; ANN, Artificial Neural Network; kNN, k-Nearest Neighbours; RMSE, Root Mean Square Error; EVOO, Extra Virgin Olive Oil; CLO, Cod Liver Oil; UFO, Used Frying Oil; FA, Fatty Acid; MUFA, Monounsaturated Fatty Acid; PUFA, Polyunsaturated Fatty Acid; SFA, Saturated Fatty Acid; RBD, Refined Bleached Deodorized..

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1. Introduction

Oils and fats are the essential components of our daily diet, since they are also capable of several bodily functions. Oils and fats are lipids derived from animals, and vegetables sources; at room temperature, fats are solid, while oils are liquid. They are complicated mixtures made up of a variety of different substances, including free fatty acids, diacylglycerols, triacylglycerols, phospholipids, and other minor components. Triacylglycerols are chemically described as glycerol esterified with fatty acids and are the most fundamental family of molecules (Mehmood et al., 2012). Oils and fats play an essential role in maintaining the quality, texture, and taste of the foods, so the amount and quality of oils and fats composition are necessary to monitor in foodstuffs (Pooja et al., 2021). Adulteration of food ingredients such as oils and fats has been a significant issue for many years, especially in national and global markets. Ensuring food authenticity is a crucial concern for all stakeholders in the food industry, including those in the oils and fats sector. As adulteration techniques become more sophisticated, there is a growing need for effective methods to detect fraudulent manipulations. (Schieber, 2018). Lipid adulteration is a significant issue that affects food safety and undermines consumer trust. Economic gain often leads to adulteration, substituting cheaper oils or fats for more expensive ones, deceiving consumers, and disrupting the market for genuine products. The consumption of adulterated oils can lead to serious public health consequences. Regulatory bodies are working to enhance food safety standards, but enforcement is complicated by inconsistent regulations and sophisticated adulteration techniques. Increased public awareness and industry vigilance are critical, with companies encouraged to conduct third-party testing and share information about adulteration practices and offenders (Everstine et al., 2013; Momtaz et al., 2023).

The global production and consumption of oils and fats have been steadily increasing in recent years. In 2024, the revenue in the oils and fats market is expected to amount to US$253.80 billion. The market is projected to grow annually by 6.44% (CAGR 2024–2029). Furthermore, the volume in the oils and fats market is expected to reach 49.35 billion kg by 2029, with a volume growth of 3.3% anticipated in 2025 (Statista., 2023). The study estimated that 22% of food products are adulterated annually, indicating a significant issue in the food supply chain. This widespread adulteration poses serious health risks to consumers and highlights the need for stricter regulatory measures (Haji et al., 2023). According to a recent report in 2023, the adulteration in fats and oils is estimated at 6.92% worldwide (Polakova et al., 2024).

This review significantly advances the existing literature by providing a comprehensive overview of analytical techniques collectively applied for detecting adulteration of edible oils and fats in food and non-food samples. Prior reviews have often focused on a single analytical method for the detection of vegetable oils (Di Pietro et al., 2020), certain kind of oil and fat (Harun, 2019; Rohman et al., 2020), techniques to detect adulteration in individual vegetable oil (Meenu et al., 2019; Mohammed et al., 2021), edible oils (Pooja et al., 2021; Salah & Nofal, 2021; Sudhakar et al., 2023), and food samples (He et al., 2021; Mortas et al., 2022; Valdés et al., 2018). In contrast, this review encompasses the chromatographic, spectroscopic, and advance technologies including electronic nose, polymerase chain reaction. These strategies recognise the complexity of the adulteration problem, which may involve a wide range of food and non-food substances. It provides a detailed understanding of problems and solutions, offering valuable insights for businesses, regulatory bodies, and researchers to ensure the authenticity and quality of oils and fats in various applications.

2. Source of edible oils and fats

The primary sources of oils and fats are animals, and plants sources.

2.1. Animal fats

Animal fat is found in the tissues and organs of animals such as cow, mutton, pig, chicken, and oily fish. It occurs in many forms, including lard, tallow, chicken, butter, and fish oil. Animal fat is composed of a mixture of unsaturated and saturated fatty acids, making it a significant source of energy in the human diet (H. Sharma et al., 2013). Animal fats have been utilized as fat components in the food industry for a long time. Research indicates that a high intake of animal fat may increase the risk of developing health problems such as heart disease and obesity, while other research suggests that moderate consumption of animal fat may not have negative impacts on health (Forouhi et al., 2018; Hariri & Thibault, 2010). Fig. 1 shows various types of animal fats derived from different animals.

Lard is commonly produced from the adipose tissue of pig fat. Lard has significant quantities of cholesterol and saturated fatty acids, which are known to be harmful to human health. For instance, the use of lard has been a serious problem for customers because of religious responsibilities and health issues, including hypercholesterolemia and coronary heart disease (Abidin et al., 2023). Due to its low cost, lard is a common adulterant in high-cost vegetable oils and cod liver oil. Oils and fats are not totally replaced by lard, but vegetable oils may effectively be combined with lard to produce margarine, shortening, and other oil-based products at a lower cost. Additionally, palm oil has identical chemical characteristics to lard due to the high amount of palmitic and oleic acids in both lard and palm oil (Hussain et al., 2023).

Mutton tallow and beef tallow are also the type of animal fat derived from the fatty tissue of sheep or goat and cattle, respectively. Fat obtained from cattle is often softer than fat derived from sheep or goat. Tallow, a versatile fat with a high smoke point, is ideal for frying and sautéing. However, its high saturated fat content can lead to health issues like high cholesterol and heart disease. Therefore, it should be consumed in moderation to maintain a healthy diet, as with all other fats (Richards et al., 2005).

Chicken fat, extracted from chicken skin and fatty tissues, is a popular ingredient in cooking and food preparation. It provides flavour, moisture, and a smooth texture to dishes. It has essential fatty acids and vitamin D. Chicken fat is used as an adulterant in various vegetable oils, such as coconut oil, sunflower oil (J. Marikkar et al., 2012; Xu et al., 2015). Butterfat, made from cow's milk, is a significant source of

Fig. 1. Various types of animal fats derived from different animals.

vitamin A and vitamin D. Other dairy products, including cheese, ice cream, whipping cream, and coffee cream, also contain butterfat (Mishra & Manchanda, 2012).

Fish oil is a dietary supplement made from the tissues of oily fish, including cod, salmon, whales, and other species. Fatty acid (FA) content in fish varies not just between different species but even within the same species. Cod liver oil (CLO) is derived from the liver of cod fish. Omega-3 fatty acids are abundantly found in CLO and are essential fats that our body cannot make on its own and must obtain through diet. Omega-3 fatty acids offer numerous health benefits such as reducing inflammation, enhancing heart health, and brain function. They are also important for eye health, reducing the risk of certain cancers, and improving mood and mental health (Indarti et al., 2005).

2.2. *Vegetable oils*

Vegetable oil is derived from various plant sources, such as seeds, fruits, or nuts. Some types of vegetable oils are high in unhealthy fats, such as saturated and trans fats, which can contribute to heart disease and other health problems. These include palm, and coconut oils, whereas olive, sunflower, mustard, safflower, sesame, and rice bran oils are low in saturated fats (Ogori, 2020). It is necessary to choose vegetable oils that are lower in unhealthy fats and consume them in moderation. Different kinds of vegetable oils are represented in Fig. 2.

Soybean and palm oils are produced from the seed of soybean plant and fruits of palm tree, respectively. Crude palm oil and palm kernel oil are two distinct types of oils produced from the palm tree, obtained by pressing the fleshy fruit and crushing the kernel, or stone, in the middle part of the fruit (Obibuzor et al., 2012). Cotton seeds are used to make cottonseed oil. This oil has a deep reddish-brown colour and a strong flavour and aroma. This oil is traded in a small amount because it is a by-product and dependent on the usage of cotton in textiles (Ogori, 2020).

Canola oil is a relatively recent kind of rapeseed plant that is used to obtain edible oil. Sunflower and safflower plant's seeds are used to make sunflower oil and safflower oil, respectively. Olive oil is extracted from the olive' s fruits. Not all olive oils are created equal in terms of quality and cost; for instance, extra virgin olive oil is the costliest than other low-priced or low-quality oils and has the highest level of adulteration occurred (Salah & Nofal, 2021). Peanut, mustard and corn oils are extracted from the peanut's seed, mustard's seed, and germ of corn (maize), respectively. These oils are a good source of healthy unsaturated fats. Peanut oil, also known as ground nut or *arachis* oil. Copra, or dried coconut meat from the *Cocos nucifera* palm, is the raw material used to make coconut oil. It is solid at room temperature but becomes liquid above 25 °C; that's why it is categorised as fat (Ogori, 2020).

Sesame and flaxseed (linseed) oils are edible oil extracted from sesame seed and flax seed, respectively. These oils are good source of omega-6 fatty acids, that is vital for human health. Sesame has a unique nutty aroma and flavour and contains antioxidants, such as sesamol and sesamin, which help to protect against oxidative damage to cells and reduce inflammation in the body. Flaxseed oil has health advantages, such as boosting heart health, reducing inflammation, and possibly reducing the risk of certain types of cancer (Mujtaba et al., 2020; Yuan et al., 2020).

Bran is the source of rice bran oil, which is the outer layer of the rice grain. It contains vitamin E and gamma oryzanol, which help to protect against free radical damage and reduce inflammation in the body (Fraterrigo Garofalo et al., 2021). Walnut and grape seed oils are extracted from walnut and grape's seed. Each grape variety's oils have a unique fatty acid (FA) composition (Akin et al., 2019; B. Li et al., 2015). Argan and avocado oils are derived from the kernel of the argan tree and pulp of avocado, respectively. These oils contain high content of vitamin E, antioxidants, and essential fatty (Lumakso et al., 2015; Mohammed et al., 2021).

Fig. 2. A list of vegetable oils from different seeds, fruits, and nuts of plants.

3. Chemistry of animal fats and vegetable oils

The main fatty acids found in animal fats are palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 ω9c), and linoleic acid (C18:2 ω6c). Lard is a type of fat that contains polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs), and saturated fatty acids (SFAs) in the percentages of 12%, 46%, and 40%, respectively. Out of all the animal sources, lard contains a higher percentage of MUFAs. The percentage of C18:1 and C18:2 in lard is higher than in tallow but almost equal to that in chicken fat. However, the percentage of stearic acid is higher in tallows (Nizar et al., 2013). Interestingly, the palmitic acid enrichment factor, which is 2-monoacylglycerol palmitic acid (2-MG), is higher in lard than other oils and fats by using pancreatic lipase hydrolysis (Al-Kahtani et al., 2014).

Vegetable oils have different percentages of SFAs, MUFAs, and PUFAs. Palm and coconut oils contain higher amounts of SFAs than other edible oils. Olive, sunflower, rapeseed, peanut, sesame, and rice bran oils contain higher percentages of MUFAs. Soybean, safflower, cottonseed, maize, linseed, walnut, and grape seed oils contain higher percentages of PUFAs. The average fatty acid composition of different oils and fats is represented in Fig. 3. The fatty acid compositions of animal fats and vegetable oils reported from various studies are shown in supplementary Tables S1 and S2, respectively.

