

**ATR-FTIR SPECTROSCOPIC ANALYSIS FOR
FORENSIC DIFFERENTIATION OF COOKING OILS**

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LIST OF ABBREVIATIONS

TAGs	Triacylglycerols
SFA	Saturated fatty acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
FTIR	Fourier Transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
PCA	Principal Component Analysis
LDA	Linear Discriminant Analysis
VOCs	Volatile organic compounds
e-nose	electronic nose
GC-MS	Gas Chromatography-Mass Spectrometry
NIR	Near-infrared
ATR	Attenuated Total Reflectance
IR	Infrared
MIR	Middle infrared region
PCs	Principal components

LIST OF SYMBOLS

cm^{-1} Wavenumber

% Percent

ABSTRACT

The accurate classification of cooking oils is important for quality control, and forensic investigations such as food fraud and adulteration. In this study, attenuated total reflectance–Fourier transform infrared (ATR-FTIR) spectroscopy combined with chemometric analysis namely PCA and LDA was used to discriminate various type of cooking oils based on their spectral profiles. The type cooking oil samples analysed in this study were (i) black seed oil, (ii) canola + sunflower oil, (iii) canola oil, (iv) castor oil, (v) coconut oil, (vi) corn oil, (vii) olive oil, (viii) perilla seed oil, (ix) palm oil, (x) rice bran oil, (xi) sesame oil and (xii) sunflower oil. The spectrum of ATR-FTIR in both fingerprint region ($1800\text{--}600\text{ cm}^{-1}$) and the C–H stretching region ($3050\text{--}2800\text{ cm}^{-1}$) was not reliable due to overlapping spectral features in both spectral regions which made it harder to differentiate different types of cooking oil. Therefore, PCA which is an unsupervised method was performed where clear clustering of the cooking oils can be seen in the scatterplot of PC2 versus PC3 especially for black seed oil, perilla oil coconut oil and palm oil in the second region. The chemometric analysis was followed by a supervised method namely, LDA where the principal component (PC) score obtained from PCA for its respective region. Overall classification accuracy for fingerprint region was only 50.8% where misclassification of samples was observed in canola, sunflower, and corn oils due to having similar chemical structure. In contrast, the second region has very high accuracy for classification of cooking with 90.0% accuracy. Sunflower oil, canola oil and the blend of sunflower and canola oil was later re-classified as vegetable oil to improve the accuracy of classification. This has greatly improved overall accuracy to 91.7%. However, sesame oil remained as the most misclassified sample due to having very similar chemical structures to other type of cooking oils. The findings in this study demonstrate that ATR-FTIR spectroscopy coupled with PCA-LDA is a rapid, non-destructive method to discriminate various fresh cooking oils.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Cooking oils are lipids made from plants, animals, or synthetic compounds which are used to fry, bake and prepare food for preparing daily meal. Three different types on lipid in cooking oils are, triacylglycerols, phospholipids, and sterols (Anderson, et al., 2022). Triacylglycerols are the main component of edible oil with more than 95% and they provide a significant source of energy. Triacylglycerols consists of three fatty acids (FA) esterified on a glycerol backbone (Zhang, et al., 2018). Phospholipids are naturally found in edible oils and can represent around 0.1-1.8% of the total extracted lipid (Meng, et al., 2014). However, phospholipids will be removed from cooking oil using degumming process because they will cause undesirable effects such as discolouration and foaming. Sterols are naturally found in cooking oils as well where vegetable oils contain phytosterols and animal fats contains cholesterol (Tsimidou, et al., 2023).

Some of the examples of cooking oil derived from plants are sesame oil, palm oil, corn oil, sunflower oil and olive oil. Each type of oil has a unique chemical composition influenced by its botanical source, processing, and storage conditions (Giacomino, et al., 2023). In forensic science, the characterisation and differentiation of oils can be very useful in case such as food fraud or adulteration. Food fraud is the intentional deception of consumers for economic gain (Lombardo, 2025), for example, by replacing a more expensive oil with a cheaper oil, which is commonly done for expensive oils such as olive oil and avocado oil. This can be done by adding the cheap oil of the same type of oil or adding another different type of oils to increase the overall bulk (Pereira, et al., 2021). This is dangerous as the other oil might trigger allergic reaction for the consumers. Apart from that, many cooking oils in the market are repackaged or falsely labelled, such as cheap oils being sold as premium brands.

However, differentiating between various types and brands of cooking oils is challenging because they share similar chemical compositions which are mainly composed of triglycerides and fatty acids. Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) can be used to discriminate various cooking oils and brands based on their spectral profiles. ATR-FTIR is a tool that can be used in non-destructive analysis to obtain molecular fingerprints of substances (Grzelec, 2024). This method is helpful in forensic analysis due to limited sample quantity. After that, ATR-FTIR data can effectively differentiate between oils based on their spectral differences using chemometric techniques such as Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA).

1.2 PROBLEM STATEMENT

Cooking oils appear very similar to each other from colour and odour. For example, canola oil is normally pale yellow in colour, while olive oil ranges from pale yellow to deep green. The refining process could affect the colour of the oil. Refined oils tend to be lighter in colour compared to unrefined oils. This is because impurities and pigments are removed from the oil during refining process producing, thus light-coloured oil. However, some oils may be bleached or deodorized to alter their colours (savorysaga, 2024). As for odour, most of the oils have neutral scent. This makes forensic identification challenging when only small amounts of samples are obtained as cooking oil looks similar and have similar odours.

