ADULTERATION DETECTION OF EDIBLE PALM OIL USING INFRARED UNTARGETED SCREENING AND TARGETED FATTY ACID DIAGNOSTIC RATIO COUPLED WITH CHEMOMETRICS

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LIST OF SYMBOLS

mg	Milligram
L	Litre
µL	Microlitre
µm	Micrometer
N	Normality
e	Random variables
x	Vector of e
α	Constant of vector of e / eigenvector
α'	Transpose of vector of e / Transpose of eigenvector
$\sum$	Covariance matrix
T_p	Predictive score matrix
P_p	Predictive loading matrix
T_o	Orthogonal score matrix
P_o	Orthogonal loading matrix
E	Residue of matrix
cm^{-1}	Per cm (wavenumber)
nm	Nanometer (wavelength)
R^2Y	Fitness of data
Q^2	Predictiveness of data

LIST OF ABBREVIATIONS

CV	Cross-validation
DR	Diagnostic ratio
GC-FID	Gas chromatography-flame ionisation detector
ID	Internal diameter
MIRs	Mid-infrared spectroscopy
NIRs	Near-infrared spectroscopy
OPLS-DA	Orthogonal partial least square-discriminant analysis
PCs	Principal components
PCA	Principal component analysis
RBDPO	Refined, bleached and deodorised palm oil
SNV	Standard normal variate
TAG	Tri-acylglyceride
TPC	Total polar compound

LIST OF APPENDICES

PENGESANAN PENGADUKAN MINYAK KELAPA SAWIT BOLEH DIMAKAN MENGGUNAKAN SARINGAN TIDAK TERTUMPU INFRAMERAH DAN NISBAH DIAGNOSTIK ACID LEMAK TERTUMPU BERGABUNG DENGAN KEMOMETRIK

ABSTRAK

Penggunaan minyak masak secara berulang kali ketika pengorengan makanan, terutamanya ‘Refined, bleached and deodorised palm oil’ (RBDPO), merupakan norma dalam kalangan rakyat Malaysia. Pengadukan RBDPO telah dikaji menggunakan simulasi pengorengan dengan daging lembu, ikan, khinzir dan kambing. Minyak masak telah digoreng pada 180 darjah selsius, sejumlah 25 kali gorengan, selama 10 minit untuk setiap gorengan. Minyak goreng dikumpulkan pada gorengan kali ke-5, ke-10, ke-15, ke-20 dan ke-25. Kemudian, minyak goreng dicampuradukkan dengan minyak tulen untuk dijadikan pengadukan mengikut peratusan, 1%, 5% dan 10%. Kesemua minyak tulen, pengadukan dan goreng dinilai dengan spektroskopi infra-merah dekat (NIRs), infra-merah tengah (MIRs) dan juga kromatrografi gas dengan flame ionisation detector (GC-FID). Spektra NIR dan MIR, dan nisbah diagnostik lemak asid (fatty acid DR) telah digunakan dalam kemometrik. Perbezaan antara minyak tulen dan minyak goreng yang digoreng dengan daging yang sama telah digambarkan dengan principal component analysis (PCA). Selain itu, semua minyak goreng yang diguna semula pada kali ke-5 menunjukkan perbezaan dengan minyak tulen dalam PCA.dan orthogonal partial least square discriminant analysis (OPLS-DA) telah digunakan untuk mendiskriminasi minyak tulen daripada minyak pengadukan dan goreng. Plot PCA berjaya menunjukkan perbezaaan antara minyak goreng kali ke-5 dan ke atas, dengan minyak tulen. Semua model OPLS-DA daripada NIR berjaya

mengenalpasti minyak goreng daripada minyak pengadukan dan minyak tulen. Manakala, terdapat satu misklasifikasi dalam model MIR daripada kumpulan daging lembu. Model nisbah diagnostic asid lemak juga menunjukkan satu misklasifikasi masing-masing daripada kumpulan daging lembu dan ikan. Dalam kajian ini, lingkungan spektra 1620 - 1670 nm dan 1830 - 1890 nm membezakan minyak tulen dengan miyak tidak tulen dalam model NIR. Di samping itu, lingkungan spektra diskriminasi yang paling penting dalam model MIR ialah 650 - 690 cm^{-1}, 2920 - 2950 cm^{-1} dan 3200-3400 cm^{-1}. Akhir sekali, dalam model nisbah diagnostic asid lemak, nisbah jumlah asid palmitic dan sterik kepada jumlah asid olik dan linolik berserta dengan nisbah asid oleic kepada asid palmitik dalam minyak tulen dan tidak tulen adalah nisbah diagnostik diskriminasi yang paling penting. Dalam kajian ini, terdapat beberapa aspek yang perlu diperhatikan. Sebagai contohnya, model diskriminasi hanya berkesan terhadap minyak tidak tulen yang digoreng dengan sejenis daging. Selain itu, saringan tidak tertumpu tidak dapat mengenal pasti kompaun yang menyebabkan perubahan dalam minyak goreng. Manakala, nisbah asid lemak hanya tertumpu kepada asid lemak tetapi kompaun lain tidak akan dikaji. Secara umumnya, saringan tertumpu dan tidak tertumpu seperti NIR, MIR dan DR berpotensi untuk dijadikan alat pengesahan bagi minyak goreng.