Olive oil contains a higher concentration of oleic acid than other fatty acids, while palm oil contains higher concentrations of palmitic and oleic acids. Coconut oil contains a high level of SFAs like methyl laurate (C12:0), methyl myristate (C14:0), palmitic acid (C16:0), and stearic acid (C18:0). Canola oil contains approximately 6% saturated fatty acids (Ogori, 2020). Corn, sunflower, soybean, and rapeseed oils contain high percentages of oleic and linoleic acids, but in comparison to these four oils, rapeseed oil has more oleic acid and less linoleic acid than others (Jabeur et al., 2014). Safflower oil is a rich source of mono- and polyunsaturated FAs, including linoleic and oleic acids. Cotton seed oil is high in polyunsaturated fats, which are considered to be healthier than saturated fats (Ogori, 2020). Peanut oil is a nutritious source of mono- and polyunsaturated FAs, vitamin E, and antioxidant properties. It is low in saturated and trans fats, making it a healthier choice compared to other oils (Zhu et al., 2017). Mustard oil is a rich source of MUFAs, and PUFAs, as well as omega-3 fatty acids, which are crucial for heart health (Mishra & Manchanda, 2012). Walnut and grape seed oils are rich in PUFA, mainly MUFA, and less SFA. Walnut oil is primarily composed of unsaturated fatty acids, including oleic, linoleic, and linolenic acids (Akin et al., 2019; Li et al., 2015).

4. Application of oils and fats

4.1. Application in food products

Edible oils and fats are essential raw materials and components for many food products, including bakery, confectionery, chocolate, sauces, ice creams, margarine, frying purposes, and other tailored products (Rios et al., 2014).

Frying is a dry-heat cooking method that involves frying food in hot oil or fat to produce a crispy and flavourful exterior while maintaining the inside moist and tender. Different types of oil and fat used affect the taste and nutritional value of the food. Animal fats like lard and tallow can be used due to their high smoke points and distinct flavour. Vegetable oils like canola, corn, peanut, and sunflower oils are commonly used. Coconut oil is used as a frying oil for margarine, milk fat substitutes, and non-dairy creamers (Ogori, 2020). Animal fats and vegetable oils are used in bakery products like biscuits and cakes, but their properties can affect the outcome of the baked goods. Animal fats, such as butter or lard, have a rich flavour and texture but have a higher melting point than vegetable oils, resulting in denser baked goods. Vegetable oils, on the other hand, have a neutral flavour and a lighter texture (Ragavendra et al., 2022).

Chocolate typically consists of animal fat or vegetable oil, with cocoa butter and milk fat being traditional ingredients. Cocoa butter is a vegetable fat derived from cocoa beans. Some producers may substitute it with vegetable oils like palm, soybean, rapeseed, ground nut, and coconut oils. Non-dairy ice cream typically contains vegetable oils like palm kernel oil or coconut oil as the base oil. Some manufacturers may use soybean oil or coconut oil in place of or in addition to cream and milk. Some manufacturers may also use vegetable fats or other substitutes (Rios et al., 2014).

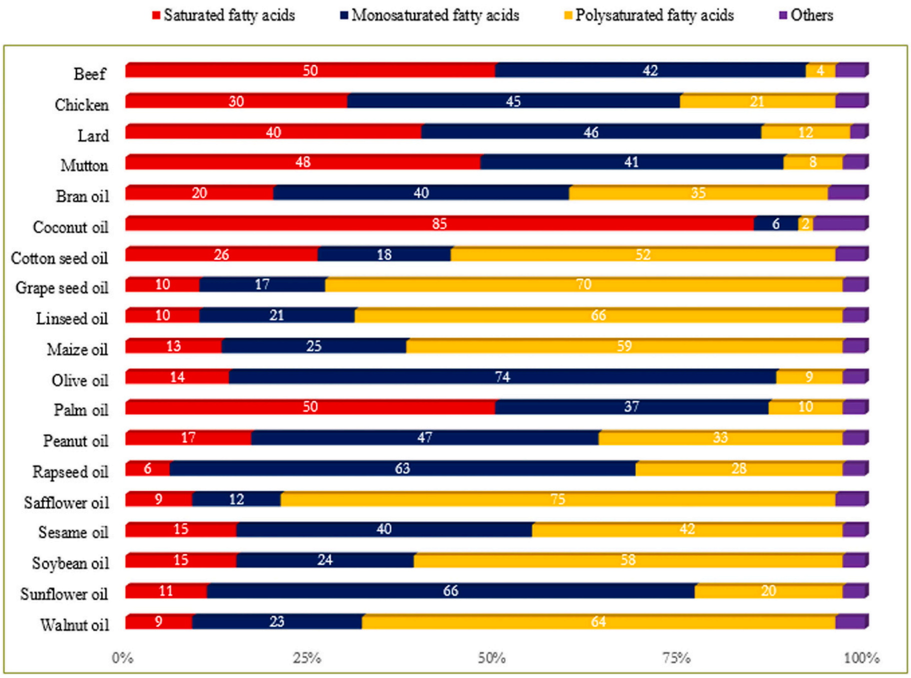

Fig. 3. A comparison of the average fatty acid percentage compositions of different oils and fats. (Data from Reeve et al. (Reeves III & Weihrauch, 1979)).

4.2. *Application in dairy products*

A wide range of food items made from or containing animal milk are called dairy products. Milk chocolate may include butter or milk, but some producers may substitute other ingredients. Ice cream contains fat, a multipurpose component that enhances flavour, colour, texture, and mouthfeel. Ice cream production traditionally involved cream and milk. Dairy ice cream typically contains cow's milk fat. In premium or high-fat ice creams, animal fats like butterfat or whey butter may also be used (Rios et al., 2014).

4.3. *Application in non-food products*

Animal fats and vegetable oils are used in personal care products and cosmetics such as moisturizers, lotions, cream, and lipstick etc. The use of personal care items and cosmetics has skyrocketed in recent years. Vegetable oils such as palm, coconut, olive, castor, soybean, almond, argan, and avocado oils are popular choices in skincare products (Athar & Nasir, 2005; Rabasco Álvarez & González Rodríguez, 2000). Pork fat or lard is frequently utilized as viscosity-increasing ingredients in several cosmetic treatments. According to the Food and Drug Administration (FDA), lard is one of the widely acknowledged safe chemicals used in cosmetics. However, certain religious groups, like those who practice Islam, forbid the use of cosmetics that contain lard (Lukitaningsih et al., 2012). The main ingredients in lipsticks include colouring chemicals, oils, fats, and waxes. Castor oil is widely used in lipstick formulations due to its high viscosity and ability to dissolve staining dye (Waskitho et al., 2016). It is important to choose high-quality animal fats and vegetable oils for cosmetic, as some products may contain additives or impurities that are harmful for the skin.

Edible oils and fats are used in pharmaceuticals as excipients or inactive ingredients to improve drug properties, manufacturing, stability, and delivery. Lard is used in suppositories, ointments, and creams. However, it may not be suitable for all patients, especially those with dietary restrictions or allergies. Different vegetable oils are also used in pharmaceuticals (Rabasco Álvarez & González Rodríguez, 2000; Thillany et al., 2020). Edible oils and fats can also be used to make soap and detergent. Tallow and lard are commonly used in traditional soap making due to their ability to produce lather and cleanse the skin. However, modern soap making frequently uses coconut, palm, castor, linseed, apricot, avocado, and almond oils (Adane, 2020; Athar & Nasir, 2005).

5. Analysis of edible oils and fats

Food is a very complicated mixture, thus it is necessary to use effective and trustworthy ways to evaluate the quality and authenticity of food. The most commonly utilized techniques for the determination of adulteration of edible oils and fats in food and non-food items can be enumerated as gas chromatography (GC), high-performance liquid chromatography (HPLC), thin layer chromatography (TLC), fourier transform infrared spectroscopy (FTIR), near infrared (NIR) spectroscopy, raman spectroscopy (RS), direct mass spectrometry, DNA polymerase chain reaction (PCR), differential scanning calorimetry (DSC), Nuclear magnetic resonance (NMR) spectroscopy, and electronic nose (*E*-Nose). A flow chart of analytical techniques is represented in Fig. 4.

Chromatography techniques are effective methods for identifying, separating, and purifying various components in a mixture and are frequently employed to identify food adulterants. The reason for their broad usage of techniques is that they range from detecting adulterated edible oils and fats to even tracing the levels of any product (Yadav, 2018). These methods provide a wealth of information to the analyst in the form of a significant volume of data. The number and amount of specific constituents like triglycerides, fatty acids, sterol ratios, and hydrocarbons in unadulterated and adulterated oils and fats are typically detected using chromatographic techniques. To identify the unknown adulterant oil, libraries for various chemicals found in all edible oils are needed (Sudhakar et al., 2023). Spectroscopic techniques such as FTIR, NIR, RS, and NMR are widely used in edible oils and fats authentication. In general, it has been noted that these methods have several benefits, including speed, non-destructiveness, and little to no sample preparation (Rifna et al., 2022). A tabular compilation of adulteration detection techniques for animal fats and vegetable oils in food and non-food samples is summarised in Table 1 and Table 2, respectively.

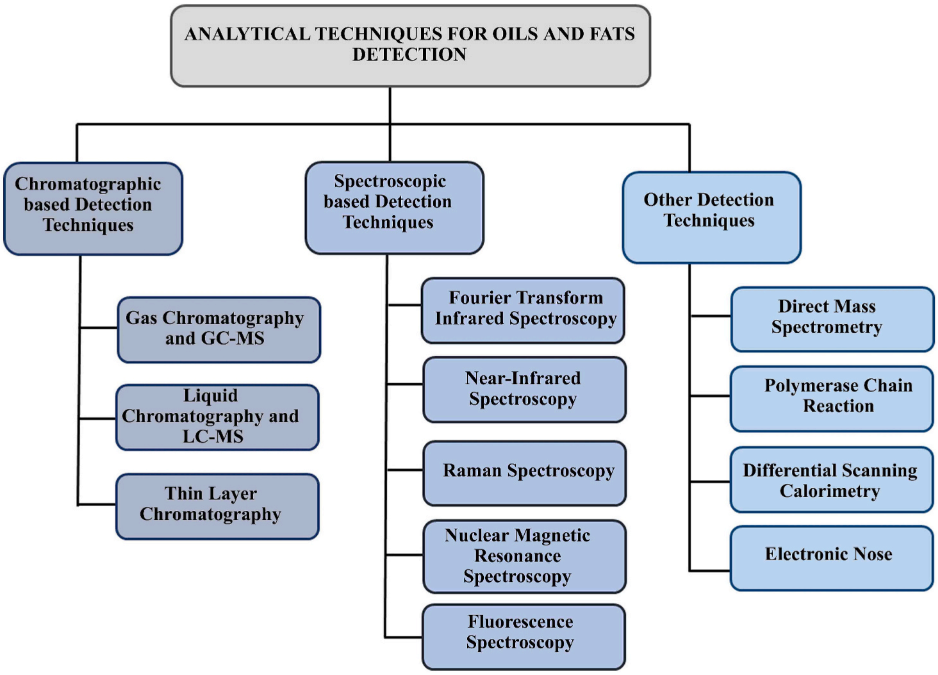

Fig. 4. A flow chart of analytical techniques for the detection of oils and fats.

Table 1
Adulteration detection techniques of animal fats in food and non-food samples.