The physical nature of cooking oil and economically motivated factor could lead to food fraud where cooking oils sold in the market are repackaged or falsely labelled for financial gain. For instance, cheap cooking oils may be repackaged and sold as premium brand oil for higher price. However, production fraud where the oil composition is changed such as peanut oil being sold as other cooking oil could lead to economic fraud. Such act could also pose serious risks to consumers with allergies after consuming food cooked using that adulterated cooking oil.

There are many analytical techniques that can be used to discriminate the composition of cooking oils such as Nuclear Magnetic Resonance (NMR), Differential Scanning Calorimetry (DSC), and High-Performance Liquid Chromatography (HPLC). However, these methods are time-consuming and destructive. Therefore, ATR-FTIR is a better choice of method because very minimal to no sample preparation is needed and it is a non-destructive method. However, the studies of differentiation of cooking oils using ATR-FTIR are still limited especially using samples from Malaysia.

1.3 RESEARCH QUESTIONS

The research questions of this research will be as follows:

1. Can ATR-FTIR spectroscopy effectively differentiate between various types of cooking oils?
2. What are the distinctive spectral features (functional group regions) that enable the discrimination of cooking oil?
3. Can multivariate analysis (e.g., PCA) enhance the differentiation and classification accuracy of these oils?

1.4 RESEARCH OBJECTIVES

The general objective is as stated below:

- To discriminate various type of cooking oils based on their spectral profiles using Attenuated Total Reflectance–Fourier Transform Infrared (ATR-FTIR) spectroscopy.

The specific objectives are as stated below:

1. To compare ATR-FTIR spectra of different types of cooking oil.
2. To discriminate fresh cooking oils using Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA).

1.5 SIGNIFICANCE OF STUDY

This study provides a rapid, simple, and non-destructive analytical approach for the differentiation of cooking oils using ATR-FTIR spectroscopy. Chemometric methods such as Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) can be used to interpret subtle spectral differences which may not be visually distinguishable. Therefore, by applying these chemometric methods, the reliability of oil identification can be improved. Apart from that, from this study, a reference spectral database for common cooking oils and brands can be created for forensic comparison of unknown samples. This plays a huge role in forensic case investigation involving food adulteration, quality control and trace residues in forensic casework which strengthens the scientific foundation for evidence interpretation.

1.6 SCOPE OF RESEARCH

This research was carried out at laboratory of School of Health Science, University Sains Malaysia. This research covers the discrimination of various type of cooking oils based on their spectral profiles and chemometric methods (PCA and LDA). In this study, only fresh cooking oils available in the Malaysia market were used. The analysis is limited to the mid-infrared region ($4000\text{--}600\text{ cm}^{-1}$) and employs standard preprocessing techniques such as baseline correction and normalisation. Chemometric analysis is confined to PCA for pattern recognition and LDA for supervised classification. The study aims solely to evaluate the potential of ATR-FTIR combined with PCA and LDA for differentiating oil types and does not attempt to determine geographical origin, fatty acid profile, or production quality.

1.7 THESIS OUTLINE

This thesis consists of a total of five chapters which consists of the introduction, literature review, methodology, results and discussion, and conclusion and recommendations. Chapter One introduces the background of the study, problem statement, research questions, research objectives, significance of study and scope of research. Chapter Two presents a comprehensive literature review, including the composition and physical examination of

cooking oils, principles of ATR-FTIR spectroscopy, and the application of chemometric techniques in forensic analysis. Chapter Three describes the methodology regarding the materials, sample preparation, instrument parameters, and data analysis methods used in this research. Chapter Four presents the results and discussion, including the physical examination of cooking oils, ATR-FTIR spectral profiles, and the outcomes of PCA and LDA and discusses the findings. Finally, Chapter Five provides the conclusions drawn from the study, limitations of this study and recommendations for future research.

CHAPTER 2

LITERATURE REVIEW

2.1 COOKING OILS AS FORENSIC AND ANALYTICAL SAMPLES

Cooking oils are very widely used ingredient in human daily life diet to prepare food. Frying improves the sensory and texture of the food by producing golden colour, crispy texture and juicy fillings (Pascual, et al., 2024). It also helps to supply energy to maintain body normal temperature. Three types of vegetable oil that have dominated the world market are palm oil, soybean oil and rapeseed oil due to their high stability.

On the other hand, perilla seed oil, evening primrose oil, and grape seed oil are not generally consumed by people due to low production rate (Tian, et al., 2023). Vegetable oils are also considered as economic sources because nutritionists recommend people to use vegetable oils for cooking instead of margarine. This is because margarines are mostly composed of fats while vegetable oils contain essential fatty acids and fat-soluble vitamins A, D, E, and K which are needed by human metabolism (Rohman, et al., 2020).

Cooking oil contains mainly triglycerides which are formed from glycerol and fatty acids. Triacylglycerol profile in cooking oils can be used to determinate the authenticity, origin and eventually evidence frauds or adulterations (Indelicato, et al., 2017). It depends on the fatty acid composition, degree of saturation and presence of pigments such as carotenoid. These functional groups influence the physical and chemical properties of the cooking oil. Some of the functional group of cooking oils may be different because it depends on the botanical origin and technological process of oil obtention (Pascual, et al., 2024).