ADULTERATION DETECTION OF EDIBLE PALM OIL USING INFRARED UNTARGETED SCREENING AND TARGETED FATTY ACID DIAGNOSTIC RATIO COUPLED WITH CHEMOMETRICS

ABSTRACT

In Malaysia, repeated use of cooking oil in deep frying, especially refined, bleached and deodorised palm oil (RBDPO), is common. The adulteration of RBDPO with used oil has been studied with frying simulation using different kinds of meats, namely beef, fish, mutton, and pork. The oil underwent frying for 25 cycles, at 180 ºC, 10 minutes per cycle. Then, used oil was sampled at the 5th, 10th, 15th, 20th, and 25th frying cycles. Admixture of pure oil with used oil was done at 1%, 5%, and 10% (mass/mass) concentrations. All the pure, admixed, and used oil were evaluated with untargeted screening analysis, near-infrared spectroscopy (NIRs), mid-infrared spectroscopy (MIRs), and targeted analysis, gas chromatography coupled with flame ionisation detector (GC-FID). NIR and MIR spectra and fatty acid diagnostic ratio (DR) composed of fatty acid abundance were obtained. The variation between pure and used oil of the same meat type was visualized with principal component analysis (PCA). Besides, the 5th frying cycle's used oil showed a significant difference to the pure oil in the PCA score plot. The orthogonal partial least square discriminant analysis (OPLS-DA) of the NIR models had successfully identified used and admixed oil from the pure oil. Next, in the MIR model, there was one misclassification in Beef. Lastly, in fatty acid diagnostic ratio models, Beef and Fish had one misclassification of pure oil, respectively. The most significant factors for the NIR model were determined to be spectral region 1620 - 1670 nm and 1830 - 1890 nm, whereas the most significant factors in MIR for pure oil was spectral region 650 - 690 cm^{-1}, whilst spectral regions

2920 - 2950 cm^{-1} and 3200-3400 cm^{-1} contributed to the used oil. On the other hand, the critical factor in the fatty acid diagnostic ratio model is the ratio of palmitic and stearic acid to oleic and linoleic acid, and the ratio of oleic acid to palmitic acid present in pure and used RBDPOs. Some aspects were not within reach of the current study. For example, the classification analysis only worked on used oil fried with the same meat type. Other than that, untargeted screening methods do not directly indicated the compounds linked to the adulteration of used oil. In terms of targeted analysis, only the changes in the composition of fatty acids were measured. Overall, untargeted screening and targeted analysis potentially be handy tools in adulteration detection of used oil in palm oil.

CHAPTER 1

INTRODUCTION

1.1 Background

This chapter provides a brief introduction to food adulteration in general and palm oil adulteration in specific. Besides, the admixing of used oil in pure oil has led to food adulteration issues. Subsequently, detection techniques that may help curb this issue will be briefly discussed in this chapter. The problem statement is then identified, and the objectives of this research are proposed. Last but not least, the structure of this thesis is conveyed.

1.1.1 Food adulteration

Food is human nourishment. It helps sustain a healthy physical body. Consumers tend to have preferences over particular food due to the health benefits, aroma, or taste. The demand for these foods always results in higher economic value. The high price of food triggers the unethical act of manipulating food constituents with cheaper material for the sake of profit. This act is considered adulteration, and it will affect the authenticity of the food.

Authenticity is the genuineness of food, where the food matches its description (Kvasnička, 2005). Authentic food may develop a deterioration in quality under some circumstances, including prolonged storage. Countless cases of food authenticity issues which include the adulteration of melamine in milk (Pei *et al.*, 2011), the horsemeat scandal in Europe (Abbots and Coles, 2013), and phthalate tainted food in Taiwan (Tsai *et al.*, 2016), have been encountered in the past.

These issues may go unnoticed by consumers most of the time as the differences between authentic and adulterated food may not be noticeable. These issues can happen as long as there is a chance to profit from altering food composition (Johnson, 2014). When the truth is revealed, the consumers may have lost confidence in the product as the money paid may not be worth the product's value. Law enforcement alone may not be enough to curb food adulteration. The help of technology, particularly the authenticity assessment and database establishment, are required.

1.1.2 Palm oil and used oil

Palm oil is a type of vegetable oil. It is also the most productive oil crop on this planet, as it can produce 3.85 tonnes of oil per hectare of land compared to the other vegetable oils (MPOC, 2020). Oil palm tree (*Elaeis guineensis Jacq*.) is a species that originated from West Africa and is cultivated across tropical countries. Palm oil can be traced in different products such as cosmetics, personal hygiene, and biofuel. Furthermore, palm oil can be found in food products such as cooking oil, bread, chocolate and others. Therefore, the application of palm oil in various food products shows the significance of palm oil.

Southeast Asia countries such as Malaysia and Indonesia are responsible for 85% of palm oil production globally. The availability and low price have made them the primary choice for cooking oil. Cooking oil made from palm oil is mainly used in the fried food industry, and fried food is famous for its crisp texture after frying at high temperatures. However, the high temperature initiates some chemical reactions and produces unwanted waste products that may be carcinogenic.

For the sake of cost-saving, cooking oil is often reused. The changes in cooking oil may not be significant appearance-wise, but the deterioration of the cooking oil quality remains unknown. The incident of the gutter oil scandal (Wee *et al.*, 2016) that happened in mainland China had caused a disturbance in our society. Gutter oil refers to waste oil originating from food processing plants and restaurants (Lu and Wu, 2014). Gutter oil was reprocessed, remixed with fresh oil, and resold in the market. If we were to allow the used oil adulteration to happen, consumers' rights could be compromised, and the reputation of the palm oil industry could be tainted.