Objective	Adulterated substance	Detection techniques	Statistical analysis	Sample Preparation Methods	Reference
Detection of lard adulteration	Biscuit	GC–MS	PCA, HCA	Soxhlet extraction (petroleum ether)	(Azizan et al., 2021)
		GC, HPLC DSC	ANOVA	FAME by sodium methoxide (CH_3ONa)	(Yanty et al., 2014)
	Butter	GC–MS	PCA	Fat extraction by rendering process	(Fadzillah et al., 2016)
		^{1}H NMR	PLS	Fat extraction by rendering process	(Fadzillah et al., 2015)
		HPLC, ^{1}H NMR	–	Solvent extraction (CD_3OD)	(Fadzillah et al., 2017)
		RS	HCA, PCA	Fat extraction by rendering process	(Taylan et al., 2020)
		DSC	–	Butter extraction a/c to AOAC Method 920.118	(Nurrulhidayah et al., 2015)
	Cocoa butter	GC-FID, HPLC, DSC	–	FAME by sodium methoxide	(Azir et al., 2017)
	Chocolate	FTIR, GC–MS	PCA, PLS	Soxhlet extraction (n-hexane) FAME by (CH_3ONa)/ methanolic boron trifluoride (BF_3)	(Suparman, 2015)
		FTIR	PLS	Not required	(Man, Syahariza, et al., 2005)
		PCR	–	DNA extraction using CTAB	(Rosman et al., 2016)
	Cake	FTIR	PLS	Fat extraction by Bligh-Dyer method Liquid-liquid extraction ($CH_3Cl/MeOH/H_2O$)	(Syahariza et al., 2005)
	Rambak crackers	FTIR	PLS, PCA	Soxhlet extraction (hexane)	(Erwanto et al., 2016)
	Pre-fried french fries	FTIR	PLS	Soxhlet extraction (petroleum ether)	(Che Man et al., 2014)
	Cod liver oil	FTIR	DA, PLS	Fat extraction by rendering process	(Rohman & Che Man, 2009a)
	Olive oil	GC–MS	PCA	FAME by methanolic potassium hydroxide (KOH)	(Heidari et al., 2020)
	Virgin coconut oil	GC-SAW, FTIR	PLS	Fat extraction by rendering process	(Tengku Mansor et al., 2011)
		DSC	–	Fat extraction by rendering process FAME by CH_3ONa	(Mansor et al., 2012)
	Palm oil	FTIR	PLS	Not required	(Ahda and Safitri, 2016)
		NIR, GC–MS	LDA, PLS	FAME by CH_3ONa	(Hussain et al., 2023)
		NIR	PLS	Not required	(Basri et al., 2017)
	Sunflower, olive, canola, coconut, and mustard oils	FTIR	PLS	Not required	(Munir et al., 2019)
	Sunflower, corn, olive, and palm oils, frozen french fries	FTIR	–	Solvent extraction (hexane)	(Al-Kahtani et al., 2017)
	Camel, beef, sheep, goat, rabbit, and chicken fats, corn, sunflower, palm, and olive oils, french fries, butter, chocolate and meat products	GC-FID	–	FAME by BF_3 in methanol	(Al-Kahtani et al., 2014)
	RBD palm olein	*E*-Nose	–	Not required	(Man, Gan, et al., 2005)
	Ghee (heat clarified milk fat)	FTIR	PLS	Ghee preparation by direct cream method Fat extraction by rendering process	(Upadhyay et al., 2018)
	Beef tallow and chicken fat	DSC	PCA	Fat extraction by Bligh-Dyer method Liquid-liquid extraction ($CH_3Cl/MeOH/H_2O$)	(Dahimi, Rahim, et al., 2014)
	Chicken, lamb, and cow fats	FTIR	PLS	Fat extraction by rendering process	(Rohman & Man, 2010)
		FTIR	–		(Che Man et al., 2001)
Discriminate lard from other animal fats	Beef tallow, and mutton tallow, and chicken fat	GC-FID, HPLC, DSC	ANOVA, PCA	Fat extraction by rendering process	(N. Marikkar et al., 2021)
		GC–MS	PCA	FAME by NaOH, and BF_3 in methanol	(Nizar et al., 2013)

(*continued on next page*)

Table 1 (*continued*)

Objective	Adulterated substance	Detection techniques	Statistical analysis	Sample Preparation Methods	Reference
		GC*GC-TOF-MS	PCA	Fat extraction by rendering process	(Indrasti et al., 2010)
		GC–MS, FTIR, DSC, EA-IRMS	PCA	FAME by CH_3ONa	(Naquiah et al., 2017)
	Beef tallow, chicken fats	GC-FID	PCA	Solvent extraction (CH_3Cl/MeOH/H_2O)	(Dahimi, Hassan, et al., 2014)
		E-Nose	–	Fat extraction by rendering process	(Latief et al., 2017)
	Beef tallow, chicken fat, duck oil	RS	–		(Lee et al., 2018)
Lard in cosmetic	Cream formulations	FTIR	PCA	Fat extraction by rendering process	(Rohman et al., 2014)
	Lotion formulation	FTIR	PLS	Liquid-liquid extraction (hexane)	(Lukitaningsih et al., 2012)
	Lipstick formulation	FTIR	PLS, PCA	Fat extraction by rendering process Saponification and Bligh and Dyer methods	(Waskitho et al., 2016)
Lard in extracted ink	Printed food packaging	FTIR	SIMCA, PCA	Fat extraction by rendering process Deinking process (NaOH)	(Ramli et al., 2015)
Lard, beef tallow, chicken fat	Sunflower oil	DSC	–	Fat extraction by rendering process	(J. Marikkar et al., 2012)
	Canola oil	GC–MS, ^{1}H NMR	PCA, PLS-DA		(Fang et al., 2013)
	milk fat	GC-FID	–	Folch method	(Kim et al., 2015)
Beef tallow, lard	Beef Sausage	FTIR	PLS, PCA	Soxhlet extraction (hexane)	(Guntarti et al., 2019)
	Meatball broth	FTIR	PCA, PLS	Fat extraction by rendering process Liquid-liquid extraction (hexane)	(Kurniawati et al., 2014)
Lard and randomized lard	Refined-bleached-deodorized palm oil	DSC	–	Fat extraction by rendering process FAME by CH_3ONa	(J. Marikkar et al., 2001)
Buffalo tallow	Cow ghee	GC, PCR	–	Ghee preparation by direct cream method Fat extraction by rendering process	(V. Sharma et al., 2018)
		HPTLC	–	Fat extraction by rendering process Saponification bymethanolic KOH	(De et al., 2013)
	Lard	LC-MS	–	Fat extraction by rendering process FAME by H_2SO_4 in MeOH	(Dugo et al., 2006)
	Butter	FTIR	–	Fat extraction by rendering process	(Nurrulhidayah et al., 2013)
Analysis of chicken	Butter	FTIR	PLS	Fat extraction by rendering process Butter extraction a/c to AOAC Method 920.118	(Ahmad Fadzillah et al., 2013)
Goat tallow	Cow and buffalo milk fat (ghee)	PCR	–	Fat extraction by rendering process DNA extraction by Stool Mini Kit (Qiagen) with modification	(Hazra et al., 2017)
Fish oil	Lard	FTIR	OPLS-DA	Direct pressed extraction method	(Windarsih et al., 2023)
			PLS-DA, OPLS-DA		(Irnawati et al., 2023)
		FTIR	PCA	liquid-liquid extraction Fat extraction by rendering process	(Zilhadia Zilhadia et al., 2024)
	beef fat	FTIR, GC-FID	PLS	Fat extraction by rendering process	(Rohman & Che Man, 2011a)
	Mutton fat	FTIR, GC-FID	PLS, DA		(A Rohman et al., 2012)
	Palm, and corn oil	FTIR	PLS, DA	Not required	(Riyanto et al., 2021)
	Lard, chicken oil, and tallow	FTIR	PLS, PLS-DA		(Gao et al., 2021)
	Lard, beef, mutton, and chicken fats	FTIR	PLS, DA	Fat extraction by rendering process	(Rohman & Che Man, 2009b)
	Beef, mutton, and chicken fats	FTIR	PLS, DA	Fat extraction by rendering process	(A Rohman et al., 2011)
	Palm oil	FTIR	PLS, PCR	Maceration extraction (n-hexane)	(Putri et al., 2019)
		FTIR	PLS, PCR	Not required Not required	(Jannah et al., 2023)

(continued on next page)

Table 1 (*continued*)

Objective	Adulterated substance	Detection techniques	Statistical analysis	Sample Preparation Methods	Reference
		Raman, IR	–		(Ahmmed et al., 2022)
	Sunflower, and canola oils	^{1}H, ^{13}C NMR, FTIR, GC-FID	ANN	Not required	(Giese et al., 2019)
	Sunflower, corn, and grape seed oil	FTIR	PLS	Not required	(A. Rohman et al., 2017)
	Canola, corn, soybean, and walnut oils	FTIR	PLS, DA	Not required	(Rohman & Che Man, 2011b)
Cow and buffalo milk fats	Soybean and sunflower oils	TLC	–	Creamery butter method Saponification by alcoholic KOH, diethyl ether	(Upadhyay et al., 2015)
Butter	Palm and coconut oils	FS	MLR models	Not required	(Dankowska et al., 2014)

6. Detection of adulteration and authentication of oils and fats using analytical techniques

6.1. Gas chromatography (GC) and its related techniques

GC is an accurate method for analysing the variation or determining the fatty acid (FA) compositions in edible oils and fats, and for analysing the alteration of the FA compositions in food items generated via various technological procedures.

The gas chromatographic analysis of oils and fats involves several steps, including accurate weighing the sample, adding a nonpolar solvent like hexane or chloroform for extraction, concentrating the extract, and potentially derivatizing the analytes to enhance their volatility and stability, most commonly into fatty acid methyl esters (FAMEs). The derivatized sample is then transferred to an injection vial, sealed, and introduced into the GC system for precise analysis. This comprehensive process enables the identification and quantification of various compounds within complex matrices. Samples can also be extracted using conventional and modern methods, including soxhlet, Folch, Bligh and Dyer, and microwave-assisted extraction (Quero-Jiménez et al., 2020). A schematic representation of GC–MS analysis of oils and fats is shown in Fig. 5.

6.1.1. GC and GC–MS techniques

Lard, a pork fat, is often used with plant oils, other animal fats, and fat-based food or non-food samples due to its low cost. However, it is difficult to detect due to its small presence in other oil and fat samples. As a result, techniques relying on the total fatty acid composition may not be effective for lard detection. Hence, it is necessary to investigate the FA distribution pattern inside the triacylglycerol (TAG) molecules. In a study, pancreatic lipase hydrolysis was used to determine the sn-2 positional distribution of fatty acids within the glycerol chain, which was the most effective method for distinguishing lard from other edible oils and fats. The palmitic acid enrichment factor, a key indicator of lard content in various animal fats, vegetable oils, dairy products, meat products, and other commercial products, increases with lard percentage (Al-Kahtani et al., 2014). GC analysis was used to identify milk fat, lard, beef tallow, and vegetable oils by monitoring their fatty acid and triacylglycerol profiles. Results showed that adulterated milk fat was rich in short- to medium-chain saturated fatty acids and C36, C38, C40, C50, C52, and C54 in TGs, which can be used as biomarkers (Park et al., 2014).