In forensic science, cooking oils may be encountered in cases such as adulteration, contamination and trace evidence. Cooking oils are non-volatile which allows them to stay on surface for a longer time which increases their potential evidential value. Adulteration is a very

common problem in food business where adulterated cooking oils are being sold to restaurants.

2.1.1 COMPOSITION OF COOKING OILS

Cooking oils contain to 98% of TAGs and phytochemicals. Fatty acids in cooking oil can be divided into several groups such as saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) (Socaciu, et al., 2022). In vegetable oils, typically only one type of fatty acid will predominate which determines the physicochemical properties such as melting point, odour and oxidative stability. For example, oleic acid is the major fatty acid found in olive oil (Tian, et al., 2023).

Phytochemicals such as tocopherols, phytosterol, squalene, carotenoids, phenolics, phospholipids are found in cooking oil as well and each of the phytochemicals brings its own benefit. Tocopherols also known as tocopherols and tocotrienols prevent the peroxidation of unsaturated lipids to produce good quality cooking oil. Phytosterol have similar function and chemical structure as cholesterol which is to provide energy to human body. From a study conducted by Tian et al. (2023), highest amount of phytosterol was found in rice bran oil followed by corn oil (Tian, et al., 2023). These minor components present in low concentration in oil and contribute to oil quality, stability, colour, and aroma.

2.1.2 VARIABILITY AMONG DIFFERENT TYPES OF COOKING OILS

The chemical composition of different types of cooking oils depends on many factors such as gene type, growth conditions, climate, and processing methods. For example, phenolic compound found in cooking oils is highly dependent on plant source agriculture treatment and technology of oil extraction (Tian, et al., 2023). Oils derived from different plant species present characteristic profiles of fatty acids, triglycerides, and minor constituents, reflecting genetic, biochemical, and environmental influences.

Cooking oils are obtained via solvent extraction process of seeds or mechanical expelling. During production, other components such as free fatty acids, unsaponifiable matters, waxes, pigments oxidation products (peroxides, aldehydes, ketones, alcohols) are produced as well. This results in production of low-quality oils with bad odour and colour. Apart from that, contamination during production could also cause variability in cooking oils. For example, pesticides used to grow the crop could be found in oil. Therefore, oils must undergo refining process to remove all these unwanted components to produce high quality oil which is more resistance to oxidation (Gharby, 2022). However, removing all these unwanted components while maintaining certain characteristics, flavour and nutritional value is very challenging (Vaisali, et al., 2015).

Chemical refining of oil involves six stages namely degumming, neutralisation, washing and drying, bleaching, dewaxing and deodorizing (Pascual, et al., 2024). Degumming is to remove phospholipids, neutralisation to remove free fatty acids, chlorophylls and so on. The third step which is washing and drying is to eliminate residuals of soap and water. Bleaching is to eliminate pigments, and dewaxing is to remove wax from oils. The last step, deodorizing helps to remove volatiles, carotenoids, and free fatty acids producing a good quality oil which is marketable (Gharby, 2022). Different types of oil require different methods of refining process which results in variability among different types of cooking oils.

Besides that, the origin of oilseed crop, the cultivation method, the storage conditions and the climate also play a role on causing variability among different types of cooking oils. From a study investigating about the component of safflower oil from Turkey, Sudan, India, and Nigeria has discovered that the samples from India and Sudan shows the highest phenolic content and the samples from Nigeria and Sudan shows the highest total for tocopherol content (Li, et al., 2025).

2.1.3 FORENSIC RELEVANCE OF COOKING OILS

Cooking oils are valued by consumers for their nutritional value such as vitamin E and for improving the taste and texture of the food. However, it raises concern to the consumers whether the cooking oils being sold at store are adulterated or not. Consumers are worried about the quality and safety of the cooking oils while the food producers are motivated to generate more income (Socaciu, et al., 2022).

Adulteration in this case refers to substituting expensive materials with cheaper materials to increase their profit. However, replacing these materials may threaten consumer health. For example, extra virgin olive oil is generally bought by consumer solely for its health benefits. For instance, the manufacturer mixing low-priced oil with olive oil and promotes it as extra virgin olive oil. As a result, consumers may face adverse side effects such as allergic reactions from consuming these adulterated cooking oils because it is nearly impossible to differentiate cooking oils with naked eye (Tan, et al., 2021).

The authenticity of oil is very important due to the significant differences in chemical structure, price and safety between cold-pressed oil and refined. For example, refined olive oil should not be sold as expensive as cold-pressed extra virgin olive oil. Most importantly, the manufacturer committing food fraud such as mislabelling refined olive oil as extra virgin olive oil to deceive consumers and for economic purposes (Indelicato, et al., 2017).

Spectroscopic techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Raman Spectroscopy, Nuclear Magnetic Resonance (NMR) Spectroscopy and so on can be used to detect adulteration in cooking oils. This is followed by chemometric techniques such as Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) to characterize adulteration in various cooking oils. This is important as part of quality control and forensic sample authentication strategies.

2.2 PHYSICAL EXAMINATION OF COOKING OILS

Physical examination of cooking oils typically involves observing the colour and the odour of cooking oil. These physical properties are influenced by the chemical composition of the oil including fatty acid content, degree of unsaturation, and presence of minor components such as pigments and oxidation products. Chlorophyll pigments and phospholipids play a role in appearance and oxidative stability of cooking oil.

