

**CHEMICAL CHARACTERISATION OF FRESH AND USED COOKING
OILS BY ATR-FTIR SPECTROSCOPY**

By

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CERTIFICATE

This is to certify that the dissertation entitled “Chemical Characterisation of Fresh and Used Cooking Oils by ATR-FTIR Spectroscopy” is the bona fide record of research work done by Ms. Camey Ho Ting Ting during the period from October 2025 to February 2026 under my supervision. I have read this dissertation and, in my opinion, it conforms to an acceptable standard of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Science in Forensic Science (Honours).

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LIST OF SYMBOLS

cm^{-1}	Wavenumber
$^{\circ}\text{C}$	Degree Celsius
%	Percentage
=	Double bond
-	Single bond

LIST OF ABBREVIATIONS

ANN	Artificial Neural Network
ANOVA	Analysis of Variance
ATR	Attenuated Total Reflectance
ATR-FTIR	Attenuated Total Reflectance–Fourier Transform Infrared Spectroscopy
AV	Anisidine Value
C=C	Carbon–carbon double bond
C=O	Carbonyl group
C-H	Carbon–hydrogen bond
FFA	Free Fatty Acids
FTIR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography–Mass Spectrometry
HCA	Hierarchical Cluster Analysis
HPLC	High-Performance Liquid Chromatography
LDA	Linear Discriminant Analysis
PAHs	Polycyclic Aromatic Hydrocarbons
PC	Principal Component
PCA	Principal Component Analysis
PLS-DA	Partial Least Squares Discriminant Analysis
PPE	Personal Protective Equipment
RSD	Relative Standard Deviation
SVM	Support Vector Machine
TAG	Triacylglycerol

ABSTRAK

Minyak masak boleh mengalami degradasi kimia apabila terdedah kepada suhu tinggi semasa proses penggorengan dan dengan kehadiran matriks makanan, yang seterusnya menyebabkan tindak balas pengoksidaan, hidrolisis dan pempolimeran. Penggunaan semula minyak goreng secara berulang, khususnya dalam sektor makanan tidak formal, menyebabkan kemerosotan kualiti kimia dan pembentukan sebatian berpotensi berbahaya, sekali gus menekankan keperluan kepada pendekatan spektroskopi bagi memantau kualiti minyak dan memastikan keselamatan makanan. Kajian ini bertujuan untuk mencirikan dan membezakan secara kimia antara minyak masak segar, minyak yang dimasak tanpa matriks makanan, serta minyak yang digoreng dengan pelbagai matriks makanan menggunakan spektroskopi attenuated total reflectance–Fourier transform infrared (ATR-FTIR) yang digabungkan dengan analisis kemometrik. Dalam kajian ini, minyak masak segar digunakan untuk menggoreng pelbagai matriks makanan termasuk makanan berasaskan tumbuhan dan haiwan, dan minyak masak yang dikumpulkan dianalisis menggunakan spektroskopi ATR-FTIR. Seterusnya, analisis komponen utama (PCA) dan analisis diskriminan linear (LDA) digunakan untuk menilai keupayaan pengelasan berdasarkan profil ATR-FTIR. Pemerhatian menunjukkan bahawa profil spektrum ATR-FTIR bagi sampel minyak masak mempamerkan jalur serapan ciri yang berkaitan dengan trigliserida. Pemeriksaan visual dan perbandingan spektrum secara langsung menunjukkan variasi yang minimum antara minyak masak yang digoreng dengan pelbagai matriks makanan, tanpa perbezaan ketara pada jalur serapan. PCA berjaya membezakan antara minyak segar dan minyak yang telah dimasak, sekali gus menunjukkan perubahan kimia yang boleh diperhatikan akibat pemanasan. Walau bagaimanapun, PCA sahaja tidak

mencukupi untuk membezakan secara berkesan antara minyak yang digoreng dengan dan tanpa matriks makanan, namun gabungan PCA-LDA menunjukkan keupayaan diskriminasi yang lebih baik dengan ketepatan pengelasan sebanyak 98.0%. Secara keseluruhan, kajian ini membuktikan keberkesanan gabungan spektroskopi ATR-FTIR dan kemometrik dalam mengenal pasti perubahan kimia akibat pemanasan dalam minyak masak serta membezakan minyak berdasarkan keadaan penggorengan. Dapatan kajian ini menekankan potensi kaedah ini sebagai alat untuk menilai kualiti minyak dan memantau tahap keselamatan makanan.