FA compositions of recycled cooking oil (RCO) can be used to identify used cooking oil in fresh palm oil. Adulterated oil samples (AO) were obtained when RCO was mixed with fresh palm olein (FPO) in a proportion of 1% to 50%. Gas chromatography-flame ionization detection (GC-FID) was used to determine the FA compositions of FPO, RCO, and AO. The FA composition of RCO showed significant changes, including short-chain, trans, odd-chain, and monounsaturated fatty acids (Lim et al., 2018). The GC–MS analysis of lard in imported chocolate revealed specific eicosadienoate 11,14-acid (C20:2 ω6) fatty acid. The existence of C20:2 ω6 is demonstrated in the presence of 100% lard oil in some formulations with a retention duration of 38.8 min (Suparman, 2015). Moreover, a study reported the use of gas chromatography to detect the presence of adulterants in olive oil, including soybean, corn, and sunflower oils (Jabeur et al., 2014).

6.1.2. Two-dimensional gas chromatography (GC*GC)

Two-dimensional gas chromatography hyphenated with time-of-flight mass spectrometry (GC *GC-TOFMS) is a method to distinguish lard from other animal fats. Methyl trans-9,12,15-octadecatrienoate, methyl 11,14-eicosadienoate, and methyl 11,14,17-eicosatrienoate constituents may be used as indicators for the presence of lard (Indrasti et al., 2010). GC*GC-TOFMS was used for a complete assessment of the FA profile of eight vegetable oils, namely rapeseed, sesame, hempseed, sunflower, mustard, olive pomace, sunflower, and extra virgin olive oils. In a comparison of one- and two-dimensional gas chromatography, the GC*GC approach produced more accurate quantification results, notably for odd-carbon and medium-chain fatty acids, which are present at only trace levels (Vyviurska et al., 2015). GC*GC-TOFMS is also used to detect adulteration of animal fats in virgin coconut oil. The study found that monitoring the cholesterol level in virgin coconut oil might be used to identify the presence of lard, chicken fat, mutton, and beef tallow and determine their combination at levels as low as 0.25% (Xu et al., 2015).

6.1.3. Use of chemometric tools

The results from GC-FID and GC–MS provide a lot of information to the analyst. For the authentication of various edible oils and fats, GC data with chemometric approaches like statistics, multivariate regression, and pattern recognition methods are valuable tools.

GC-FID or GC–MS techniques alone may not be suitable for the differentiation of animal fats, whereas GC combined with multivariate methods like PCA can be employed to distinguish lard from chicken fat and beef tallow. Stearic, oleic, and linoleic acids were identified as the most discriminating factors in animal fat clustering, as shown by the results of Principal component analysis (PCA) (Nizar et al., 2013). Dahimi and co-worker found that lard has a higher C18:2 cis and lower C16:0 fatty acid content than chicken fat and beef tallow. The proportions of C4:0, C14:0, and C18:0 are similar across all edible fats. PCA can detect lard in blends of lard-beef tallow and lard-chicken fat with as low as 0.5% lard (Dahimi, Hassan, et al., 2014). The metabolomics approach using GC–MS results in conjunction with PCA provided a simple and accurate way to differentiate and identify olive oil, sunflower oil, sesame oil, lard, and lard-adulterated samples. This method successfully demonstrated the identification and quantification of lard in other samples. The quantitative determination of lard adulteration, even at low percentages of 5% *w/w*, was utilized by particular discriminant markers like methyl myristate and methyl stearate (Heidari et al., 2020).

Random forest and PCA were used to differentiate between pure

Table 2
Adulteration detection techniques of vegetable oils in food and non-food samples.

Adulterated oils	Adulterant substances	Detection techniques	Statistical analysis	Sample Preparation Methods	Reference
Olive oil	Soybean, sunflower, and corn oils	GC-FID, HPLC	–	FAME by methanolic KOH	(Jabeur et al., 2014)
		FTIR	PCA, SLLE, PLS	Not required	(Sun et al., 2015)
		RS	LS-SVM, PLS	Not required	(Dong et al., 2012)
		RS	SVM	Not required	(X. F. Zhang et al., 2011)
		FTIR	–	Not required	(Mashodi et al., 2020)
	Canola, corn, sunflower, and soybean oils	NIR	PCA	Not required	(Vanstone et al., 2018)
			PCA, PLS	Not required	(Borghi et al., 2020)
		ESI-MS	PCA, HCA	Liquid-liquid extraction (MeOH/H_2O)	(Mitsutake et al., 2015)
			PLS	Liquid-liquid extraction (MeOH/H_2O/0.1%HCOOH)	(J. Alves et al., 2014)
			PLS-DA		(J. O. Alves et al., 2013)
	Canola, corn, sunflower, peanut, and soybean oils	DSC	PCA, KNN, SVM	Not required	(Huang et al., 2023)
	Grape seed, soybean, canola, palm, corn, sunflower, and walnut oils	FTIR	PLS, PCA, DA, PCR	Not required	(Rohman et al., 2020)
	Grape seed, rice bran, walnut, and canola oils	FTIR	DA, PLS	Not required	(Rohman et al., 2013)
	Sunflower, corn, soybean and hazelnut oils	FTIR	LDA	Not required	(Lerma-García et al., 2010)
		NIR	PLS	Not required	(Kasemsumran et al., 2005)
	Sunflower, soybean, canola, and hazelnut oils	RS	SIMCA, PLS-DA	Not required	(Vali Zade et al., 2023)
	Sunflower, soybean, cotton, sesame, corn, safflower, walnut, canola, rapeseed, hazelnut, almond, peanut and mustard oils	GC-FID, HPLC	–	Not required	(Christopoulou et al., 2004)
	Soybean, peanut, corn, sunflower, rapeseed, sesame and grapeseed oils	FTIR, RS	kNN, PLS-DA, OCPLS, SVM—C, SIMCA	FAME by CH_3ONa in methanol/TBME (tert-butyl methyl ether) mixture	(Jiménez-Carvelo et al., 2017)
	Sunflower, soybean, garlic, sesame, rapeseed, sunflower, walnut, safflower, bean, wheat germ, and flaxseed oils	RS	PCA, PLS	Not required	(El-Abassy et al., 2009)
	Corn, soybean, linseed, and sunflower oils	FS	PLS-R	Not required	(Lia et al., 2018)
	Corn, peanut, rapeseed, and sunflower oils	GC–MS	PLS-LDA	Not required	(Yang et al., 2013)
	Soybean, sunflower, and rapeseed oils	FS	–	Not required	(Dankowska et al., 2013)
	Sesame, peanut, maize, sunflower, and hazelnut oils	PCR	–	DNA extraction by NucleoSpin Plant and CTAB	(Vietina et al., 2013)
	Hazelnut oil	FTIR, RS	SIMCA, PLS-DA	Not required	(Georgouli et al., 2017)
		DSC	–	Not required	(Chiavaro et al., 2008)
		^{1}H NMR, ^{13}C NMR	–	Not required	(García-González et al., 2004)
		^{1}H NMR, ^{31}P NMR	CDA, CBTs	Not required	(Agiomyrgianaki et al., 2010)
		MALDI-MS, LC-MS	–	Liquid liquid extraction (hexane/ACN)	(Calvano et al., 2010)
		MALDI-MS	–	Extraction by precipitation, and in solution trypsin digestion/CHCA	(De Ceglie et al., 2014)
		MALDI- MS	–	Modified Bligh-Dyer method/Ionic liquid composed by TBA, and CHCA	(Calvano et al., 2012)
	Corn oil	MALDI- MS	UHC, Pearson's correlation analyses, PCA	Not required	(Di Girolamo et al., 2015)
	Sunflower oil	MALDI- MS	PCA	Not required	(Jergović et al., 2017)
		RS	–	Not required	(Philippidis et al., 2017)
		FS	PCA, PLS	Not required	(Ali et al., 2018)
		DSC	–	FAME by potassium methylate in methanol	(Chiavaro et al., 2009)
	Peanut oil	FTIR	PCR, PLS-R, LDA	Not required	(Vasconcelos et al., 2015)
	Soybean oil	RS	PLS-R	Not required	(Yildiz Tiryaki & Ayvaz, 2017)
	Rapeseed and sunflower oils	GC–MS, *E*- Nose	PCA	Not required	(Mildner-Szkudlarz & Jeleń, 2010)
	Soybean, and sunflower oils	FTIR	PLS-DA	Not required	(Oussama et al., 2012)
	Corn and sunflower oils	NIR	GILS	Not required	(Özdemir & Öztürk, 2007)

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Table 2 (*continued*)