Some volatiles with functional group such as aldehydes, ketones and alcohols may cause unpleasant odour and flavour (Endo, 2018). Colour of cooking oil is typically a yellowish to greenish hue in unrefined oils due to carotenoid and chlorophyll pigments. These physical properties can be used as a preliminary screening to determine the type of cooking oil. However, most of the cooking oils share similar colour and odour which makes it not reliable. Therefore, analytical techniques such as spectroscopic and chemometric methods can be used for reliable differentiation among different types of cooking oil.

2.2.1 COLOUR AND APPEARANCE

Cooking oils typically appear pale yellow in colour when freshly prepared after refining process. However, the colour of cooking oil may gradually darken due to the oxidation which occurs during transport, storage and consumption. Consumers generally prefer light coloured oil because as they are considered fresh and high quality. The gradual colour change in oil is known as colour reversion. Colour reversion can be slowed down by maintaining the optimum temperature during refining process. Apart from that, pretreatment of oilseeds and proper storage conditions (Chen & Sun, 2023).

Carotenoids and chlorophylls are the natural pigments present in cooking oils which influences its colour. Carotenoids are responsible for red, yellow, and orange colour while chlorophylls are responsible for green colours of vegetable oils. There are two main forms of chlorophylls which are chlorophylls a and chlorophylls b. Chlorophylls a produces blue-green

colour while chlorophylls b produces yellow-green colour (Abedi, et al., 2025). The concentration and type of these pigments lead to visible colour differences among oils such as olive, corn, and palm oil.

Processing pigments is used to improve the appearance of the cooking oil. For example, oxidative polymerisation of monoacylglycerols is used to fix the colour of rice bran oil during refining process to make it marketable. However, visual examination can be influenced by factors such as transparency, lighting conditions, and observer bias. This makes it unreliable for identification or classification of oil type. Moreover, many different types of oil have similar colour due to the similar refining profile and processing pigments added to the cooking oil.

2.2.2 ODOUR CHARACTERISTICS

The aroma of cooking oil is an important characteristic for differentiating different types of cooking oil. Each type of cooking oil has specific aroma depending on the oilseed and refining process (Hu, et al., 2014). Volatile compounds such as aldehydes, ketones, alcohols, esters, and hydrocarbons are produced from lipid oxidation, thermal degradation, and enzymatic reactions (Zhang, et al., 2019). These compounds are responsible to create distinctive aroma among different types of cooking oil. For example, sesame seed undergoes Maillard reaction producing roasted nutty aroma which is widely used for food preparation (Chen & Sun, 2023).

Improper storage of oils may cause the development of unpleasant odour and flavour. For example, oil is stored in environment exposed to light, heat and air promote the presence of moisture, bacteria and enzymes in the oil which leads to degradation of oil. When cooking oils are exposed to high temperature, the deterioration of fatty acids can produce unpleasant odours. Additionally, oxygen in the atmosphere reacts with lipids and other compounds in oil

leading to structural degradation which produces bad odour of cooking oil. Microbial decomposition could lead to undesirable odours too (Okparanta, et al., 2018).

Apart from that, headspace gas chromatography studies have demonstrated that different types of vegetable oils can be discriminated based on their aroma profiles due to each type of cooking oil possessing characteristic volatile organic compounds (VOCs). In another study, an electronic nose (e-nose) was used to detect the differences in VOC emissions from different type of oil samples. The e-nose is highly capable of recognizing complex odours which can be used for detection and identification of adulterated vegetable oils. This method provides a rapid, non-destructive and low-cost approach for identification of adulteration (Aghili, et al., 2023).

The application of e-nose itself has showed a promising outcome in differentiating between adulterated and unadulterated oils without the use of gas chromatography-mass spectrometry (GC-MS). However, the use of expert human sensory is not reliable as everyone have different smell and could produce potential bias and lack reproducibility. Moreover, it is costly and time-consuming to hire an expert human sensory (Aghili, et al., 2023).

2.3 SPECTROSCOPIC TECHNIQUES FOR COOKING OIL ANALYSIS

Cooking oils are one of the most adulterated food products in many countries. Physical examination alone is insufficient to confidently differentiate different types of cooking oils or to detect adulteration (Nunes, 2014). Therefore, spectroscopic techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Near-infrared (NIR), Raman and gas chromatography-mass spectrometry (GC-MS) are widely used for detection of adulterated cooking oil. These methods allow verification of oil origin and purity by identifying the functional groups present in the oil (Ozen, et al., 2024).

Numerous studies have been conducted which have demonstrated success in authentication of edible oil by using FTIR, Raman and NIR (Ozen, et al., 2024). Among all these methods, FTIR spectroscopy coupled with Attenuated Total Reflectance (ATR) is preferred to differentiate cooking oil due to its minimal sample preparation and its ability to produce characteristic spectral fingerprints of oils (Rohman, et al., 2020). The principles, applications, and advantages of these spectroscopic techniques are discussed in detail in the following sections.

2.3.1 OVERVIEW OF ANALYTICAL TECHNIQUES

Several analytical techniques are widely applied in oil analysis where each method has its own principles, advantages and limitations. Gas Chromatography coupled with Mass Spectrometry (GC-MS) is a standard method used to identify and quantify fatty acids and volatile compounds (Socaciu, et al., 2022). This technique involves the separation of volatile compounds in a sample based on their different volatilities and interactions with column material (Raut, 2023). One of the limitations of using GC-MS is that several stationary phases are required to separate TAGs of different types of oils. Moreover, according to a study conducted by Indelicato, et al. (2017) GC analysis can face issues such as column deterioration which is caused by high temperature and chemical degradation of analytes (Indelicato, et al., 2017). Besides that, GC-MS requires extensive sample preparation, longer analysis time and higher cost.