ABSTRACT

Cooking oils may undergo chemical degradation when subjected to high temperatures during frying and with the addition of food matrices, leading to oxidation, hydrolysis and polymerisation reactions. The repeated reuse of frying oil, particularly in informal food sectors, causes chemical degradation and the formation of potentially hazardous compounds, emphasising the need for a spectroscopic approach to monitor oil quality and ensure food safety. The aim of this work was to chemically characterise and distinguish between fresh cooking oil, cooked oil without food matrices, and oils fried with different food matrices utilising attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy combined with chemometric analyses. In this study, fresh cooking oil were used to fry the various food matrices, including plant-based and animal-based diets, and the collected cooking oils were analysed using ATR-FTIR spectroscopy. Subsequently, principal component analysis (PCA) and linear discriminant analysis (LDA) were used to evaluate the classification capabilities based on the ATR-FTIR profiles. Through observation, the ATR-FTIR spectral profiles of the cooking oil samples showed distinctive absorption bands associated with triglycerides. Visual inspection and direct spectral comparison revealed negligible variations between cooking oils fried with various food matrices with no significant spectral differences. PCA successfully differentiated between fresh and cooked oils, demonstrating observable chemical changes caused by heating. Although PCA alone was insufficient to effectively distinguish between oils fried with and without food matrices, a PCA-LDA had enhanced discrimination capabilities with 98.0 % classification accuracy. To conclude, this study demonstrates the effectiveness of ATR-FTIR spectroscopy combined with chemometrics in determining heat-induced

chemical changes in cooking oils and discriminating the cooking oils based on frying circumstances. The findings emphasised this method's potential for assessing oil quality and monitoring food safety level.

CHAPTER 1

INTRODUCTION

In this chapter, the background and scientific context of cooking oil degradation and the relevance of FTIR-based analysis are explained. The problem statement is also described, followed by the determination of the objectives of study. The significance of the research is also delineated.

1.1 Background of Study

Cooking oils are widely used in households, food industries and restaurants. Cooking oils are edible oils, serving as an integral part of human nutrition and food preparation, as cooking media and in food processing (Jamwal *et al.*, 2021). Chemically, edible oils mainly consist of triacylglycerols (TAGs), along with other minor compounds such as sterols, phospholipids, pigments and antioxidants, contributing to their oxidative stability and nutritional values (Jamwal *et al.*, 2021). The main constituents of both fats and oils are fatty acids and TAGs. TAGs are the primary component of plant oils, typically accounting for about 92% (Bunaciu *et al.*, 2020).

Repeated heating and prolonged use of cooking oils may cause chemical degradation, producing oxidation products, free fatty acids and toxic compounds. Such produces could lead to the variations in the chemical composition between fresh and used cooking oils. During high-temperature cooking or frying, these oils may undergo physicochemical changes including oxidation, hydrolysis and polymerisation, leading

to the formation of free fatty acids, aldehydes, and peroxides (Rahmania *et al.*, 2014). Such degradation not only reduces the sensory quality of the oil but may also generate harmful compounds linked to various health risks such as cardiovascular diseases and carcinogenic effects.

Previous literatures suggested that prolonged or repeated use of cooking oil significantly altered its chemical profile. Factors such as frying temperature, duration and the type of food fried may influence the extent of degradation (Guillén and Cabo, 2000). Monitoring these changes is crucial to ensure food quality, consumer safety and compliance with health regulations. Conventional analytical methods such as gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been used for oil characterization; however, these techniques could be time-consuming, require extensive sample preparation and utilise hazardous solvents (Jamwal *et al.*, 2021). Therefore, there is an increasing demand for rapid, non-destructive and environmentally friendly analytical methods for assessing oil quality.

Fourier Transform Infrared (FTIR) spectroscopy is a vibrational spectroscopic technique frequently used in food authentication (Pauliuc *et al.*, 2021). It is increasingly employed to track changes in edible oils, determine these changes and detect unauthorised modifications (Rohman *et al.*, 2021). FTIR is valued because it is a non-destructive, accurate, and inexpensive method. It is also characterised as a “fingerprint analytical technique” (Lestari *et al.*, 2025). FTIR spectroscopy, particularly when coupled with attenuated total reflectance (ATR), has emerged as a powerful technique for the qualitative and quantitative analysis of edible oils. FTIR could detect molecular vibrations and provided characteristic spectral fingerprints of

oils based on the absorption of infrared radiation by chemical bonds within fatty acid molecules (Rahmania *et al.*, 2014). Specific absorption bands in the mid-infrared region (4000-650 cm^{-1}), such as those corresponding to the stretching vibrations of C-H, C=O, and C-O groups, were highly sensitive to compositional and structural changes caused by oxidation and thermal degradation (Rosman *et al.*, 2019). Such characteristics made ATR-FTIR an efficient and reproducible method for differentiating between fresh and used cooking oils, as well as among oils exposed to various food matrices.