Adulterated oils	Adulterant substances	Detection techniques	Statistical analysis	Sample Preparation Methods	Reference
		LIFS	PLS-R	Not required	(Hamdy & Mohammed, 2023)
	Peanut, and soybean oils	LIFS	–	Not required	(Zhang et al., 2022)
	Rapeseed, and corn oils	RS	–	Not required	(de Lima et al., 2020)
	Canola, sunflower oils	PCR	–	Not required	(Kumar et al., 2011)
	Canola, grapeseed oils	RS	–	Not required	(Farley III et al., 2017)
	Waste cooking oil	RS	iPLS, SiPLS	Not required	(Li et al., 2018)
	Paraffin oil	FTIR	–	Not required	(Bunaciu et al., 2022)
	Lard, chicken fat, beef tallow, and mutton tallow	GC *GC–MS	–	Not required	(Xu et al., 2015)
Coconut oil	Paraffin oil	FTIR	PCA, LDA, PCR, PLS-R	Soxhlet extraction (hexane)	(Jamwal, Kumari, et al., 2020)
	Canola, corn, sunflower and soybean oils	FTIR	SIMCA	Not required	(Neves & Poppi, 2020)
	Grape seed and soybean oils	FTIR	PLS	FAME by NaOH, BF_3 in methanol	(Rohman et al., 2019)
	Sunflower, soybean, canola, corn, sesame, castor bean, peanut, palm kernel, mineral, and vaseline oils	RS	MCR-ALS	Not required	(De Géa Neves & Poppi, 2018
	Corn and sunflower oils	FTIR	–	Not required	(Rohman & Man, 2011)
	Palm kernel olein	FTIR	PLS, DA	Not required	(Manaf et al., 2007)
		E-Nose, GC-FID	PCA	FAME by sodium methoxide (CH_3ONa)	(A. M. Marina et al., 2010)
	Palm kernel oil, and soybean oils	DSC, GC-FID	–		(Marina et al., 2009)
Palm oil	Palm, and mustard oils	FTIR	PCA, DA	Not required	(Pandurangan et al., 2017)
	Recycle cooking oil	GC-FID, FTIR	PLS	FAME by Toluene, H_2SO_4	(Lim et al., 2018)
	Inedible plastic	FTIR	PCA	Not required	(Prinsipal, 2021)
	Lard	NIR, GC–MS	LDA, PLS	Fat extraction by rendering process FAME by CH_3ONa	(Hussain et al., 2023)
	Beef tallow	NIR	LDA, PLS	Not required	(Khir, 2018)
Canola oil	Lard and beef tallow	^1H NMR, GC–MS	PCA, PLS-DA	Fat extraction by rendering process FAME by BF_3 method	(Fang et al., 2013)
	Palm oil	NIR	LDA	Not required	(Hussain et al., 2014)
Mustard oil	Argemone oil	FTIR	LDA, PCR, PLS-R	Soxhlet extraction (hexane)	(Jamwal, Amit, et al., 2020)
Hazelnut oil	Canola, soybean, corn, sunflower, sesame, walnut, and peanut oils	FTIR	PLS	Not required	(Ozen & Mauer, 2002)
Edible oils	plasticizer	SERS	–	Not required	(Wang et al., 2023)
	Used frying oil	FTIR	DA, PLS	Not required	(Kou et al., 2018)
Corn, peanut, rapeseed and soybean oils		FTIR	DA	Not required	(Q. Zhang et al., 2012)
		LF-NMR	–	Not required	(Zhang et al., 2013)
Rapeseed oil	Waste cooking oil	FTIR	PLS	Not required	(Wu et al., 2015)
Rapeseed, peanut, corn and olive oils	Waste frying oil	LIFS	PCA, PLS	Not required	(Hao et al., 2019)
Grape seed oil	Soybean oil	FTIR	PCA, LDA, SIMCA, PLS-R	FAME by methanolic KOH	(Akin et al., 2019)
		FS	PCA, SIMCA, PLSR	Not required	(Elmas et al., 2019)
Sesame oil	Rapeseed, palm, soybean, sunflower, and cottonseed oils	GC-FID	PCA, SVM	FAME by methanolic NaOH	(Peng et al., 2015)
	Hazelnut, canola, and sunflower oils	FTIR, GC-FID	PLS	Saponification by KOH/ hexane	(Ozulku et al., 2017)
	Corn oil	FTIR	PLS-R, LDA	Not required	(Fadzlillah et al., 2014)
	Corn, rapeseed, rice, peanut oils	NIR	CARS, ECR, PLS	Not required	(Chen et al., 2018)
	Maize oil	E-Nose	LDA, PCA	Not required	(Hai & Wang, 2006)
Avocado oil	Sunflower, canola and soybean oils	FTIR	SIMCA, PLS	Not required	(Quiñones-Islas et al., 2013)
	Soybean and corn oils	FTIR	PLS	Not required	(Lumakso et al., 2015)
	Palm, and canola oils	FTIR	PLS-R, PCA	Solvent extraction (hexane)	(Rohman et al., 2016)
Flaxseed oil	Cottonseed, maize, soybean, rapeseed, and sunflower oils	NIR	OPLS-DA	Not required	(Yuan et al., 2020)
Walnut oil	Soybean, sunflower rapeseed, and peanut oils	FS, FTIR	SIMCA, PLS	Not required	(B. Li et al., 2015)
	Sunflower oil	FS	PLS	Not required	(Ge et al., 2014)
		RS	PLS	Not required	(Becze & Simedru, 2020)
Sunflower oil	Mineral oil	NIR	PLS	Not required	(Picouet et al., 2018)
	Lard, beef tallow, and chicken fat	DSC	–	Fat extraction by rendering process	(J. Marikkar et al., 2012)
Peanut oil	Sunflower, corn, soybean, cotton seed, and rapeseed oils	FS	PCA, LDA	Not required	(Huyan et al., 2018)
	Soybean oil, rapeseed, and palm oils	LF-NMR	DA, PCA, ANOVA	Not required	(Zhu et al., 2017)
Argan oil	Sunflower oil	E-Nose	PCA, DFA, SVMs	Not required	(Bougrini et al., 2014)

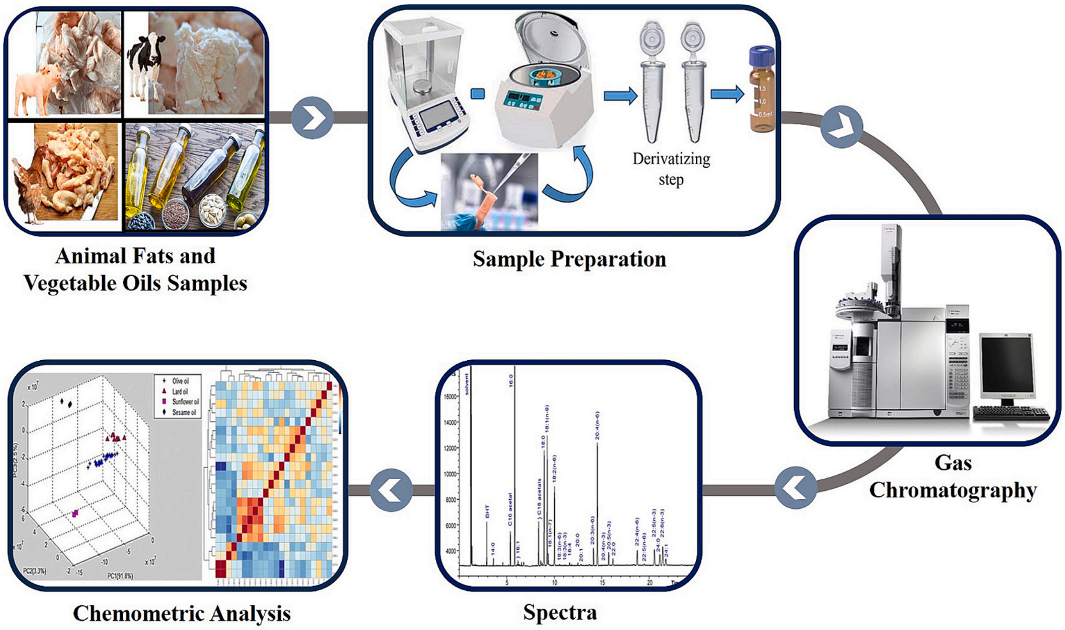

Fig. 5. Schematic representation of GC–MS analysis of oils and fats.

wheat biscuits and adulterated biscuits with lard. Random forest had a higher prediction accuracy of 1.0. Lard identification was indicated by the fatty acids C16:1 and C18:3 ω6, with mean decrease accuracy *v*alues of 0.0825 and 0.0423, respectively. C18:3 ω6 is proposed as a biomarker for differentiating lard-adulterated and unadulterated biscuits (Azizan et al., 2021). Butter and lard may be distinguished using GC and PCA. The amount of FA compositions of 12:0, 14:0, and C16:0 in butter has decreased, and the level of C18:0, C18:1, and C18:2 in butter has increased as a result of the addition of lard, ranging from 1% to 80% (*v/v*), respectively (Fadzlillah et al., 2016). Peng et al. used GC and multivariate data analysis to detect adulteration in sesame oil, revealing that at least 5% of adulterated oil could be detected, with root mean square errors for prediction ranging from 1.19 to 4.29% (Peng et al., 2015). Numerous studies have reported the adulteration of olive oil with various vegetable oils, such as soybean, corn, sunflower, sesame, cottonseed, safflower, walnut, canola, almond, rapeseed, peanut, hazelnut, and mustard oil (Al-Ismail et al., 2010; Christopoulou et al., 2004).

6.2. *High-performance liquid chromatography (HPLC) and its related techniques*

High-performance liquid chromatography-mass spectrometry (HPLC-MS) is a powerful analytical technique used to separate, identify, and quantify constituents in mixtures. In the analysis of edible oils and fats, this technique is used to separate and identify triglycerides (TG), and other lipid components.

To prepare a sample for HPLC analysis of oils and fats, you must first weigh the sample and dissolve it in a suitable solvent for the extraction. After centrifugation and filtration, the extracted sample is diluted to fall within the HPLC's linear range. Standards are prepared for calibration, ensuring accurate quantification of TAG content. Overall, the use of HPLC and its related techniques like mass spectrometry provides a powerful tool for analysis of complex lipid mixtures in animal fats and vegetable oils due to their high resolution, sensitivity, and selectivity.

HPLC was used to determine the TAG compositional changes in the oil and fat samples before and after adulteration. Various ratios of animal fats, including lard, cow tallow, and chicken fat, were added to vegetable oils, notably canola, palm kernel, and palm oils. Results showed that a visual comparison of TAG profiles could detect lard in palm kernel oil but was ineffective for palm and canola oils. However, the distinct categorization of lard-adulterated samples in all three oils was made possible by the combination of LC data and multivariate techniques (J. Marikkar et al., 2005). HPLC was also used to investigate the TAG composition of lard, cod liver oil, beef, mutton, and chicken fats. The main TAGs composed of lard are palmitooleoolein (POO), palmitooleopalmitin (POP), and palmitooleostearin (POS), which account for 21.55%, 5.10%, and 14.08%, respectively. PCA is also used to classify lard and other animals (Rohman et al., 2020). HPLC combined with mass spectrometry was used to study the TAG composition of lard, tallow, and their mixture. By using multidimensional HPLC to analyse the sn-POP/sn-PPO ratio, it was possible to detect tallow in lard. This parameter could be a marker for quality assurance and authenticity, especially in nations where lard is frequently used (Dugo et al., 2006).

HPLC is used to analyse the TAG composition of lard adulterated with butter or cocoa butter as a marker. If lard concentration is increased in cocoa butter from 1 to 30%, the amount of disaturated TAGs (POS, SOS, and POP) is decreased, and the overall amount of triunsaturated TAGs rises from 0.63% to 3.65% (Azir et al., 2017; Fadzillah et al., 2017).

6.3. *Thin layer chromatography (TLC)*

TLC serves as an effective method for distinguishing between animal fats and vegetable oils in food analysis. The fats are extracted from the food sample and then applied onto a TLC plate to detect the components. The separation occurs as the mobile phase, usually a nonpolar solvent, migrates through the stationary phase. Different lipid components, such as fatty acids and triglycerides, exhibit distinct migration patterns, enabling the differentiation between animal fats and vegetable oils. TLC provides a rapid and cost-effective means for quality control and authentication in the food industry. High-Performance Thin Layer Chromatography (HPTLC) is an advanced form of TLC with improved resolution, speed, and sensitivity.

Various studies were reported to detect adulteration in cow/ buffalo ghee (clarified butter) and milk fat with soybean oil, sunflower oil, and other animal fats by using TLC and HPTLC (De et al., 2013; Upadhyay et al., 2015).

6.4. Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy is a fast, no require extensive sample preparation, non-destructive analytical method for analysing edible oils and fats. It measures the absorption of IR light by a sample and analyses the resulting spectrum in the mid-infrared (4000–650 cm^{-1}) regions to identify its chemical composition. FTIR is used to determine the quality and purity of oil and fat samples and is commonly used to identify functional groups like esters, alcohols, and fatty acids. When used with the attenuated total reflectance (ATR) sampling technique, FTIR is more attractive due to its minimal sample preparation and non-destructive nature and eliminates the use of chemical reagents that are dangerous to people and the environment. So it is considered green chemistry (Rohman et al., 2020). A schematic representation of detecting oils and fats using FTIR is shown in Fig. 6.