Vibrational spectroscopy, such as Raman spectroscopy can also be used for classification of cooking oils. The preferred spectral wavenumbers for Raman spectroscopy are 950–650 cm^{-1} (Rohman, et al., 2020). The advantage of using Raman spectroscopy is that identifies the functional groups and can also provide the skeletal structure of the compounds present in the sample. This technique is rapid, accurate, simple and non-destructive for the samples under examination. However, according to the study conducted by Valaso, et al. (2021), FTIR was considered more reliable technique and the results produced

by Raman Spectroscopy were found to be unsuccessful for differentiating oils after chemometric analysis (Valasi, et al., 2021). Raman spectroscopy is weak in detecting vibration from polar groups and bands of bending vibrations which could affect the accuracy of the results (Nunes, 2014).

Nuclear Magnetic Resonance (NMR) spectroscopy is another technique successfully used to study the triacylglycerols (TAGs) in cooking oils. NMR provides detailed information on molecular structures and dynamics by measuring the magnetic properties of atomic nuclei. Although, NMR is less sensitive compared to GC, but it can provide the same structural and compositional information for mixture of TAGs in oils. This technique also does not require extensive sample preparation (Indelicato, et al., 2017). The limitation of NMR is the expensive instrumentation and its maintenance cost and therefore becomes not commonly available (Tan, et al., 2021).

Fourier Transform Infrared (FTIR) spectroscopy coupled with attenuated total reflectance (ATR) is a preferred technique for differentiating different types of oil because this technique non-destructive, very minimal sample preparation and fast (Rohman, et al., 2020). FTIR detects molecular functional groups by measuring their absorption of infrared radiation and produces rapid spectral fingerprints (Tan, et al., 2021). However, ATR-FTIR is less sensitive to compounds which are present in low concentrations.

Overall, several analytical techniques as mentioned can be used for the differentiation of cooking oils. The method to be used depends on the objective of the examination. GC-MS can be used to identify specific structures and nutritional value in cooking oil. ATR-FTIR is suitable for detection of oil adulteration and to differentiate different types of cooking oil. Therefore, the choice of method depends on analytical objectives, sample size and available resources, as well as the case scenarios.

2.3.2 ATTENUATED TOTAL REFLECTANCE–FOURIER TRANSFORM INFRARED (ATR-FTIR) SPECTROSCOPY

FTIR spectroscopy employs transmission with attenuated total reflectance (ATR) measurement methods. The sample which is placed between the diamond and the surface undergoes internal reflection when infrared (IR) light strikes.

A portion of light will be absorbed by the sample, and this interaction produces a spectrum representative of the sample's chemical composition (Ozen, et al., 2024). ATR-FTIR detects both stretching vibrations and bending vibrations. Stretching vibrations tend to appear in high wavenumber region and shorter wavelength. Bending vibrations changes bond angles within molecules which can be divided into rocking, scissoring and twisting (Ozen, et al., 2024). The spectral range of middle infrared region (MIR) which are $4000 - 400 \text{ cm}^{-1}$ is commonly used to fingerprint specific functional groups. This region can be used to authenticate, identify or classify fats and various types cooking oil (Socaciu, et al., 2022). The technique can capture absorbance bands associated with functional groups common in oils—such as C–H stretches from aliphatic chains and carbonyl (C=O) stretches from ester groups—directly from the sample matrix, providing high-quality spectral fingerprints with minimal interference (Ozen, et al., 2024).

The major advantages of using ATR-FTIR is this method has high reproducibility, simple operation and easy cleaning of instrumentation (Dong, et al., 2020). FTIR spectra are complex and not easy to objectively interpret under untrained eyes. Therefore, chemometric analysis such as Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) can be used to interpret the data (Rohman, et al., 2020).

2.4 ATR-FTIR SPECTRAL CHARACTERISTICS OF COOKING OILS

ATR-FTIR Spectroscopy is a powerful analytical tool used to analyse the molecular composition of materials. In this method, ATR sampling method is combined with the principles of infrared spectroscopy to determine the chemical bonds, and functional groups present in a sample. An IR laser beam is directed through the crystal, undergoing internal reflection where part of the light is absorbed by the sample and the rest is reflected to the detector. In FTIR spectroscopy the absorption of radiation by the molecules occurs due to presence of bond vibrations involving a change in dipole moment. ATR-FTIR has been successfully used to identify a range of functional group in cooking oil samples (Valasi, et al., 2021).

In cooking oil analysis, fingerprint region ($1800\text{--}900\text{ cm}^{-1}$) and the C–H stretching region ($3000\text{--}2800\text{ cm}^{-1}$) are used for qualitative interpretation and chemometric (Rohman, et al., 2020). The spectral characteristics and analytical relevance of these regions are discussed in detail in the following subsections.