Recent developments have combined FTIR spectroscopy with chemometrics and multivariate statistical approaches to enhance classification accuracy and interpretation of complex spectral data (Jamwal *et al.*, 2021). Among these, principal component analysis (PCA) is widely used for exploratory analysis and dimensionality reduction, revealing inherent groupings or variations within the spectra of oils. PCA can simplify large datasets and highlights the main spectral features responsible for differences between fresh and thermally degraded oils. Meanwhile, linear discriminant analysis (LDA) serves as a supervised classification method, capable of constructing predictive models that can categorise oil samples based on their spectral characteristics. Integrating PCA and LDA may allow for efficient discrimination between oil samples used for frying different food types, providing deeper insights into compositional changes and degradation patterns (Rosman *et al.*, 2019). Therefore, in this study, ATR-FTIR spectroscopy combined with PCA and LDA was used to chemically characterise and classify fresh and used cooking oils.

1.2 Problem Statement

There are serious ethical and food safety issues with the widespread practice of reusing frying oil and reselling or repackaging it as 'fresh' oil. The cooking oil's chemical structure deteriorates with repeated heating, producing potentially hazardous chemicals. This approach erodes confidence in food supply systems and puts consumer health at risk. The Singapore Food Agency claims that constantly heating oil produces dangerous substances including aldehydes and polycyclic aromatic hydrocarbons (PAHs), which are connected to long-term illnesses like cancer and heart disease (Singapore Food Agency, 2024). Similar frying practices have been reported in several countries, including Malaysia, particularly within small-scale food businesses and street food operations, where economic considerations may encourage prolonged oil reuse (Azman *et al.*, 2019). In Malaysia, the high consumption of fried foods and the informal nature of certain food preparation sectors raise comparable food safety concerns related to reused cooking oils (Ministry of Health Malaysia, 2019). According to a more technical analysis, the oxidative and polymerisation breakdown of TAG in heated oil produces polymers, which lower the oil's nutritional values (D Kmiecik *et al.*, 2019).

Apart from that, additional contaminants are introduced when oil comes into touch with food residues such as moisture, proteins and other organic matter during frying. In the meantime, a complex combination of degradation products could be produced by chemical reactions such as oxidation, hydrolysis and polymerisation that are fuelled by high temperatures. These alterations decrease oil quality and may be harmful to human health. (Jhaumeer-Laull *et al.*, 2001). Therefore, the use of ATR-

FTIR spectroscopy in conjunction with chemometric analysis provides a non-destructive and efficient method for monitoring oil quality and distinguishing oils subjected to different frying conditions, thereby aiding in food safety assessment and regulatory enforcement.

1.3 Objectives

This study is hoped to determinate if there are any chemical changes that could have occurred in cooking oil after frying different food matrices and to determine whether these changes could be distinguished using ATR-FTIR spectroscopy supported by chemometric analysis. Therefore, the general and specific objectives are outlined.

1.3.1 General Objective

The general objective of this study is to characterise and discriminate between fresh and used cooking oils fried with different food matrices through the application of ATR-FTIR spectroscopy combined with chemometric techniques, specifically PCA and LDA.

1.3.2 Specific Objective

To achieve the general objective, three specific objectives are constructed as follows:

- i. To compare the ATR-FTIR spectral profiles of fresh and used cooking oils

- ii. To compare the ATR-FTIR spectral profiles of cooked oil with and without matrices.
- iii. To distinguish the ATR-FTIR spectral profiles of used oils that were cooked with various food matrices through chemometrics.

1.4 Significance of the Study

This study is important as it can advance our knowledge of how various food matrices affect the chemical quality and patterns of cooking oil deterioration during frying. Fewer studies have explicitly examined the special interactions between food components, such as moisture, proteins, and starch and the oil, which could significantly speed up oxidation, hydrolysis and the formation of degradation products, whereas many earlier studies have mostly concentrated on the effects of repeated heating.

This study can offer a quick and non-destructive method for identifying molecular-level alterations in frying oils. The study may also enhance the capacity to distinguish and categorise cooking oils according to the food matrices they were exposed to when paired with chemometric methods, providing a more objective way to evaluate oil's quality.

By comparing the ATR-FTIR spectral patterns of fresh and used cooking oils, this study could possibly demonstrate the chemical changes caused by heating. Identifying these alterations at the molecular level is critical for understanding early-stage oil deterioration that may be undetectable by eye inspection alone. This could

also improve the oil quality assessment and food safety monitoring by demonstrating the sensitivity of ATR-FTIR spectroscopy in identifying any possible heat degradation.