FTIR can be used to analyse oils, fat-based food, and non-food products, such as cooking oils, cosmetics, lard, etc. Several studies have focused on distinguishing lard from other oils and fats, examining indicators of adulterated oils and fats, and determining the authenticity of oils and fats. FTIR is a technique used to detect lard blended with chicken, mutton, and beef fats. It records and interprets spectral bands associated with these fats and their lard blends. Qualitative differences are proposed to differentiate between pure animal fats and their blends. A semi-quantitative approach measures lard percentage in blends using wavenumber in specific regions of 3009–3000 cm^{-1}, 1418–1417 cm^{-1}, 1385–1370 cm^{-1}, and 1126–1085 cm^{-1} and 966 cm^{-1} (Che Man & Mirghani, 2001). FTIR has been utilized to detect lard adulteration in vegetable oils and food products like frozen french fries, with levels as low as 1% detected in all mixtures, using spectral regions at 1405–1365 cm^{-1}, 1260–1198 cm^{-1}, 935–910 cm^{-1}, 877–857 cm^{-1}, and 857–833 cm^{-1} (Al-Kahtani et al., 2017). The study used ATR-FTIR spectroscopy to analyse the adulteration of EVOO with other edible oils like canola, corn, sunflower, and soybean oils. The main functional groups were identified using wavenumbers around 3500 cm^{-1}, 2925 cm^{-1}, 3006 cm^{-1} and 1745 cm^{-1} (Mashodi et al., 2020).

6.4.1. Detection through FTIR with chemometric analysis

Chemometrics is a field that uses statistical and mathematical methods to extract chemically important information from data generated by chemical experiments. Principal component analysis, partial least squares, principal component regressions, cluster analysis, and discriminant analysis are numerous chemometric techniques that are used to analyse oils and fats. It has a significant role in improving the efficiency of FTIR spectroscopy (Rohman et al., 2020).

FTIR spectroscopy is a potential analytical technique for distinguishing lard from lamb fat, cow fat, and chicken fat due to its ability as a fingerprint technique. Lard was blended with beef, lamb, and chicken samples in a 10–50% *v/v* ratio to obtain adulterated samples. In this study, two regions of difference (RoD) at 1700–1800 cm^{-1} and 2800–3000 cm^{-1} were identified for all samples in the FTIR spectrum. A multiclass support vector machine (M-SVM) is also used to classify pure and adulterated samples (Siddiqui et al., 2021). Lard adulteration in various vegetable oils was detected and measured using FTIR spectroscopy and PLS (Munir et al., 2019). Functional group vibrations and comparative wavenumbers of lard and beef fat, and lard and vegetable oils are summarised in Table 3.

FTIR was used to identify spectral bands associated with lard, cocoa butter, and their mixtures in chocolate formulations. The amount of lard in blends could be determined using a semi-quantitative approach. A lard-specific difference was observed at 3006.8 cm^{-1}, 1118.84 cm^{-1}, and 1097.42 cm^{-1}. Lard can also be identified in cocoa fat using PCA and PLS calibration models (Man, Syahariza, et al., 2005; Suparman, 2015). Using FTIR, various studies have established being able to distinguish between butter that has been adulterated with beef fat, and chicken fat (Ahmad Fadzillah et al., 2013; Nurrulhidayah et al., 2013).

Lard is frequently used as an emulsifier, emollient, and viscosity-increasing ingredient in cosmetics. Additionally, lard and lard-derived compounds are utilized in cosmetics such as lipstick formulation, lotion, cream, and other skin care products. Lard has been analysed using an FTIR spectroscopic approach in cosmetic products that contain coconut, olive, castor, and palm oils (Rohman et al., 2014; Waskitho

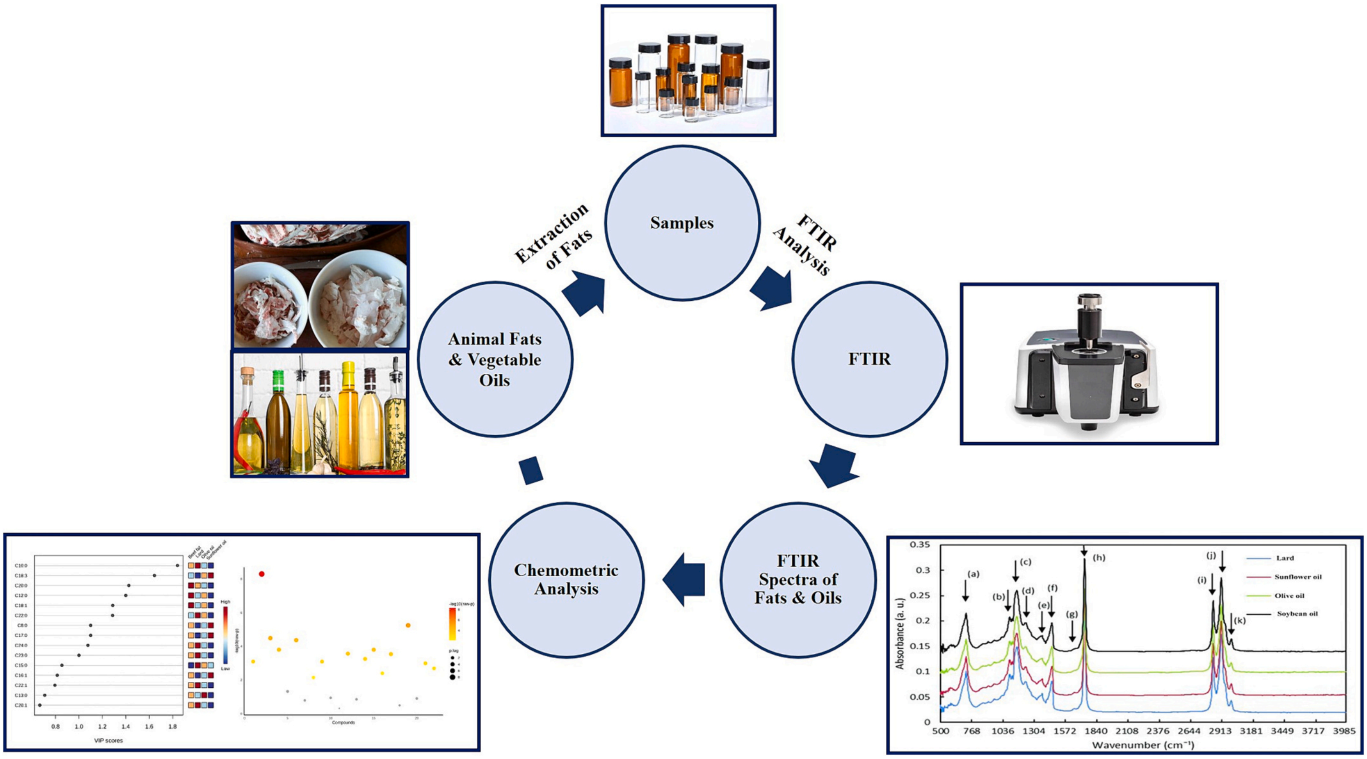

Fig. 6. Schematic representation of FTIR spectroscopy-based analysis of oils and fats.

Table 3
Functional group vibrations and comparative wavenumbers of lard and beef fat, and lard and vegetable oils (Kurniawati et al., 2014; Rohman & Che Man, 2011c).

Vibration Wavenumbers (cm^{-1})		Functional group vibrations
Lard and beef fat	Lard and vegetable oils	
3007	3005	cis C=CH stretching
2970	2954	Asymmetric stretching vibration of methyl (-CH_3) group
2925	2924	Asymmetric and symmetric stretching vibration of methylene (-CH_2) group
2875	2852	Symmetric stretching vibration of methylene (-CH_2) group
1715	1743	Carbonyl (C=O) from the ester linkage of triacylglycerol
1650	1654	cis C=C
1462	1465	Bending vibrations of the CH_2
1418	1417	Rocking vibrations of CH bonds of cis-disubstituted alkenes
1375	1377	Symmetric bending vibrations of CH_3 groups
1226	–	C–O (ether) stretching
1160	–	C–O (ether) stretching
1117 and 1098	1111 and 1097	-CH bending and -CH deformation vibrations of fatty acids
1031	1033	C-O stretching
962	962	bending vibration of CH functional groups of isolated trans-olefin
	914	Bending vibration of cis -HC-CH-
	721	Overlapping of the methylene (-CH_2) rocking vibration and to the out of plane vibration of cis-disubstituted olefins

et al., 2016).

Some studies have reported that the detection of adulteration in various fish oils with lard (Irnawati et al., 2023; Windarsih et al., 2023) and vegetable oils including sunflower, canola, palm, corn, and grape seed oils was accomplished using FTIR and chemometric analysis (Giese et al., 2019; Jannah et al., 2023; Putri et al., 2019; Riyanto & Rohman, 2021; Rohman et al., 2017).

FTIR spectroscopy was utilized to detect the adulteration of hazelnut oil with seven other oils and the adulteration of olive oil with hazelnut oil. According to the results of the chemometric analysis, 2% and 25% detection levels of adulteration were found in hazelnut oil and olive oil, respectively (Ozen & Mauer, 2002). Several studies were conducted to determine the authenticity and classification of olive oil adulteration with different oils using FTIR and multivariate analysis as already mentioned in Table 2. Ozulku and co-workers used ATR-FTIR spectroscopy along with chemometrics to identify hazelnut, sunflower, and canola oils as adulterants in cold-pressed sesame oil. They conclude that ATR-FTIR might be a quick, non-destructive tool for detecting sesame oil adulteration (Ozulku et al., 2017). Antora et al. developed an optical sensing technique using FTIR spectroscopy, which demonstrated significant potential in detecting 5–40% (*v*/*v*) of soybean oil and palm oil as adulterants in rice bran and sunflower oil, respectively (Antora et al., 2019). Moreover, FTIR with chemometrics was also utilized to detect vegetable oils tainted with used frying oil (Kou et al., 2018; Zhang, Liu, et al., 2012).

6.4.2. Near-infrared spectroscopy

Near-infrared spectroscopy measures the absorption of electromagnetic radiation in the 750–2500 nm (12,000–4550 cm^{-1}) range and consists of the light source, wavelength selector, sample and optical detectors, and data analysis/processing system. The process records transmitted and reflected radiation, with pre-treatment procedures and computer-based evaluation. NIR offers numerous benefits including rapidity, simplicity, non-destructiveness, ease of use (no sample pretreatment is required), instrument calibration, remote applications, minimal solvent usage, and simultaneous measurement of many chemical compounds (González-Pereira et al., 2021).

NIR spectroscopy in the shortwave range (950–1650 nm) is a promising method for detecting adulterated palm oil with lard. Chemometric analysis with SIMCA and PLS regression can distinguish pure and contaminated samples with model accuracy and a coefficient of determination (R^2) of more than 0.95 and 0.998, respectively (Basri et al., 2017). Hussain et al. used long-wave NIR spectroscopy (1350–2450 nm) and chemometrics analysis to detect and quantify lard in palm oil. They found that linear discriminant analysis (LDA) in Open-source R software could detect a minimal level of adulteration as low as 0.5%. PLS calibration produces good results, displaying a linear correlation with an R^2 coefficient of 0.9987 for quantification. The results supported the viability of long-wave NIR spectroscopy as an effective technique for lard adulteration detection (Hussain et al., 2023).

Multiple research studies have focused on the use of NIR spectroscopy for detecting adulteration in vegetable oils. Ozdemir et al. utilized NIR and multivariate calibration to identify adulteration in olive oil. They used the genetic inverse least squares (GILS) technique to generate multivariate calibration data that determine the amount of adulterants present in the olive oil samples, resulting in prediction errors ranging from 1.42 to 6.38% for sunflower, corn, and olive oil mixtures, and 2.49–2.88% for sunflower and olive oil mixtures (Özdemir & Öztürk, 2007). Moreover, NIR spectroscopy coupled with chemometrics was also used for detecting adulterants in sesame and sunflower oils (Chen et al., 2018; Picouet et al., 2018). Hussain and co-workers investigated to determine the palm oil adulteration with canola oil as low as 3.23% adulteration using NIR with multivariate analysis (Hussain et al., 2014).