2.4.1 FINGERPRINT REGION ($1800\text{--}900\text{ cm}^{-1}$)

The fingerprint region of an FTIR spectrum contains absorption bands which arises from complex molecular vibrations. These molecular vibrations are associated with functional groups present in cooking oils. Two important functional groups that appear in this region are stretching vibration of the carbonyl ($\text{C}=\text{O}$) of the ester bonds, and to the asymmetric stretching vibration of the C–O ester bonds (Vaisali, et al., 2015). The ester carbonyl ($\text{C}=\text{O}$) stretching vibration band arises from the ester linkages that bond fatty acid chains to the glycerol backbone and serves as a strong marker for lipid content.

Table 2.1 shows FTIR wavenumber regions of vegetable oils, correlated with the mode of vibration for specific functional groups for fingerprint region (Socaciu, et al., 2022)

Range (cm ⁻¹)	Functional Group	Mode of Vibration
1795–1677	-C=O (ester, acid)	Stretching
1486–1425	-C–H (CH ₃ , CH ₂)	Bending
1425–1409	=C–H(cis)	Bending (rocking)
1396–1371	-C–H (CH ₃)	Bending
1290–1211	-C–O; -CH ₂ -	Stretching; Bending
1211–1147	-C–O; -CH ₂ -	Stretching; Bending
1147–1006	-C–O	Stretching
1006–885	-HC=CH-(trans/cis)	Bending (out of plane)
885–802	=CH ₂	Wagging
802–701	-C–H; -HC=CH- (cis)	Bending (out of plane)

According to the study conducted by Dong, et.al (2020), this region is capable for differentiating various edible oils. After chemometric analysis, different types of oil can be differentiated based on botanical origin, processing conditions, and adulteration levels (Dong, et al., 2020).

2.4.2 C–H STRETCHING REGION (3000–2800 cm⁻¹)

There are significant differences in peak intensity in the C-H stretching region that covers (3000–2800 cm⁻¹). The triglyceride absorption peaks, as major components of edible oils were dominant, with absorptions 2922–2924 cm⁻¹ (C–H asymmetric stretching), 2852 cm⁻¹ (C–H symmetric stretching), 1454 cm⁻¹ (C–H bending (scissoring), 1166 cm⁻¹ (C–O stretching and C–H bending), and 719–721, 790 cm⁻¹ (C–H and -HC=CH- bending) (Socaciu, et al., 2022). The peaks observed at 2922–2924 cm⁻¹ are due to the stretching vibration of the C-H bonds of the non-aromatic methylene group.

The intensity of CH₂ and CH₃ peaks in this spectral region depends on the fatty acid chain length and degree of saturation. Oils with long chain saturated fatty acids tend to have stronger CH₂ absorptions due to a higher proportion of methylene groups. Oils with higher unsaturated fatty acids can exhibit subtle shifts in band positions which causes the changes

in intensity of the peak. Therefore, this region is also suitable for distinguishing between different cooking oils based on their fatty acid composition (Valasi, et al., 2021).

Table 2.2 shows FTIR wavenumber regions of vegetable oils, correlated with the mode of vibration for specific functional groups for C-H stretching region (Socaciu, et al., 2022)

Range (cm ⁻¹)	Functional Group	Mode of Vibration
3029–2989	=C–H (trans and cis)	Stretching
2946–2782	-C–H (CH ₃ , CH ₂)	Stretching (asym/sym)

2.5 CHEMOMETRIC TECHNIQUES IN SPECTROSCOPIC ANALYSIS

Spectroscopic techniques such as ATR-FTIR generate complex datasets consisting of absorbance values across hundreds to thousands of wavenumbers for each sample. Visual interpretation of the spectra may not very accurate and unreliable due to the overlapping of peaks. The comparison of more than two spectra becomes different. Therefore, chemometric techniques are used to compare the data obtained, and thus provide a more objective interpretation.

Chemometrics refers to the use of mathematical and statistical methods for handling, interpreting and predicting chemical data (Indelicato, et al., 2017). Chemometric methods are typically used for evaluating vibrational spectral data such as data from FTIR spectroscopy by reducing dimensionality and enhancing relevant variance. Chemometric analysis typically involves the use of machine learning algorithms, pattern recognition, and predictive modelling techniques (Ozen, et al., 2024).

The use of ATR-FTIR spectroscopy along with chemometrics has been widely used for authentication, discrimination between various oil types and modelling physicochemical properties of cooking oils (Arslan, 2018) (Shiv, et al., 2024). The use of both ATR-FTIR and chemometric methods also can be used to analyse large datasets of commercialised oils (Socaciu, et al., 2022).

In cooking oil analysis, chemometrics is applied to spectral data related to triacylglycerols (TAGs) to extract maximum chemical information from the samples. Through chemometric analysis, hidden information such as chemical composition of cooking oil from the sample can be extracted which is very helping in detecting adulteration (Indelicato, et al., 2017). Chemometrics is also used to classify unknown samples with similar characteristics and determining the type of adulterant present in the cooking oil (Hu, et al., 2014).