1.1.3 Untargeted screening and targeted analysis

There are two main categories in adulteration analysis: untargeted screening and targeted detection analysis. Untargeted screenings consider numerous undetermined compounds, usually termed 'fingerprint', to detect adulteration. Such analysis is qualitative and multivariate statistics is essential to analyse and interpret the outcome. The shortcoming of untargeted screening is that the application of such a model is sophisticated for non-statisticians (Brereton, 2014) and model validation is a must. On the other hand, untargeted screening offers an advantage as this technique compares the ‘fingerprint’ of unknown samples with the ‘fingerprint’ of authentic samples. Any samples with significant variation from the authentic samples will be considered adulterated samples (Limm *et al.*, 2018). This approach will reduce the risk of misidentification of adulterated samples adulterated with different adulterants.

Fourier transform infrared spectroscopy (FTIRs) and near-infrared spectroscopy (NIRs) have been applied in previous food adulteration detection research. Infrared spectroscopy is well known for its fingerprinting technique in the authentication of food products. It offers minimal sample preparation, fast spectral acquisition, is easy to operate and can handle different food samples. Both of these analyses require the help of multivariate statistical analysis to interpret the relevant information from the raw data.

The targeted analysis is the mainstream analysis. A specific marker compound is targeted, and its concentration is compared to the established limit to determine adulteration in food. These analyses work well on known adulterants and are supported by matrix-matched certified reference material, a requirement for accreditation (Ballin and Laursen, 2019), which can be used as legal evidence in the court of law. The analyses provide a clear indication of food adulteration in one aspect but not the others. In some circumstances, the targeted analysis may be inefficient to detect adulteration, significantly when many potential adulterants can be admixed into food products. One of the examples is the adulteration of melamine in milk powder (Pei *et al.*, 2011). The protein content of milk was estimated based on the total protein content without justifying the quality of the protein. The combustion-based protein analysis (Moore *et al.*, 2010), widely used in official control to determine protein content, failed to detect melamine adulteration. The other example is the determination of organic produces using nitrogen isotopes analysis. This technique can hardly differentiate between crops cultivated using legume-based green manure and synthetic fertiliser, which may cause inconvenience to the producers (Ballin and Laursen, 2019).

To date, mass spectroscopy (Hong *et al.*, 2011), nuclear magnetic resonance (NMR) (Miyake *et al.*, 1998), and gas chromatography flame ionization detector (GC-FID) (Godswill *et al.*, 2014) have been applied in the determination of palm oil adulteration by detecting a few parameters such as iodine value, fatty acid composition, and polar compound. These targeted analyses offer quantitative measurement and direct comparison to detect adulteration, with some shortcomings such as time-consuming and costly (Christopoulou *et al.*, 2004). Gas chromatography flame ionization detection (GC-FID) is the most commonly used technique to assess fatty acid profile, which are the main constituents of palm oil.

1.2 Problem statement

Used oil is cooking oil that has been subjected to frying at high temperatures in either the food processing plants, restaurants, or homes (Singh-Ackbarali *et al.*, 2017). The degradation of used oil depends on the frying condition, including food fried, temperature, cooking oil type and others. One of the most significant causes of degradation is the repetitive use of used oil, which may have happened in food processing plants or restaurants, mainly due to cost-saving. Other than degradation, the quality of used oil may be affected by the contamination of fried food. For instance, the used oil may have been contaminated with animal fats during frying. The contamination is a concern from a particular perspective of religion as consumption of pork is prohibited in Islam while consumption of beef is restricted in Hinduism. Then, a common indication to discard degraded used oil is when the oil starts to smell bad or form foam.

The commonly accepted regulation limit for the disposal of used oil is when 25% of the total polar compound (TPC) is reached. The task of monitoring the compliance of TPC limit in the food and beverage industry is done by the authority such as the Ministry of Health, Malaysia. TPC is usually measured using a ‘testo 270’ cooking oil tester where this instrument generates instant results by direct contact with the oil samples. Nonetheless, the testo 270 is specifically designed to detect the changes in the polar compound but not other compounds. The reuse of used oil may be unintentional, however, used oil consumption potentially cause a negative impact on health because of the impurities and the chemical changes in the used oil. Hence, the combination of reasons above signifies the necessity to develop other measures against the used oil adulteration.

The conventional screening method for palm oil adulteration is mainly on targeted analysis, such as gas chromatography. These techniques provide precision in determining the concentration of particular compounds such as aromatic compounds, hydroperoxides, and others. The diagnostic ratio of saturated to unsaturated fatty acid in detecting vegetable oil adulteration has been suggested (Lee *et al.*, 1998) and investigated in previous research (Park *et al.*, 2010; Strashnov *et al.*, 2019). All these researches aimed to detect adulteration between different vegetable oils. Nevertheless, these techniques may not recognise any changes in the used oil other than the targeted compounds.

On the other hand, untargeted screening such as infrared spectroscopy is another approach to used oil adulteration in palm oil. The combination of chemical fingerprint of oil and multivariate analysis can help detect changes in palm oil through the profile of palm oil rather than a specific marker. Under different circumstances, untargeted screening and targeted techniques can prove to be helpful as well as complimentary. For instance, an untargeted screening technique can be applied when screening through many suspected samples with unknown adulterants. In contrast, if the type of adulterant was known, then a targeted technique can quantitatively assess the adulteration level in the particular sample.