6.5. Raman spectroscopy

Raman spectroscopy is a non-invasive, non-destructive method for identifying molecular vibrations in a food substance induced by an incident light source. The characteristics of the sample and the light's (visible, infrared, or UV) wavelength affect the interactions between the sample molecules and the light. It may be used rapidly to analyse animal fats and vegetable oils. Since it is easy to use and requires no sample preparation (González-Pereira et al., 2021). The fingerprint's spectral feature range, which comprises highly distinctive and identifying characteristics of the compounds under investigation, is predicted to be in the 400–2000 cm^{-1} range (Taylan et al., 2020).

Different fatty acids have distinct features in raman spectra to identify the presence of adulterant in oils and fats, which are crucial for understanding their nutritional and health properties (Velioglu et al., 2016). For instance, the addition of lard, lower-quality and waste cooking oils in other animal fats and high-quality edible oils (Lee et al., 2018; X. F. Zhang et al., 2011). Another way to use the waste cooking oil is as an adulterant in olive oil, and samples with varying concentrations (2.5–50%) were analysed using raman spectroscopy and chemometrics. The results showed that spectral data after SNV processing is best for synergy interval partial least square (SiPLS) modelling and forecasting, with a root mean square error (RMSE) of validation and calibration of 0.0485 and 0.0503, respectively (Y. Li et al., 2018).

Surface-enhanced raman spectroscopy (SERS) is a sensitive analytical technique used to detect and analyse molecules. It is an extension of raman spectroscopy, which measures the vibrational modes of molecules. SERS enhanced raman signals when molecules are adsorbed onto nanostructured surfaces, typically made of noble metals like gold or silver, due to localized surface plasmon resonance (LSPR) (Demirel et al., 2018). Phthalate ester (PAE) plasticizers, a frequently used industrial addition of plastic products, can easily contaminate food and endanger human health. SERS has been utilized for the fast, precise, and reliable detection of PAE plasticizers in edible oils. A two-dimensional (2D) silver plate was developed to detect potassium hydrogen phthalate (PHP), a hydrolysate of a PAE plasticizer. SERS utilized nanostructured surfaces to amplify the raman scattering signal of molecules attached to them, enabling the sensitive detection of contaminants in oil and fat samples even at low concentrations (Wang et al., 2023).

6.6. Fluorescence spectroscopy

Fluorescence spectroscopy is a valuable technique for detecting adulteration in foods such as edible oils and fats. It enables the characterization of the sample by absorbing light energy and reemitting it at longer wavelengths. Edible oils and fats contain various fluorophores like tocopherols, chlorophylls, and polyphenols, depending on the botanical source and extraction technology. It is a non-invasive technique that does not require sample preparation and is trustworthy enough to provide an accurate and genuine analysis. Differences from the predicted profile might be a sign of mixing or adulteration. Moreover, FS enables quantitative analysis by measuring fluorescence intensity. Refined oils are more susceptible to oxidation due to reduced vitamin E and chlorophyll concentrations. The emission fluorescence spectra of refined oils show oxidative changes in fatty acids, with a broad peak for excitation radiation at 365 nm (SádeCká & TóThoVá, 2007). Single fluorescence spectroscopy and synchronous fluorescence spectroscopy (SyFS) can be performed with a fluorescence spectrometer. While SyFS is better for examining complicated multicomponent systems. Conventional fluorescence spectroscopy can reveal specific emission peaks when a precise excitation wavelength is selected (Dankowska et al., 2014).

FS is commonly used to verify the authenticity of extra virgin olive oil (EVOO). It is used to investigate the adulteration of sunflower oil in pure EVOO using PCA analysis and a PLS regression model. The spectra show chlorophyll and carotenoids as fingerprints, distinguishing EVOO from sunflower oil. Adulterated EVOO shows visible changes with increased sunflower oil concentration (Ali et al., 2018). Adulterated EVOO with seed oils, such as soybean, sunflower, and rapeseed oils, can be detected using synchronous fluorescence spectra. According to the lowest limit of detectability and RMSE for fluorescence intensity at a wavelength interval of 30 nm, this method proved successfully in detecting adulteration (Dankowska et al., 2013). LED-induced fluorescence spectroscopy can determine if EVOO has been mixed with soybean or peanut oil. By using regression and pre-processing techniques, a prediction model can be created that shows how much of the oil has been adulterated and also provides a linear relationship between the actual and expected adulteration concentrations (T. Zhang et al., 2022). Fluorescence combined with chemometric analysis is used to examine EVOO adulterated with corn, soybean, linseed, and sunflower oils. The study found that synchronised PLS-R models for detecting EVOO percentage in vegetable oil blends had a lower root mean square error and a higher R^2 value compared to PLS-R models used on the entire spectrum. An additional technique for identifying adulterated olive oil is artificial neural network (ANN). The type of data input and cross-validation method affect the ANN model's performance (Lia et al., 2018). The detection of adulterated walnut and peanut oils with various vegetable oils and waste frying oils was accomplished using fluorescence spectroscopy (Ge et al., 2014; Hao et al., 2019; Huyan et al., 2018).

6.7. Nuclear magnetic resonance (NMR) spectroscopy

NMR spectroscopy is the study of the absorption of radio frequency radiation by a nucleus. NMR (^{1}H, ^{13}C, ^{31}P) is a powerful, rapid, non-destructive technique used to study the structure and composition of organic molecules, including animal fats and vegetable oils. NMR is a crucial tool in the application of food, lipids that is acquiring more and more uses because of the bounty of information available (Guillén & Ruiz, 2001). It provides information on mixture composition in a single spectrum without derivatisation or pre-treatment, requires minimal sample preparation, and is quantitative, allowing for molar concentration determination. It is efficient in fatty acid characterization, determining fatty acid profile, classifying edible oils, monitoring oxidation, assessing purity, and detecting adulteration. There are numerous methods for using NMR, including proton nuclear magnetic resonance (^{1}H NMR), low-field NMR, diffusion NMR, time domain NMR, and ultrafast 2D NMR. The NMR spectra produced are too complicated to be classified visually; hence, the use of NMR in conjunction with chemometric analysis was carried out for the classification (Di Pietro et al., 2020).

Butter adulteration with lard is also a fraudulent practice. ^{1}H NMR spectroscopy can identify all proton-bearing components of tainted butter with distinctive characteristics in the 2.60–2.84 ppm range. NMR and PLS regression were used for analysis, which provide simultaneous quantification of all compounds. PLS calibration showed the lowest root mean square error calibration (RMSEC) of 0.991 and the highest regression coefficient (R^2) of 0.998 (Fadzillah et al., 2015; Fadzillah et al., 2017).

NMR spectroscopies, including ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR, were used to identify adulteration of olive oil with other vegetable oils, including soybean, corn, cottonseed, sunflower, and hazelnut oils. ^{1}H NMR was used to identify fatty acids and iodine numbers, whereas ^{31}P NMR was used to quantify minor chemicals like phenolic compounds, diacylglycerols, sterols, and free fatty acids (free acidity). Canonical discriminant analysis (CDA) and classification binary trees (CBTs) were used to categorize refined oils, effectively distinguishing between hazelnut and olive oils. Subsequent NMR data with CDA enabled the detection of hazelnut oil in olive oil at greater than 5% (Agiomyrgianaki et al., 2010). ANN has been developed to detect hazelnut oil in olive oil using ^{1}H NMR and ^{13}C NMR data. The model was tested on olive oil, hazelnut oil, and blended olive oil with 2–20% hazelnut oil. A blend and pure sample sets were also used as an external validation of this mathematical model. The model's detection threshold was about 8% (García-González et al., 2004). Moreover, the adulteration of edible oils with used frying oil (UFO) and other oil samples was also detected using low-field NMR spectroscopy (Q. Zhang et al., 2013; Zhu et al., 2017).

6.8. Direct mass spectrometry

Mass spectrometry (MS) is a technique used for identifying and characterizing lipid profiles in food products, including quality control, authenticity testing, and detecting adulteration. It generates ions from samples and measures their mass-to-charge ratio, allowing for the identification and quantification of components. MS offers advantages over chromatography-based methods, such as simplicity and speed, eliminating complex separation steps and reducing sample preparation errors. It is an efficient and user-friendly alternative for detecting adulterants in food and non-food samples (Ahamad et al., 2022).

6.8.1. Matrix-assisted laser desorption/ ionization (MALDI) mass spectrometry

MALDI-MS is a fast and reliable method for detecting food fraud due to its ease of use and speed in collecting data from complex samples. It works by using a pulsed laser beam to hit the sample, which consists of co-crystals of the matrix and the analyte. It releases ions that form charged analyte molecules. A TOF mass analyser separates these ions based on their m/z ratios. The linear geometry of TOF analysers is used for high molecular weight molecules, while a "reflectron" configuration balances initial velocities for smaller molecules. MALDI mass spectrometers can generate spectra, profiles, and fingerprints from food matrices with simple or no sample preparation. The potential of MALDI in combating food fraud is well-known, with most applications focusing on evaluating food quality and detecting adulterations (Zambonin, 2021).

High-resolution mass spectrometry such as MALDI-MS could also be used for the detection of olive oil adulterated by vegetable oils including hazelnut, corn and sunflower oils. The adulteration of extra virgin olive oil (EVOO) with hazelnut oil (HO) poses a significant issue for both oil suppliers and consumers due to the high similarity between the two oils in terms of triacylglycerol, total sterol, and fatty acid profiles. Phospholipids (PLs) are typically present in seed oils. A sample pretreatment procedure for phospholipid extraction involved modifying the Bligh-Dyer extraction method by introducing an ionic liquid made from the combination of tributylamine (TBA) and α-cyano-4-hydroxycinnamic acid (CHCA). The extracted samples were then analysed using MALDI TOF-MS. This technique, combined with specific extraction and enrichment of phospholipids, proved effective in detecting even a 1% adulteration of HO in EVOO (Calvano et al., 2012). Additionally, the adulteration of EVOO with corn oil (CO) was also successfully detected phospholipids using MALDI-MS combined with advanced statistical techniques, including unsupervised hierarchical clustering (UHC), principal component analysis (PCA), and Pearson's correlation analyses. These methodologies enhanced the detection capability, revealing corn oil adulterations in EVOO at remarkably low levels, down to 0.5% (Di Girolamo et al., 2015). The study developed a fast protocol for detecting adulteration of sunflower oil (SoO) in extra virgin olive oil (EVOO) by mass spectrometry fingerprinting of triacylglycerol (TAG) profiles based on MALDI-MS. This technology, coupled with statistical analysis, was proven useful for the detection of adulteration in EVOO at a rate down to 1% (Jergović et al., 2017).

6.8.2. *Electrospray ionization mass spectrometry (ESI-MS)*

ESI-MS is a sensitive and specific technique used to detect adulteration in lipids. It creates a distinct lipid profile for each sample, enabling the detection of unauthorized substances and the quantification of specific lipid species. ESI-MS requires minimal or no sample preparation and is particularly effective for polar molecules. (Catharino et al., 2005).