The comparison of cooked oil with and without food matrices could demonstrate the importance of food-oil interactions in accelerating or altering oil breakdown pathways. While earlier research has focused solely on repeated heating, this study demonstrated that the presence of food introduces new factors such as moisture, proteins and other organic substances that influence oxidation and hydrolysis reactions.

The use of chemometric approaches to differentiate oils cooked with different food matrices reveals how multivariate analysis may uncover minor but relevant chemical changes from highly similar ATR-FTIR spectra. Although visible spectrum differences were small, the application of PCA and LDA allowed for statistical discrimination depending on treatment history. This emphasises the significance of chemometrics in increasing the interpretability of spectroscopic data and supports its use as a quick and objective screening method.

CHAPTER 2

LITERATURE REVIEW

This chapter analyses the existing literature on the composition and degradation of edible oils during frying, the impact of food matrices on oil quality, and the use of spectroscopic and chemometric techniques for oil analysis. The chemical mechanisms that cause oil deterioration, such as oxidation, hydrolysis, and polymerisation, are highlighted, as are the limits of standard assessment methods. Furthermore, earlier investigations using ATR-FTIR spectroscopy and multivariate analysis are discussed to lay the scientific groundwork for the approach used in this study and to identify research gaps filled by the current work.

2.1 Composition and Chemical Structure of Edible Oils

Edible oils are an important part of the human diet and are frequently employed in both household and industrial food preparation due to their functional and sensory qualities. Edible oils are complex lipid systems made up predominantly of TAGs, with a variety of minor ingredients that influence their nutritional value, oxidative stability and thermal processing behaviour (Gunstone, 2004). Understanding the fundamental composition and molecular structure of edible oils is critical for interpreting their chemical changes during heating and explaining the spectrum patterns detected by infrared-based analytical techniques.

2.1.1 Major Components of Edible Oils

TAGs constitute approximately 95-98% of the total composition of most edible oils (Gunstone, 2004). A TAG molecule is made up of a glycerol backbone that has been esterified with three fatty acid chains of varying length and unsaturation. The ester functional groups (-COO-) and long hydrocarbon chains dominate the chemical structure of oils and are responsible for their distinct infrared absorption bands, particularly in the mid-infrared region (Guillén and Cabo, 2000).

Minor components of edible oils include free fatty acids, mono- and diacylglycerols, phospholipids, sterols, tocopherols, colours, and trace metals (Shahidi, 2012). Despite their modest concentrations, these chemicals play crucial roles in oil quality and stability. Tocopherols, for example, are natural antioxidants, but free fatty acids influence oil acidity and hydrolytic processes (Choe and Min, 2006). The percentage of these minor elements varies according to the oil source and processing conditions.

Most spectroscopic examinations of edible oils are structurally based on the predominant presence of ester linkages and long aliphatic chains in TAGs. In FTIR spectroscopy, vibrational modes related with C-H stretching, C=O stretching, and C-O stretching are especially useful as markers of oil composition and structural integrity (Sinelli *et al.*, 2010).

2.1.2 Role of Fatty Acid Saturation in Thermal Stability

The fatty acid makeup of edible oils is an important aspect in determining their chemical stability, especially under heat circumstances. Fatty acids are categorised into

three types based on their degree of saturation, namely saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids. Saturated fatty acids lack carbon-carbon double bonds, while monounsaturated and polyunsaturated fatty acids have one or more double bonds (Gunstone, 2004).

The existence of double bonds creates structural weaknesses that are more vulnerable to chemical reactions. Polyunsaturated fatty acids, such as linoleic and linolenic acids, are especially sensitive due to the existence of numerous double bonds that can easily participate in oxidation reactions (Choe and Min, 2006). Saturated fatty acids, on the other hand, have higher thermal and oxidative stability because of their fully hydrogenated carbon chains. As a result, oils high in saturated or monounsaturated fatty acids are more resistant to chemical deterioration than oils high in polyunsaturated fatty acids (Shahidi, 2012).

From a structural standpoint, the degree of unsaturation influences the vibrational behaviour of fatty acids, which can be determined by infrared spectroscopy. Unsaturated fatty acids exhibit a characteristic olefinic $=C-H$ stretching vibration in the region of $3006-3010\text{ cm}^{-1}$, while saturated hydrocarbon chains primarily contribute to strong aliphatic $C-H$ stretching bands at 2922 cm^{-1} and 2853 cm^{-1} (Guillén and Cabo, 2000). These spectral features provide useful information about oil saturation levels and serve as the foundation for later monitoring of chemical changes throughout processing.