As far as the author's knowledge is concerned, only two researches had been done on the quality assessment of used palm oil using GC-FID (Khor *et al.*, 2019) and FTIR (Lim *et al.*, 2018). Therefore, there is room for an investigation into the application of untargeted (NIR and MIR) screening and targeted (fatty acid diagnostic ratio) analysis on the used oil adulteration assessment of palm oil.

1.3 Aim and objectives

This study aims to investigate the adulteration detection analysis that can differentiate between pure and non-pure oil. The non-pure oil in this context refers to the used oil and admixed oil (pure oil adulterated with used oil). Three specific objectives are listed below:

1. To determine the spectral regions and diagnostic ratios that contributed to the differences of pure and used oil using exploratory statistical techniques.
2. To develop discriminant models based on untargeted screening and targeted techniques to assess spectral regions and diagnostic ratios that contributed to pure and non-pure oil differentiation.
3. To evaluate the effectiveness of untargeted screening and targeted techniques in the discrimination of used oil adulteration in pure oil.

1.4 Scope of the study

The scope of this study is focused on the adulteration detection of used oil in pure RBDPO. Firstly, the pure RBDPO was obtained from the refinery plant of Sime Darby Plantation Bhd. In this study, pure oil refers to pure RBDPO, and used oil refers to used frying oil after 5, 10, 15, 20, and 25 frying cycles. Admixed oil refers to the admixtures of used oil and pure oil at 1%, 5% and 10% (mass/mass). The untargeted screening will be done using benchtop FTIRs and NIRs, while targeted analysis will be done using GC-FID. IR spectra of near and mid-range, 9090-4000 cm^{-1} and 4000-600 cm^{-1}, respectively, will be included in this study.

Meanwhile, the fatty acid abundance will be plotted as the fatty acid diagnostic ratio (fatty acid DR) and assessed their suitability in discriminating used oils. As for multivariate statistical analysis, principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA) will be performed using Sartorius Umetric SIMCA® software. PCA will be applied to explore the variation between pure and used oil subjected to different frying conditions without prior class definition. The contributing factors for the differences between pure and used oil will be correlated to functional groups represented by the absorbance band. In the targeted analysis, the contributing aspects will be explained using the fatty acid DRs. Meanwhile, OPLS-DA can help to distinguish pure oils from non-pure oils by identifying the most significant classification factor.

1.5 Thesis outline

This thesis is arranged into chapters comprising Introduction, Literature reviews, Methodology, Results and discussions, and Conclusion and Future recommendations.

Firstly, Chapter 1 gives an overview of the palm oil industry and food adulteration. The edible oil scandal is the starting point where people started to be aware of the unethical act in the edible oil industry. Hence this research is initiated to study the available techniques in detecting used oil adulteration in palm oil.

Next, Chapter 2 discusses the origin of palm oil, how it is processed, the applications and its importance. Additionally, the deep-frying mechanism and deterioration of used oil are explained. The subsequent countermeasures such as the untargeted screening and targeted detection technique for adulteration detection and evaluation of used oil and the application of multivariate analysis in the previous works are discussed.

The methodology in Chapter 3 outlines the four stages of this study: the simulation of used oil, adulteration of pure oil, evaluation of oil using untargeted screening and targeted techniques, and finally, multivariate statistical analysis. Simulation of used oil will be done by frying the oil with different meats with different frying cycles. The admixture of used oil with pure oil will be done at 1%, 5%, and 10% of concentration (m/m). The oil samples will be evaluated using untargeted screening (IR) and targeted (GC-FID) techniques, and the data obtained (IR spectra and fatty acid DR) will be analysed with chemometrics.

In Chapter 4, unsupervised multivariate statistical analysis will be applied to explore the variation between the pure, admixed, and used oils subjected to the same frying object. Besides, the differences between pure and used oil of different frying cycles will be studied. Next, discriminant models will be developed to classify pure oil from the admixed and used oils. Contributing factors responsible for the differences in oil will also be identified.

Lastly, in Chapter 5, the conclusions of the project findings will be highlighted and further recommendations will be suggested.

1.6 Summary

As a summary, this chapter discusses the risk posed by used oil and the adulteration of used oil. Palm oil, the most readily available edible vegetable oil in Malaysia, is susceptible to adulteration with used oil. Given the potential impact of this matter, this study was initiated to identify the science behind adulterated and used oils’ changes and differentiate pure oil from non-pure oil. Then, the scope of this study is focused on untargeted screening and targeted analysis. Next, a deeper understanding of the topics is needed to identify the gap between past research and current studies. Hence, the details are discussed in the next chapter.

CHAPTER 2

LITERATURE REVIEW

In this chapter, the study object, palm oil, is discussed thoroughly, including its origin, extraction, properties, consumption trend, and applications. Palm oil can be applied as cooking oil, and the chemical changes involved during deep frying and their potential health effects are discussed as well. The research gaps in detection analyses for used oil, from the perspective of untargeted screening and targeted analysis, in combination with multivariate data analysis, are reviewed as well.

2.1 Chemical properties of RBDPO

Palm oil has triacylglycerol (TAG) as the predominant component, similar to other vegetable oils. TAG consists of a glycerol backbone and is attached to three fatty acid chains. Palmitic acid (44%), oleic acid (40%), linoleic acid (10%) and stearic acid (5%) are the significant components in palm oil. The fatty acid constituent of palm oil, which consists of a balanced proportion between saturated and unsaturated fatty acids, is the critical difference from other vegetable oils. In terms of physical appearance, palm oil is light yellow and almost indistinguishable from other types of vegetable oils.