Numerous studies have reported the successful detection of adulteration of olive oil (OO) adulterated with hazelnut, sunflower, corn and soybean oils using FTIR with chemometric method (Arslan, 2018). A study has reported that chemometric methods based on vibrational spectroscopy was very effective to detect adulteration and produces classification accuracy close to 100% (Nunes, 2014). Among the various chemometric approaches, Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) are very widely applied in spectroscopic studies of edible oils. According to another study, chemometric analysis namely PCA showed ease clustering between the authentic edible fats and oils and adulterated ones while LDA provides the predictive ability of adulteration levels (Rohman, et al., 2020)

2.5.1 PRINCIPAL COMPONENT ANALYSIS (PCA)

PCA is an unsupervised chemometric technique widely used to interpret data obtained from vibrational spectroscopy. PCA is primarily conducted for visualization and to reveal differences between each sample. By running PCA, information from large datasets is condensed into a smaller number of known as principal components (PCs), such as PC1, PC2, and PC3, without significant loss of important information (Yuan, et al., 2023) (Shiv, et al., 2024). PCA models for FTIR spectroscopy is typically conducted on fingerprint region ($1800\text{--}900\text{ cm}^{-1}$) and C–H stretching region ($3000\text{--}2800\text{ cm}^{-1}$) where variations in absorbance or transmittance peaks reflect compositional differences among cooking oils (Arslan, 2018).

The first few PCs contain most of the important variation which reduces the complexity of spectral information while retaining its most significant features. PCs are responsible for forming smaller groups from samples by searching the differences between variables. The grouping of PCs depends on the similarity of the variables (Rohman, et al., 2020). PCA also acts as a preliminary evaluation of the sample classification before LDA is supervised methods such as LDA is conducted (Socaciu, et al., 2022).

PCA can highlight the similarities between cooking oils based on the TAGs composition. Samples with similar composition are clustered into one group. In a study conducted by Indelicato et al. (2017), adulterated olive oil can be detected by looking at the distance from the olive oil cluster in the PCA plot. By plotting scores on the first two or three principal components, natural groupings among different oil samples can be visualised, allowing identification of similarities related to botanical origin, processing conditions, or adulteration without relying on prior class labels. (Indelicato, et al., 2017).

In a study by Karim et al. (2015), seven different types of cooking oil were successfully classified from their PCA scores. However, there is a possibility that samples are classified according to their level of moisture or to their particles size instead of being classified according to their chemical composition. Therefore, it is better to pair PCA with LDA differentiating defined classes because PCA only describes the overall variation in the data (Karim, et al., 2015).

Furthermore, a study by Zhang et al. (2026), PCA was employed to screen cluster of sampling and fatty acid distribution into five different types of cooking oil. In this study, all cooking oils were successfully classified into five groups where rape seed and peanut oils are far from sunflower, sesame and soybean oils (Zhang, et al., 2026) (Hu, et al., 2014).

2.5.2 LINEAR DISCRIMINANT ANALYSIS (LDA)

LDA is typically performed as the next step following PCA because sample classification in PCA is unsupervised. LDA is a linear model used for classification for fatty acid composition in cooking oils (Hwang, Choi, Jeong, & Lee, 2024). Unlike PCA, LDA is a supervised classification technique where the number of categories and the samples belonging to each category are predefined (Arslan, 2018). In LDA, the size of dataset is reduced while retaining only the most relevant discriminatory information (Nagy, et al., 2005). LDA maximises the between-class variance while minimising the within-class variance, resulting in effective discrimination in a reduced multivariate space.

In a study conducted by Arslan (2018), 100% of cooking oil samples were correctly classified using LDA models constructed with 5 predictors. The five predictors were extra virgin olive, hazelnut, cottonseed, sunflower, and soybean oils. Another study stated that, LDA successfully classifies 100% of olive oil samples according to their cultivar which proves that LDA can predict unknown samples for mixture in cooking oils. LDA is also preferred for TAG analysis in cooking oils as it is simple and requires shorter processing time (Indelicato, et al., 2017). Another study by Rohman et al. (2020) also reported that reusing the same variables as in PCA provides a better discrimination results of cooking oil samples (Rohman, et al., 2020).

2.6 LIMITATIONS OF PREVIOUS STUDIES AND RESEARCH GAP

Although ATR-FTIR combined with chemometric techniques is widely used to differentiate various types of cooking oils and detect adulteration, there were several limitations in the previous studies. Firstly, most of the studies only rely on one spectral region which is the fingerprint region ($1800\text{--}900\text{ cm}^{-1}$) without evaluating other regions. Only a limited number of studies have explored the use of another region of spectral region which is the C–H stretching region ($3000\text{--}2800\text{ cm}^{-1}$). The use of two spectral regions could enhance the discrimination and provide better results for chemometric analysis. One study has

demonstrated that the C-H stretching region has successfully used to detect adulterant in cooking oils. This overlooks the usefulness of spectral features that could differ in different types of oil (Ye & Meng, 2022). Therefore, in this study, both fingerprint region and C-H stretching regions are interpreted using chemometric analysis.

Secondly, most studies have focused on quality control and nutrition rather than forensic applications. Many studies focus on the nutritional value of the cooking oil after adulteration but do not address the identification of the specific adulterant mixed with the original oil. Besides that, very limited number article has reported forensic applications such as trace oil residues. Therefore, this remains a gap in applying and validating ATR-FTIR chemometric approaches within forensic contexts because the forensic standards are seldom discussed in the previous studies (Rohman, et al., 2020).

Finally, there are some methodological challenges as well. In FTIR spectroscopy, overlapping of spectral bands can be seen between various types of cooking oil due to having the same functional group. Although, two spectral regions with prominent peaks can be seen but most of the studies only interpret one spectral region which may reduce the reliability of discrimination (Ozen, et al., 2024).