Overall, edible oils' chemical composition and molecular structure, notably the dominance of TAGs and fatty acid saturation, have a significant impact on their

physicochemical behaviour and analytical properties. A thorough understanding of these fundamental features is required for interpreting oil degradation pathways and using spectroscopic and chemometric approaches to evaluate oil quality under various frying circumstances.

2.2 Frying Process and Oil Degradation Mechanisms

Frying is a high-temperature cooking method in which edible oils are typically heated to temperatures ranging from 160°C to 190°C. Cooking oils are exposed to heat, air, moisture and food-derived chemicals all at the same time, generating an environment conducive to a series of complicated chemical reactions (Choe and Min, 2006). These processes gradually change the chemical structure of oils, resulting in the creation of degradation products that impact oil quality, safety and nutritional value. Thermal oxidation, hydrolysis and polymerisation are the three major degradation mechanisms that occur during frying.

2.2.1 Thermal Oxidation of Cooking Oils

Thermal oxidation is a major breakdown mechanism in cooking oils during frying. At high temperatures, unsaturated fatty acids quickly react with ambient oxygen, triggering a free-radical chain reaction that leads to lipid oxidation (Guillén and Cabo, 2000). This process starts with the abstraction of hydrogen atoms next to double bonds, resulting in lipid radicals that quickly react with oxygen to form lipid hydroperoxides, which are considered the primary oxidation products (Choe and Min, 2006).

Although hydroperoxides are initially produced in large quantities, they are thermally unstable at frying temperatures and disintegrate into a range of secondary oxidation products, such as aldehydes, ketones, alcohols and short-chain carboxylic acids (Dobarganes and Márquez-Ruiz, 2015). These chemicals contribute to off-flavours, disagreeable odours and lower oil quality. Aldehydes, in particular, are of concern because of their possible toxicity and link to negative health effects (D. Kmiecik *et al.*, 2019).

From an analytical standpoint, oxidation-induced structural changes cause changes in functional groups such as carbonyl (C=O) and unsaturated C=C bonds, which can be identified with mid-infrared spectroscopy. As oxidation continues, the intensity and shape of absorption bands associated with ester carbonyls and unsaturation vary, giving useful indicators for monitoring oil degradation (Guillén and Cabo, 2000).

2.2.2 Hydrolysis Reactions During Frying

Hydrolysis is another key degradation mechanism that happens during frying, which is predominantly caused by the presence of water. Moisture enters the oil from the fried food, either by surface water or steam produced during cooking (Andrikopoulos *et al.*, 2002). At high temperatures, water molecules cleave TAG ester linkages, forming free fatty acids, monoacylglycerols, diacylglycerols and glycerol (Choe and Min, 2006).

The concentration of free fatty acids raises the acidity of the oil and decreases its smoke point, hastening further degradation during subsequent heating (Shahidi, 2012). Hydrolytic reactions also make oils more susceptible to oxidation, as free fatty acids oxidise faster than esterified fatty acids due to their increased exposure to oxygen (Dobarganes and Márquez-Ruiz, 2015). Hydrolysis-induced structural changes affect the chemical makeup of oils without generating evident visual damage, particularly during the early frying phases. These small molecular changes are difficult to identify by normal sensory evaluation, but they can be detected using spectroscopic techniques, notably in the infrared fingerprint region, where C-O stretching and glycerol-related vibrations occur (Guillén and Cabo, 2000).

2.2.3 Polymerisation and Second Oxidation Products

In addition to oxidation and hydrolysis, polymerisation processes contribute significantly to oil deterioration during extended or repeated frying. Polymerisation is the process by which oxidised fatty acid radicals and subsequent oxidation products combine to produce dimers, trimers and other high-molecular-weight molecules. These polymeric compounds have a major impact on the physical properties of oils, including increased viscosity, foaming, colour darkening and decreased heat transfer efficiency.

Polymerised triglycerides are particularly associated with oils high in unsaturated fatty acids, as many double bonds provide reactive sites for cross-linking processes (Zhang *et al.*, 2012). Polymerised chemicals are undesirable in terms of food

safety since they lower digestibility and have been linked to adverse biological effects in animal and human studies (Dobarganes and Márquez-Ruiz, 2015).

Polymerisation and secondary oxidation events produce complex combinations of polar chemicals, which are widely utilised as indicators of oil degradation in regulatory and quality control systems. These high-molecular-weight and polar compounds cause minor but detectable changes in infrared absorption patterns, notably in the fingerprint region, where overlapping bands represent cumulative chemical changes (Sinelli *et al.*, 2010).