2.2 Processing and refining of RBDPO

2.2.1 Processing of palm fruits

There are various scales in processing palm fruits, namely the traditional method, small-scale mechanical units, medium-scale mills, and large-scale mills (Poku, 2002). The processing depends on the number of palm fruits. Firstly, the fresh fruit bunch has to be processed within 24 hours after harvesting to prevent the development of free fatty acids (FFA). Then, the ripened fruits were sterilized by heat rendering and moisture absorption. This step is crucial to break down the lipolytic enzyme, the culprit for FFA formation in palm oil (Syahro *et al.*, 2016). FFA is one of the criteria for determining the authenticity of palm oil, and the concentration limit should be less than 5%. Then, the fruits were stripped from their bunches to proceed with the digestion of fruits.

2.2.2 Extraction of crude palm oils

During the digestion stage, the oil palm fruit cells were further broken down to accelerate the oil extraction process (Owolarafe and Faborode, 2008). The extraction methods can be classified as mechanical press and solvent extraction. The mechanical press could be divided into three categories: the hydraulic press, screw press, and centrifugation. In a continuous extraction system, the screw press is more applicable. In a batch or semi-batch extraction system, the hydraulic press is more suitable (Poku, 2002). The pressed liquor is a mixture of oil, water, fruit debris, and dirt. A clarification process is needed to increase the oil’s purity and reduce water concentration. The waste product formed after the process is called palm oil mill effluent (POME). On the other hand, solvent extraction is done by using a medium (water) to extract the oil from milled fruits. When the palm fruits are heated with hot steam, the oil is leached out. The hydrolysation of starch, resin, gums and coagulation of proteins also occurred during the extraction process and is removed in the clarification stage. At the end, the CPO was clarified by centrifugation and drying.

2.2.3 Refining of palm oils

The pure CPO is turned into refined, bleached and deodorised palm oil (RBDPO) through physical or chemical refining. In chemical refining, the first step is alkaline refining. Then, the CPO is washed with sodium hydroxide or sodium carbonate to remove the FFA and lipids. However, there are cons to using chemical refining. High energy consumption, loss of neutralized triacylglycerol, time-consuming, expensive equipment, and many pollutants produced are the disadvantages (Čmolík and Pokorný, 2000). Due to these factors, physical refining is favoured. Physical refining involves steam distillation of oil under high temperatures and vacuum. In the degumming process, the purpose is to isolate the hydratable gum (aqueous solution of phosphoric acid) from non-hydratable gum by heating it with water. Bleaching and deodorisation of CPO are done by removing pigments with activated clay and volatile oxidation products by steam distillation. (Dian *et al.*, 2015). The end product is bland, odourless, yellowish liquid or semi-solid oil at room temperature.

2.2.4 The grade of RBDPO

The RBDPO's qualities can be classified into different grades, measured by the purity and FFA content. Low purity and high FFA palm oil are usually used for non-edible products such as candles, while high purity and low FFA palm oil are used in food products, for instance, cooking oil (Henson, 2012). The subsequent fractionation was done by cooling the RBDPO under a controlled environment to separate the TAG based on their melting points. The fractionation is essential for products such as cooking oil in temperate regions to prevent crystallisation. Palm olein and palm stearin are among the final products of this process. Palm olein is dedicated to cooking oil, shortening, margarine, and also industrial frying oil. In contrast, palm stearin is used for shortening, margarine, fats for confectionery, soap, and oleochemical processes.

2.3 Consumption trend

The global consumption of palm oil increased tremendously due to its compatibility with various food products and affordable prices. Palm oil of different grades with different chemical and physical properties makes them suitable for multiple applications such as candles, toothpaste, cosmetics, ice cream, lubricants, and biodiesel. Furthermore, the advancement in fractionation technology has helped reduce the intake of trans-fatty acids as palm stearin could substitute solid fat without undergoing hydrogenation (Kellens *et al.*, 2007). It is estimated that 90% of palm oil production is applied in edible products, while 10% goes into the non-edible product industry (MPOC, 2020). Palm oil has been leading the global vegetable oil production since 2005 by replacing soybean oil. The global production of palm oil has increased by 27.7%, while global domestic consumption increased by 25.5% from 2015 to 2019. Palm oil production has the highest individual weightage, with 73.9 million metric tons or 36.4% of all vegetable oils production worldwide (USDA, 2020). Malaysia is the third-largest vegetable oil supplier, producing 23.24 million metric tons of oils, while India, China and the European Union are the largest importers of palm oil.

European Union has emphasized reducing reliance on non-renewable fuel by developing biofuel using palm oil several years ago. The increase in demand for palm oil also stimulated the growth of the used oil industry in Malaysia, as used cooking oil is a good material for biofuel. Recently, the Indonesian government also focused on developing biodiesel as they are the largest producer of palm oil, reducing the reliance on fossil fuel. There is much space for the growth of the palm oil sector as palm oil is a renewable source, capable of mass production, and widely applicable to a massive range of products.