Although the intensity of the peak may differ, but it is still not enough to identify and classify cooking oils. Therefore, chemometric analysis are conducted after ATR-FTIR spectroscopy to interpret the data. However, there are many different types of chemometric analysis such as PCA, LDA, Partial Least Squares–Discriminant Analysis (PLS-DA) and Hierarchical Cluster Analysis (HCA). The selection and use of chemometric analysis depends on the objective of the study (Ozen, et al., 2024).

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

This chapter describes the flow of the study, materials, instrumentation, and experimental procedures employed in this study for the forensic differentiation of cooking oils using ATR-FTIR spectroscopy combined with chemometric analysis, namely the PCA and LDA. It also outlines the methodology for spectral acquisition, data processing and statistical analysis. The procedures were designed to ensure reproducibility, reliability and suitability for forensic comparison of various types of cooking oil samples.

3.2 FLOWCHART

A flowchart as in Figure 3.1 shows the overall process flow of the study. In brief, the process began with sample collection where 12 different types of cooking oils were collected in a clean vial. In this project, 87 samples were collected which consisting of (i) black seed oil, (ii) canola + sunflower oil, (iii) canola oil, (iv) castor oil, (v) coconut oil, (vi) corn oil, (vii) olive oil, (viii) perilla seed oil, (ix) palm oil, (x) rice bran oil, (xi) sesame oil and (xii) sunflower oil. This was followed by physical examination where the colour ranging from blackish brown in colour to colourless and the odour were observed. ATR-FTIR analysis was then conducted. The spectral data obtained from ATR-FTIR analysis was used to conduct statistical analysis which were the PCA and LDA using Minitab® software. These chemometric analyses were applied to evaluate the differentiation and classification of the cooking oils.

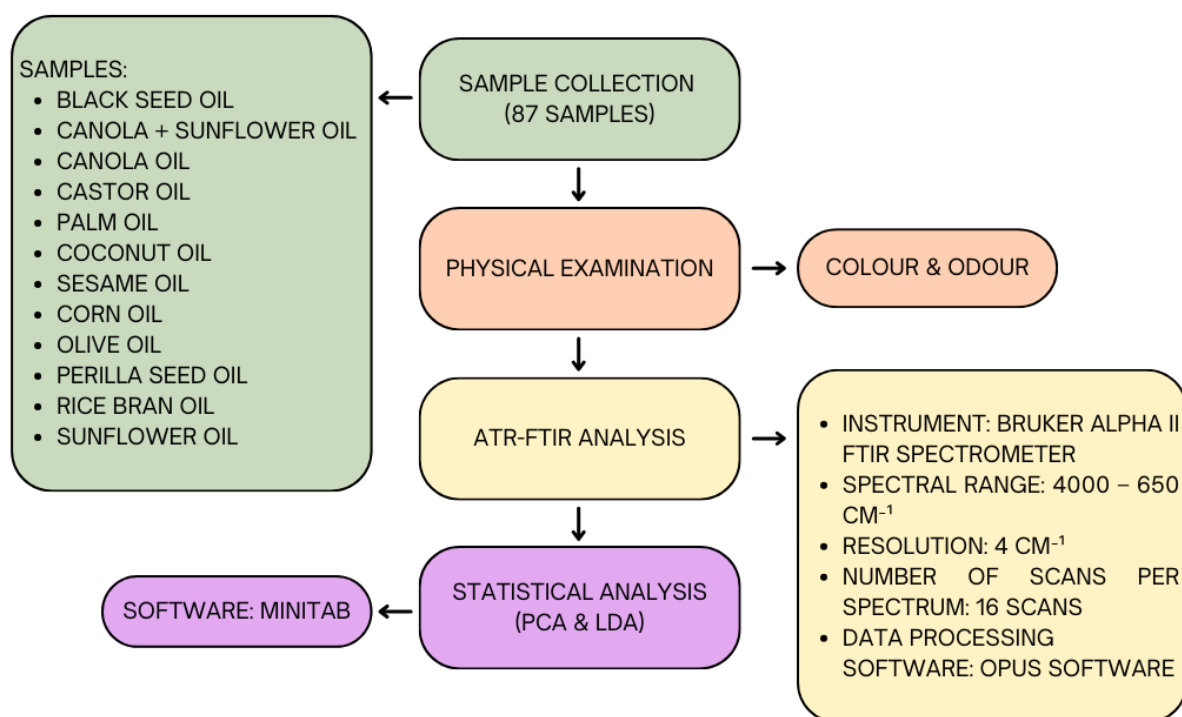


Figure 3.1 The flowchart of the methodology for differentiation of cooking oils using ATR-FTIR spectroscopy and chemometric analysis.

3.3 SAMPLE COLLECTION

A total of 87 cooking oil samples were collected from various sources (individual) and analysed in this study (Table 3.1). The oil types (i) black seed oil, (ii) canola + sunflower oil, (iii) canola oil, (iv) castor oil, (v) coconut oil, (vi) corn oil, (vii) olive oil, (viii) perilla seed oil, (ix) palm oil, (x) rice bran oil, (xi) sesame oil and (xii) sunflower oil. Not only that, but multiple oil samples were also collected for each type of oil, depending on the availabilities. This was because each type of oil from different brand could have variability arising from brand differences, processing methods, and formulation, and this allowed for sample comparison. All samples were stored at room temperature upon collection and were not exposed to heat and light.

The number samples for each type of cooking oil collected was different because the sample collection depends on the market availability of the cooking oil. The cooking oils which are commonly used such as palm oil, olive oil, and sesame oil were collected in larger