Overall, the frying process causes a series of oxidative, hydrolytic, and polymerisation events that gradually degrade cooking oil quality. While these mechanisms occur at the same time, their proportional contributions vary depending on temperature, frying time, oil composition, as well as the moisture and oxygen levels. Understanding these degradation mechanisms is crucial for understanding spectroscopic data and using chemometric approaches to discriminate oils under different frying settings.

2.3 Influence of Food Matrices on Oil Degradation

Cooking oil does not degrade in isolation while frying but rather interacts with the heated food matrix on a constant basis. Food matrices contribute moisture, proteins, carbs, and endogenous lipids into the oil, altering breakdown pathways and speeding up chemical changes when compared to heated oil alone (Gervajio *et al.*, 2020). The level and character of oil degradation are thus heavily determined by the composition

of the item being cooked. Understanding these interactions is critical for interpreting chemical changes in frying oils and explaining differences in spectrum profiles found with analytical techniques such as ATR-FTIR spectroscopy.

2.3.1 Moisture Transfer from Food to Oil

Moisture transfer is one of the most important elements that influence oil degradation during frying. Most food matrices contain different levels of water, which is quickly released as steam when in contact with heated oil. This moisture stimulates hydrolytic processes by cleaving ester linkages in TAGs, resulting in the generation of free fatty acids, monoacylglycerols and diacylglycerols (Choe and Min, 2006). Diets having a high moisture content, such as vegetables and mushrooms, tend to hasten hydrolysis more than low-moisture diets.

The presence of water indirectly promotes oxidative deterioration. Free fatty acids produced by hydrolysis are more vulnerable to oxidation than esterified fatty acids, hastening the generation of secondary oxidation products (Dobarganes and Márquez-Ruiz, 2015). As a result, oils used to cook high-moisture foods frequently see faster quality deterioration than oils heated without food matrices. Moisture-induced chemical changes may not be visible, but they can generate minor changes in the fingerprint area of infrared spectra. Changes in C-O stretching vibrations and glycerol-related functional groups are indirect indicators of hydrolytic breakdown (Guillén and Cabo, 2000).

2.3.2 Protein-Oil Interactions During Frying

Protein-rich foods, such as meat and poultry, inject nitrogen-containing molecules into frying oil via heat denaturation and breakdown. During frying, proteins undergo structural breakdown and Maillard reactions, resulting in amino acids, peptides and other degradation products that may move into the oil phase (Zhang *et al.*, 2012). These molecules can interact with lipid oxidation products, resulting in complicated secondary products that modify the oil's chemical characteristics.

Protein-derived molecules can potentially affect oxidation kinetics by acting as pro-oxidants or antioxidants, depending on their structure and concentration (Choe and Min, 2006). For example, some amino acids can chelate metal ions, whereas others can catalyse oxidative processes. Oils used to cook protein-rich foods like chicken and beef undergo more complex chemical changes than oils heated without food matrices. Although protein residues are present in relatively low concentrations, their interaction with lipid degradation products can cause subtle but detectable differences in spectral patterns, particularly in the fingerprint region, where overlapping bands reflect the cumulative chemical changes (Sinelli *et al.*, 2010).

2.3.3 Lipid Migration from Food Matrices

In addition to moisture and proteins, lipid migration from food matrices has a substantial influence on oil composition during frying. Animal-based foods, such as chicken skin and beef, contain endogenous fats, which are released into the frying oil when heated. These lipids frequently change in fatty acid composition from the

original cooking oil, especially in terms of saturation and chain length (Zhang *et al.*, 2012).

An addition of animal fats to frying oil increases the complexity of the lipid combination and may modify its oxidative activity. Saturated fatty acids released from animal tissues may improve thermal stability, while unsaturated fatty acids may increase susceptibility to oxidation, depending on the type of food matrix involved (Shahidi, 2012). As a result, oils used to fry animal-based foods frequently have chemical profiles that differ from those used to fry plant-based foods or to cook without food. Such lipid movement causes changes in functional group distribution, which can be observed spectroscopically as alterations in C-H stretching and ester carbonyl absorption bands. However, these distinctions are often modest, necessitating multivariate analysis for accurate differentiation.

2.3.4 Differences Between Plant-Based and Animal-Based Food Matrices

Plant-based and animal-based dietary matrices affect oil degradation via distinct main processes. Plant-based foods, such as vegetables and mushrooms, have a high moisture content and low lipid levels, which promotes the hydrolytic breakdown of frying oils. In contrast, animal-based diets provide both moisture and endogenous lipids, resulting in a mixture of hydrolysis, oxidation and lipid migration effects (Gervajio *et al.*, 2020).