2.4 Deep frying

Deep frying is favoured in food preparation as this method is time-saving, and the products have organoleptic properties. A few origins have been proposed, where Rossell (Rossell, 2001) suggested that deep-frying was invented by ancient Chinese, while others proposed that this method was mainly confined to the Mediterranean (Fillion and Henry, 1998) but is now becoming popular in every part of the world. Vegetable oils are the medium for deep frying, and hence part of the overall cost is the function of the vegetable oils' turnover rate and price. For the sake of cost-saving, vegetable oils are repeatedly used throughout the frying process. Deep frying is usually done by heating the oil to a high temperature, between 150 °C to 190 °C. Then, the food is immersed into the oil with air and moisture until the food is cooked (Choe and Min, 2007). During deep frying, the heat and mass exchange between the oil and the food are the key contributions to the texture and taste of fried food. The type of food, frying oil, and temperature play a role in the changes in frying oil composition.

Deep frying is usually done by immersing food into the hot oil, resulting in intense bubbling and drying at the superficial level. This phenomenon is caused by the sudden evaporation of moisture that remains on the food. The vaporisation rate of moisture from the core of food to the surface may outpace the removal of steam by the oil via convection, forming a heat resistance layer between the oil and the food. The intense bubbling also increases the contact area and the rate of heat transfer between the oil and the air, leading to oxidative degradation of oil (Costa *et al.*, 1999). As the frying time increases, the bubbling effect will reduce. Food's moisture forms a layer of steam over the oil surface and reduces the airflow in the headspace, decreasing the contact between oil with air and the oil's oxidation rate.

In deep frying, heat is transferred through convection and conduction between the food and the oil. Evaporation of moisture from the food causes more heat to move into the core of the food, which causes temperature and pressure increase of water content in the food. The process continues as the water relocates from the core of the food to the surface (Vitrac *et al.*, 2000). The transfer of moisture cools down the food surface during the initial stage of frying and preventing burning. However, prolonged frying will reduce the water content to a rate where the cooling effect has vanished, and hence the food is overcooked.

2.4.1 Fried food & fat exchange

A variety of food can be subjected to frying, including meat, vegetables, and fruits. These foods contain a different percentage of carbohydrates, protein, fibre, fats, and other constituents. Plant-based food such as potatoes contains very little fat, which is usually found within the lipid membrane. Therefore, the transfer of fat from plant-based food into the frying medium is insignificant. On the other hand, meat with little fat content, such as chicken breast, will absorb the frying fat. The most commonly consumed meat such as beef, fish, pork, and mutton have fatty acid profiles that differ from frying oils. The differences in fatty acid content make the fatty acid exchange between the food and the frying oil possible (Pokorný and Réblová, 1999).

2.4.2 Frying mechanism

Generally, deep frying can be divided into continuous and intermittent frying. The type of frying has a different effect on the frying oil. The intermittent frying has an interval for cooling where the frying process is paused temporarily, then reheated again to the desired temperature for frying operation. The cooling effect of frying oil allows more oxygen to dissolve. Therefore, the heating, cooling and reheating of frying oil increases the rate of deterioration of frying oil compared to continuous heating (Das *et al.*, 2013; Perkins and van Akkeren, 1965). Similar findings were found in another research, where the loss of oil, foaming and colour changes were more significant in intermittent frying (Peers and Swoboda, 1982). Another study found that the formation of cytotoxic secondary lipid peroxide is caused by the duration of frying rather than the type of frying (Christine and Csallany, 2006). Urbančič *et al.* (2014) reported that the frying oil reaches the 25% total polar compound limit after ten frying cycles. The FFA also shows an increasing trend of up to 20 frying cycles. The changes in fatty acid composition were studied by Chung *et al.* (2004), where linolenic acid decreased as the number of frying increases.

The temperature of deep frying is also crucial to the frying oil. The rule of thumb for a frying temperature range is between 175-195 ºC. According to the guideline provided by USDA (USDA, 2013), the recommended deep-frying temperature is 160-190 ºC. German Society for Fat Science set the rule for deep frying temperature not exceeding 180 ºC as it will accelerate the deterioration of frying oil (Matthäus *et al.*, 2012). Intermittent frying will cause instability of oil and formation of the aromatic compound when the oil is cooled to below 120 ºC and reheated again. Physical changes of oil such as colour and viscosity, and chemical changes, namely an increase of FFA, a decrease of peroxide, and iodine value, were observed when the frying temperature was raised (Tyagi and Vasishtha, 1996). Higher frying temperatures may suit frying operations with a high turnover rate of oil or food with a thin structure (Mehta and Swinburn, 2001).

2.4.3 Chemistry of deep-frying

Chemical reactions (oxidation, hydrolysis, polymerization, and isomerization) occur and cause changes in the oil composition. The chemical changes may be triggered by the operation of frying (temperature, frying duration, turnover rate), food content (lipid composition, main or minor constituent), or the oil itself (degree of unsaturation). Recurrent frying will eventually lead to lipid peroxidation (Choe and Min, 2007). The chemical reaction in the oil simultaneously affects oils' physical properties, including viscosity, colour, and flavour.