The adulteration of extra virgin olive oil (EVOO) by adding cheaper vegetable oils was detected using ESI-MS, and the mass spectrometry data was analysed using principal component analysis (PCA) and hierarchical cluster analysis (HCA). Authentic and adulterated samples were classified into different groups using these methodologies, suggesting potential alternatives for the quality control of EVOO. The developed methodologies were simple, fast, and precise, capable of detecting adulteration ranging from 1 to 20% (*w/w*) (Mitsutake et al., 2015). Some other studies have also demonstrated the use of direct infusion electrospray ionization (ESI) and chemometric analysis for monitoring olive oil adulteration (J. Alves et al., 2014; Alves et al., 2013).

6.9. *Polymerase chain reaction (PCR)*

PCR is a molecular biology technique used to amplify specific DNA sequences, particularly for detecting edible oils and fats. PCR-based assays are highly sensitive, precise, and effective in detecting adulterated oils and fats. PCR analysis targets nuclear or mitochondrial DNA genes (nDNA and mtDNA) to identify the adulterating species, and it cannot provide information about the amount or proportion of adulterant present in the sample. Other methods, such as chromatography, may be needed for more detailed information. nDNA is larger and contains more sequence variation, while mtDNA is commonly used for species identification in food analysis due to its intact nature during food processing, minimising DNA degradation, and containing no introns (Vaithiyanathan & Kulkarni, 2016).

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of a template sequence in the mt cyt b gene has been established as a tool for species identification from pig fat samples. This work demonstrates that mt-DNA is suitable as a PCR template and for routine cyt b detection. These findings suggest that PCR-RFLP analysis of cyt B is a reliable, simple, and verified methodology for species identification (Aida et al., 2005). Lard is commonly used by food companies in place of oil since it is more affordable and widely accessible. DNA analysis is a quick and affordable method to identify plant oils. The extraction of DNA is not possible from refined vegetable oils. In fact, according to author, DNA is removed throughout the processed plant oils (Pauli et al., 1998). Other claims that DNA is generally retained during refining processes, so PCR makes it feasible that DNA is retrieved from a significant test portion (Gryson et al., 2004).

Real-time PCR was used to identify lard in chocolate, but it failed. To assess the inhibitory effects of chocolate constituents, milk powder, sugar, cocoa powder, and cocoa butter were mixed with lard and tested using a porcine-specific real-time PCR. The results showed that cocoa powder prevented fat DNA extraction from chocolate, but no significant inhibition was found, confirming the cocoa powder's inhibitory effect on lard DNA extraction from lard-adulterated chocolate (Rosman et al., 2016).

Several investigations employing DNA-based methods for olive oil authenticity have produced promising outcomes. For example, molecular markers contain sequence repeats, single nucleotide polymorphisms, and amplified fragment length polymorphisms use to identify representatives of a species. These markers are known as an organism's genetic material. These indicators can assist in determining the species and variety from which olive oil was derived, as well as information on the varietal origin of the oil. They are also capable of identifying any vegetable oil combined with olive oil. As a result, utilizing one of these markers in DNA analysis is a viable method for identifying adulterated olive oil (Kalaitzis & El-Zein, 2016). Real-time PCR combined with high-resolution melting analysis were used to compare the DNA melting curves and test the adulteration of EVOO with various vegetable oils including maize, sesame, sunflower, peanut, and hazelnut oils. An adulterant in a sample at a rate of less than 10% can be detected using DNA analysis. This technique is quick, adaptable, and economical for identifying the olive oil adulterated with another vegetable oils (Vietina et al., 2013). A rapid, simplified genotyping methodology is developed to identify adulteration in olive oil with sunflower, and canola oils using a combined approach of molecular biology and bioinformatics. This method uses specific SNPs found in conserved DNA sequences of the plastid genomes, allowing for fast detection of up to 5% adulteration in olive oil (Kumar et al., 2011).

Zhang et al. employed the capillary electrophoresis single-strand conformation polymorphism (C*E*-SSCP) approach based on PCR to discriminate between the six commercially available edible oils, including olive, peanut, soybean, sesame, sunflower, and maize oils. Different plant oils have distinct forms of the chloroplast rbcL gene encoded by the ribulose 1,5-bisphosphate carboxylase that utilized to identify the type of oil in the sample. The SSCP analysis demonstrated a distinct, unique amplicon peak for each of the six oils and allowed for complete separation between all oil plant species. Consequently, the results showed that the PCR approach is a powerful method to identify adulteration in edible oils, with a ratio ranging from 10 to 30% (Zhang, Wu, et al., 2012).

6.10. *Electronic nose (E-nose)*

An electronic nose is a device that uses sensors to detect and identify odours or volatile organic compounds (VOCs) in the air. It is designed to mimic the sense of smell and to analyse the chemical composition of air samples. It has been asserted that the operational concept of *E*-Nose mimics that of the human nose. An electronic nose can be used to detect animal fat adulteration by analysing the VOCs that are released from the sample. Fatty acids and other compounds in animal fats produce characteristic VOCs that can be detected by the electronic nose sensors (Latief et al., 2017). Consumers are unaware of the adulteration of edible oil with less expensive oils and fats. Many studies have been conducted on the use of the electronic nose to detect adulteration in edible oils are represented in Table 2.

The rapid gas chromatography-surface acoustic wave (GC-SAW) is a kind of an E-Nose device that uses the fundamental GC concept and data information obtained by visual profiling of the volatile components in the forms of fragrance amplitude of radial plots. Rapid GC-SAW technique was used for the detection of virgin coconut oil (VCO) adulteration with lard and palm kernel olein at varied concentrations ranging from 1 to 50% (*v/v*) and 1–20% (*wt/wt*), respectively (A. M. Marina et al., 2010; Tengku Mansor et al., 2011). It was concluded that it is a quick, reliable, minimal sample preparation needed, chemical-free, and non-destructive technique.

6.11. *Differential scanning calorimetry (DSC)*

DSC is a technique used to identify adulterated animal fats and vegetable oils in food and non-food samples by analysing thermal behaviour. This method offers benefits over classical detection techniques, such as rapidity and no sample preparation or solvent use. It can be used as a fingerprint to differentiate between pure animal fats and other oils or fats used in food products (Ahad & Nissar, 2017).

DSC is a thermo-analytical device used to fingerprint the initial crystallization and transition behaviours of TAGs molecules. TAGs of pure and adulterated animal fats were also studied for their crystallization and melting parameters using PCA. DSC coupled with PCA was used to distinguish between lard adulteration in beef and chicken fats at a dose of less than 0.5% (Dahimi, Rahim, et al., 2014). DSC can also be used to detect butter adulteration with lard. DSC provides a direct estimation of the total enthalpy change of lard transitions as well as a distinctive thermal profile for butter and lard (Nurrulhidayah et al., 2015). The application of DSC to the detection of adulteration in edible oils, animal fats, and fat-based products has been explored in several papers. For example, DSC has been successful in determining animal fat adulteration in canola, sunflower, and palm oils (J. Marikkar et al., 2012; Yanty et al., 2014); lard in virgin coconut oil (Mansor et al., 2012), and RBD palm oil (Marikkar et al., 2001); extra virgin olive and virgin coconut in other seed oils (Chiavaro et al., 2008; Chiavaro et al., 2009; Marina et al., 2009).

7. A comparative study of various analytical approaches for the detection of edible oils and fats

Comprehensive comparisons of analytical techniques for determining adulteration in edible oils and fats reveal a diverse toolkit with distinct advantages and limitations. Chromatography methods, such as GC and HPLC, offer high precision and sensitivity for quantitative analysis. LC-MS and GC–MS provide structural information for identifying specific fatty acids and lipids. However, these methods can be resource-intensive and require extensive sample preparation. Spectroscopic techniques like FTIR, NIR, Raman, fluorescence, and NMR offer non-destructive and rapid analysis, but these methods might not have the resolution needed for comprehensive compositional analysis. Chromatography techniques are better than spectroscopic techniques in several ways. For one, they have better detection limits, which means they can detect smaller amounts of substances, and they also offer greater precision and accuracy when analysing a sample. One of the most significant advantages of chromatography is that the results are not affected by the presence of water, which can be a big issue when using spectroscopy. Contrarily, HPLC and GC techniques are expensive and time-consuming because samples must first be prepared before being analysed, require a significant amount of unfriendly solvents, and require skilled chemical analysts; as a result, their application for authenticating food is inadequate (Sudhakar et al., 2023).

Rapid screening methods using direct mass spectrometry are the preferred choice due to their simplicity and elimination of chromatographic steps. DNA-based methods for oil authentication and traceability have gained attention in recent years. PCR-based techniques require sample preparation steps, such as DNA extraction, which can lead to low yield and poor quality due to DNA degradation during food processing at high temperatures. The extraction procedure takes more time, resources, and equipment and might provide inaccurate findings and make it harder to repeat the research. *E*-Nose technology provides a distinctive method for sample discrimination and succeeds at differentiating odorous chemicals. DSC is a direct and easy-to-use method for studying the thermal properties of lipids, unlike chromatographic techniques that require sample derivatization and solvent use. DSC represents thermal events associated with lipids through both cooling and melting thermograms.

The combination of FTIR spectroscopy and chemometrics has proven to be very effective in the authentication analysis of oils and fats. Chemometrics combined with other analytical methods is a potent tool for adulterant and quality evaluation.

8. Conclusion and future perspective

Edible oils and fats are fundamental components of various culinary preparations worldwide. Adulterated oils and fats are illegal in almost every country and are a common practice. Profit-driven behaviour has led to the use of adulterants in food and non-food samples that have adverse impacts on human health. There has also been a growing concern among consumers about the authenticity of halal food products. Oil and fat adulteration has existed since the beginning of time, and research to address it is still ongoing. The current market is still in need of a straightforward, quick, trustworthy, and affordable technology or technique that can quickly identify adulterants in any sample. Product authenticity is so important for consumers, leading to the development of simple, rapid, cheap, and advanced techniques for the detection of adulteration. This review article provides a comprehensive overview of analytical approaches for determining adulteration of animal fats and vegetable oils in food and non-food samples. Several adulteration detection techniques like chromatography, spectroscopy, direct mass spectrometry, DSC, *E*-Nose, and DNA-based studies have been applied to investigate the adulterants. The diversity of these methods highlights the sophistication and versatility of modern analytical tools in this field. Several positive traits are emerging as we look to the future. Improvements in instrumentation and automation are likely to improve the accuracy and speed of analysis, making it more practical for everyday quality control in sectors like food processing. Overall, the importance of analytical methods in ensuring the integrity and safety of food and non-food products containing animal fats and vegetable oils is underscored. In the future, research should concentrate on establishing standardized protocols and methodologies for identifying adulteration in animal fats and vegetable oils using analytical methods like GC–MS and MALDI-MS to ensure uniformity and reproducibility across studies. In addition, there is a necessity to investigate rapid, non-destructive screening techniques such as advanced spectroscopic methods, which can enable fast on-site detection of adulteration. Furthermore, integrating advanced statistical analyses and machine learning algorithms can enhance the interpretation of intricate data sets and improve the accuracy of detecting subtle adulteration patterns.

CRediT authorship contribution statement

Nayab Kanwal: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Syed Ghulam Musharraf:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.140786.

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