These compositional changes explain why oils cooked with different food matrices have similar overall spectral properties but show modest differences in band

intensity and shape. However, the cumulative effects of moisture transfer, protein interaction and lipid migration can cause tiny chemical alterations detectable using spectroscopic techniques in conjunction with chemometrics (Neves et al., 2020).

Overall, the influence of food matrices is crucial in changing oil degradation pathways during frying. The intricate interactions between oil and food components highlight the necessity for sensitive analytical methods that can identify tiny chemical changes. This provides excellent rationale for using ATR-FTIR spectroscopy in conjunction with multivariate chemometric approaches to distinguish between oils treated to different frying conditions.

2.4 Conventional Methods for Assessing Cooking Oil Quality

Cooking oil quality and safety are usually assessed using a variety of physicochemical characteristics that reflect the degree of chemical degradation that occurs during preparation and use. These traditional methods are commonly used in regulatory monitoring and quality control because they provide quantifiable indicators of oxidation, hydrolysis and polymerisation. However, despite their widespread use, such technologies have numerous drawbacks when used in complicated frying systems, particularly those incorporating multiple food matrices.

2.4.1 Physicochemical Parameters

The peroxide value (PV), which measures the concentration of hydroperoxides generated during the early phases of lipid oxidation (Shahidi and Wanasundara, 2002),

is one of the most often used markers of oil oxidation. While PV is good at detecting early oxidative changes, it becomes less reliable at later stages of frying because hydroperoxides are unstable at high temperatures and rapidly breakdown into secondary oxidation products (Dobarganes and Márquez-Ruiz, 2015).

The anisidine value (AV) is commonly employed alongside PV to evaluate secondary oxidation products, particularly aldehydes. The combination of PV and AV provides a more comprehensive assessment of oxidation status; nevertheless, both metrics necessitate chemical reagents and are time-consuming (Firestone, 2006). Furthermore, AV predominantly reflects aldehydic chemicals and does not encompass the complete range of oxidation products produced during frying.

The free fatty acid (FFA) concentration, also known as acid value, is often used to assess hydrolytic deterioration in frying oils. A rise in acid value suggests the breaking of ester bonds in TAGs, which leads to the generation of free fatty acids (Choe and Min, 2006). Elevated FFA levels are related with poor oil quality, a lower smoke point and an increased susceptibility to oxidation. However, acid value alone cannot distinguish between oxidation- and hydrolysis-induced degradation, and it provides only limited information about the oil's overall chemical composition.

Total polar compounds (TPC) are commonly accepted as an accurate predictor of overall oil deterioration, particularly in frying applications. TPC testing assesses the buildup of polar molecules including oxidised triglycerides, free fatty acids, mono- and diacylglycerols as well as the polymerised products (Dobarganes and Márquez-Ruiz, 2015). Many regulatory organisations utilise TPC limits to establish the discard

point for frying oils. Despite its efficiency, TPC analysis necessitates specialised equipment and extensive sample preparation.

2.4.2 Limitations of Conventional Analytical Methods

Although standard physicochemical approaches provide useful quantitative data, they have numerous drawbacks when used to complicated frying systems. Most approaches require chemical reagents and significant sample preparation, making them unsuitable for rapid or routine screening (Firestone, 2006). Furthermore, each indicator often reflects just one aspect of oil degradation, necessitating the use of many tests to provide an accurate quality assessment.

Another key restriction is that the current approaches might not be able to detect small chemical changes that occur early in the degradation process or are caused by diverse dietary matrices. Oils cooked with different foods may have identical physicochemical properties but unique molecular-level variations that are not represented by single-parameter studies (Shahidi, 2012). As a result, standard analyses may fail to distinguish between oils treated to various frying settings, particularly when deterioration is modest. Furthermore, conventional methods are frequently ineffective for real-time monitoring in food service situations due to their time-consuming nature and reliance on skilled workers. This may have limited their use in small-scale food operations and routine quality control situations where quick, non-destructive evaluation is necessary.

These constraints underscore the need for analytical methods that are quick, non-destructive, and possible to extract detailed chemical information from cooking oils. Spectroscopic techniques, particularly FTIR spectroscopy combined with chemometric analysis, have thus gained popularity as excellent instruments for assessing oil quality and distinguishing oils based on frying history and exposure to food matrices.

2.5 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy is a frequently used analytical technique for determining the chemical composition of organic materials using molecular vibrational characteristics. In food analysis, FTIR spectroscopy is becoming increasingly used as a quick, non-destructive tool for determining lipid quality and tracking degradation processes in edible oils. The approach is especially useful for cooking oil analysis because it offers detailed chemical information about the functional groups associated with triacylglycerols and their breakdown products.