The TAG reacts with oxygen in two ways, autoxidation and thermal oxidation. The oxidative reaction happens through the free radical mechanism of chain reaction, triggered by the oxidation of the unsaturated fatty acyl group. Autoxidation occurs at low temperatures, while thermal oxidation occurs at high temperatures. The chemical mechanism of autoxidation is similar to thermal oxidation but at a slower rate (Choe and Min, 2007). The overall autoxidation process can be simplified into the initial, propagation and termination stages (Şahin and Sumnu, 2008). At lower temperatures, the reaction between unsaturated fatty acids and oxygen leads to unsaturated hydroperoxide formation (ROO*). The weakest carbon-hydrogen bond (C-H) is broken upon heating to form a carbon-centred alkyl radical (R*). Polyunsaturated fatty acids (PUFA) contain more unsaturated bonds with lower dissociation energy (C-H bonds) than monounsaturated fatty acids (MUFA). Hence, PUFA is more readily undergoes oxidation. The oxidation process continues with hydroperoxide (ROOH) formation as their primary oxidation products (Márquez-Ruiz *et al.*, 1996). The hydroperoxides formed are relatively unstable and subject to form radicals, which enter the loop of another cycle of hydroperoxide formation until all the lipid molecules are depleted. The presence of heavy metal traces (from frying utensils) also contributes significantly to lipid oxidation. Metal initiates the redox reaction with lipid and hydroperoxide to form a metal-oxygen transition complex or metal hydroxide complex (Choe and Min, 2007). Cyclisation may occur throughout the process and lead to the formation of hydroperoxyl-epidioxide.

During frying, thermal oxidation is influenced by other aspects such as the degree of unsaturation in oil and thermal exposure time. TAG, such as triolein, is more susceptible to oxidation, forms mono-hydroperoxides, and can be further oxidised to become bis and tris-hydroperoxides. It was found that the replacement of linoleic acid with oleic acid could substantially strengthen the oil’s thermal stability (Smith *et al.*, 2007).

At high temperatures, the thermal reaction occurs in the absence of oxygen; simultaneously, the oxidation rate decreases. Consequently, the hydroperoxides are oxidized through homolysis of peroxide bond and become hydroxy radicals and alkoxy radicals due to the unstable nature of hydroperoxides under high temperatures. The alkoxy radicals react with other alkoxy radicals or turn into other non-radical compounds at the termination stage. The end products are usually compound with low polarity as there is a lack of excessive oxygen in the compounds.

TAG dimers and polymers are the major decomposition products of used oil. Polymerization compounds start to occur after the accumulation of oxidized monomers (Márquez-Ruiz *et al.*, 1996). Peroxy-dimers are formed from the peroxy-radicals and alkyl peroxy. Hydroperoxy-epidioxide, TAG dimers, oligomers, triglyceride monomers, and volatile compounds are found at the end of the reaction (Şahin and Sumnu, 2008).

The amounts of polymers formed are proportional to the increase in frying temperature and the number of frying cycles. Besides, the amounts of cyclic polymers formed are dependent on the linoleic acid’s concentration in the oil. 20% of linoleic acid is the critical level for the formation of cyclic polymers. Overall, cyclic compounds formed in deep-frying are relatively lower than non-volatile compounds, dimers, and polymers (Dobarganes *et al.*, 2000). The formation of polymers eventually leads to oil degradation, such as foaming of oil, colour changes, increased viscosity, and oil absorption by the food during deep frying.

Hydrolysis is a reaction that involves the lipolytic enzyme and moisture. This reaction is common as the majority of the food contains water. It is the only reaction that breaks down the TAG into di-acylglycerol and FFA. During deep frying, steam is formed from the moisture. Water is a type of weak nucleophile, and it causes the breakage of ester linkage in the TAG. The role of water in hydrolysis is more significant than steam in the oil rather than in the water-oil interface (Lascaray, 1949). Therefore, the area of contact of the aqueous phase of food with oil increases the rate of hydrolysis. The increase in total polar compound (TPC) and FFA in oil due to food's high moisture content was observed in previous research (Bhattacharya *et al.*, 2008). Long and saturated fatty acids have a lower solubility in water and, therefore, are less readily hydrolysed than short and unsaturated fatty acids. At the end of the reaction, di- and mono-acylglycerol, glycerol, and FFA are produced. The formation of FFA causes changes in the oil's physical and chemical properties, such as decreased smoke point, reduced interfacial tension, and extra volatile and flavour compounds.

2.5 Adulteration and health effects

Adulteration means mixing or adding something with erroneous intention, while food provides energy and nutrients to all life forms. Therefore, food adulteration will cause the degradation of food quality. The purpose of adulteration has always been to maximize profit by using an inferior substance to substitute food. The Malaysian government has a particular act specialized for food adulteration, the Food Act 1983, section 13B. This act defines the conditions that will commit to food adulteration and the legal consequences of violating such regulation.

Edible oils and fats are among the highest number of adulteration cases from 1980 to 2010 (Moore *et al.*, 2012). This statistic shows that edible oils and fats are more susceptible to adulteration. Cooking oil adulteration happened a few years back and caused a riot in the Asian region (Taiwan and mainland China). Restaurants were found to be using used oil or gutter oil in preparing food. The used oil or gutter oil is waste oil meant to be discarded from restaurants, slaughterhouses, or processing plants. Those oils were reprocessed and resold due to their lower price and high similarities between the used oil and the pure oil. The fact that the market in China is vast has contributed to the adulteration of cooking oil. China needs to import cooking oil, although China is the second-leading oil and fats producer globally. Among the oil consumption in China, palm oil has a weightage of 18% (Jamet and Chaumet, 2016). The demand for palm oil increases as its price is relatively lower than soybean oil, and the oil is suitable for multi-purposes such as processed food and snack frying. Cooking oil adulteration could happen in Malaysia since Malaysia is a significant palm oil producer, and palm oil has a considerable market presence.