2.5.1 Principles of FTIR Spectroscopy

FTIR spectroscopy is based on the absorption of infrared radiation by molecules, which causes vibrational changes of chemical bonds. When infrared light travels through or reflects off a sample, it absorbs specific wavelengths based on the types of bonds and functional groups present. The resulting spectrum is a molecular "fingerprint" of the sample, with absorption bands representing typical vibrational modes such chemical bond stretching and bending (Coates, 2000).

FTIR equipment use an interferometer to direct a wide variety of infrared wavelengths. The resulting interferogram is then statistically converted using a Fourier transform to provide a spectrum expressed as absorbance or transmittance vs wavenumber (cm^{-1}) (Stuart, 2004). The mid-infrared range, ranging from 4000 to 400 cm^{-1} , is very useful for organic molecules as it includes fundamental vibrational modes of C-H, C=O, C-O and C=C bonds present in lipids. FTIR spectroscopy can identify numerous functional groups simultaneously in edible oils, allowing for thorough chemical characterisation without the requirement for substantial sample preparation or chemical reagents.

2.5.2 ATR-FTIR and Its Advantages

Attenuated total reflectance (ATR) is a sampling technique that is often combined with FTIR spectroscopy to improve its applicability to liquid and semi-solid samples, such as culinary oils. In ATR-FTIR, infrared radiation is channelled through a crystal with a high refractive index, such as diamond or zinc selenide, and reflected internally at the crystal-sample contact. An evanescent wave penetrates the sample for a brief distance, allowing absorption without the requirement for transmission (Stuart, 2004).

ATR-FTIR has various advantages over standard transmission FTIR techniques. First, it requires little to no sample preparation, making it ideal for quick examination. Second, it improves reproducibility, especially for liquid samples, by reducing variability in path length. Third, ATR-FTIR is non-destructive, which allows samples to be kept and used for future study (Sinelli et al., 2010).

These benefits make ATR-FTIR ideal for routine oil quality monitoring and comparative investigations involving numerous oil samples subjected to varying frying conditions. The methodology is also suitable for producing big spectral datasets that may be properly analysed utilising chemometric methods.

2.5.3 Key Infrared Bands in Edible Oils

The FTIR spectra of edible oils are dominated by absorption bands related to TAGs and their fatty acid components. The faint absorption band at $3006\text{--}3010\text{ cm}^{-1}$ indicates the stretching vibration of =C-H bonds in unsaturated fatty acids (Guillén and Cabo, 2000). This band indicates the degree of unsaturation in the oil. The absorption bands at 2922 cm^{-1} and 2853 cm^{-1} are caused by asymmetric and symmetric stretching vibrations of aliphatic C-H bonds in CH_2 and CH_3 groups, indicating the long hydrocarbon chains of fatty acids (Coates, 2000). These bands are often consistent and noticeable in both fresh and thermally processed oils.

The most distinctive band of edible oils comes near $1743\text{--}1745\text{ cm}^{-1}$, corresponding to the ester carbonyl (C=O) stretching vibration of TAGs. This peak is especially useful for monitoring oil degradation since variations in its intensity or shape can signal oxidation or hydrolysis of ester bonds. The fingerprint region, between 1500 and 800 cm^{-1} , has many overlapping absorption bands related to C-O stretching, C-C skeletal vibrations as well as bending modes of CH_2 and CH_3 groups. Although this region is complex and difficult to comprehend visually, it contains useful information about small chemical changes and is frequently used in conjunction with chemometric approaches for oil classification and discrimination (Sinelli *et al.*, 2010).

Overall, FTIR spectroscopy, especially when combined with ATR sampling, provides an effective analytical platform for complete chemical characterisation of food oils. Its capacity to capture both significant functional group information and minor chemical alterations makes it ideal for studying oil degradation processes and facilitating advanced multivariate analyses.

2.6 Chemometrics in Spectroscopic Oil Analysis

Chemometrics is the use of mathematical and statistical approaches to extract important chemical information from complex datasets. Chemometric approaches are critical in spectroscopic analysis for interpreting high-dimensional data obtained by instruments such as FTIR, where spectra frequently contain overlapping bands and minor fluctuations that are difficult to assess visually (Brereton, 2018).

Chemometrics improves the ability to detect compositional variations, classify samples based on treatment history, and identify deterioration patterns caused by heating, oxidation and food-oil interactions. When paired with ATR-FTIR spectroscopy, chemometric methods allow for objective, reproducible, and sensitive differentiation of oil samples, even when chemical differences are modest (Rohman et al., 2021).

2.6.1 Principal Component Analysis (PCA)

PCA is an unsupervised multivariate statistical technique that is commonly used for exploratory data analysis and dimensionality reduction. PCA reduces a huge