The detrimental effect on human health upon consuming contaminated oil or used oil is hard to estimate as it does not give rise to immediate adverse effects. However, research has shown that repeatedly heated palm and soy oil consumption could increase blood pressure, lipid peroxidation, and adverse effects on endothelial function (Leong *et al.*, 2010). The damage to human health can reach the cellular and genetic level (Adam *et al.*, 2009; Farag *et al.*, 2010; Mariam *et al.*, 2010; Sülzle *et al.*, 2004).

2.6 Detection of used oil adulteration

2.6.1 Physical parameters

One of the most commonly used methods for laypeople to examine the adulteration of palm oil is checking the oil's physical properties. Used oil tends to change appearance, such as changes in colour and foaming (Chang *et al.*, 1978). The darkening in colour may be related to oxidation, polymerization, and other chemical reactions (Bansal *et al.*, 2010). Aladedunye and Przybylski (2009) also reported that the frying temperature contributes to the darkening of oil. The reflective index has also been reported to increase with frying duration (Aniołowska and Kita, 2015; Hashem *et al.*, 2017). Besides, the change in viscosity in frying oil is associated with the degradation of frying oil. Viscosity is more reliable than colourimetric value and refractive index as an oil degradation parameter, as viscosity is not affected by frying conditions (Bansal *et al.*, 2010).

2.6.2 Untargeted screening analysis

The untargeted screening analysis aims to include as many compounds or features as technically possible, or in other words, the whole sample matrix (Riedl *et al.*, 2015). The data generated from the instruments are unique to individual samples as if the fingerprint differs from one another (Zhang *et al.*, 2011). The profile of an authentic sample will help to reveal or detect abnormalities in the samples. Many techniques can be included under the scope of untargeted screening analysis, namely nuclear magnetic resonance (NMR), infrared spectroscopy, Raman spectroscopy and others. NMR had been applied in the study of origin authentication of butter, margarine, animal fats, and 23 types of vegetable oils (Guyader *et al.*, 2018). Besides, the application of Raman spectroscopy in adulteration screening of hazelnut oil in extra virgin olive oil was studied (López-Díez *et al.*, 2003). Vibrational spectroscopy, especially infrared (IR), is a prominent methodology to assess food adulteration, as IR spectroscopy requires the slightest or no sample preparation, fast spectra acquisition, non-destructive and easy to operate. IR utilizes the interaction between the infrared wave with the functional group of food and creates the spectrum that reflects the properties of food. Fourier Transform Infrared spectroscopy (FTIRs) and Fourier Transform Near-infrared spectroscopy (FTNIRs) are the most commonly known instruments. The spectrometry method can assign the functional groups present in the samples and predict the compound present in a sample. FT-IRs or mid-infrared spectroscopy (MIRs) utilises the electromagnetic spectrum of range 4000 – 400 cm^{-1} (2500 – 25000 nm) and are based on the fundamental vibrations and associated rotational-vibrational effects.

Meanwhile, NIRs use a spectrum range of 12500 - 4000 cm^{-1} (800 - 2500 nm). The NIR spectrum consists of much more complex spectrums than MIRs, including the molecular overtone and combination vibrations. The application of MIR includes raw milk adulteration (Cassoli *et al.*, 2011), pork adulteration in beef meatballs (Rohman *et al.*, 2011), and inverted beet sugar adulteration in honey (Sivakesava and Irudayaraj, 2001). In contrast, the applications of NIR in food-related studies are pesticide residue in pepper (Sánchez *et al.*, 2010), apricot fruit quality (Bureau *et al.*, 2009), and adulteration in Chinese lotus root powder (Xu *et al.*, 2013). To date, there are very few studies on the adulteration screening of palm oil with different edible oils or fats, using MIR (Che Man *et al.*, 2014) and NIR (Basri *et al.*, 2018). There are studies on the evaluation of used oil other than palm oil, based on MIR (Goburdhun *et al.*, 2001; Zhang *et al.*, 2012) and NIR (Ng *et al.*, 2007). One study was done on the adulteration of palm oil with recycled used oil using fatty acid composition and FTIR (Lim *et al.*, 2018). There is room for an investigation into the potential of implementing NIR to detect oil adulteration of palm oil with used oil. The recent studies of untargeted screening analysis have been summarised in Table 2.1. Then, the band assignment for edible oils in MIR and NIR from different literature reviews have been summarised in Tables 2.2 and 2.3.

Table 2.1 The summary of the study on untargeted screening analysis

Analytical approach	Food products	Objective	Reference
MIR	Raw milk	Adulteration detection	Cassoli *et al.*, 2011
MIR	Beef meatball	Pork adulteration detection	Rohman *et al.*, 2011
MIR	Palm oil	Lard adulteration detection	Che Man *et al.*, 2014
MIR	Soybean oil	Used oil quality evaluation	Goburdhun, 2001
MIR	Corn, peanut, rapeseed, soybean	Used oil adulteration detection	Zhang *et al.*, 2012
MIR	Palm oil	Used oil adulteration detection	Lim *et al.*, 2018
NIR	Pepper	Pesticide residue detection	Sánchez *et al.*, 2010
NIR	Apricot	Quality assessment	Bureau *et al.*, 2009
NIR	Chinese Lotus Root Powder	Starch powder adulteration detection	Xu *et al.*, 2013
NIR	Palm oil	Chicken fat adulteration detection	Basri *et al.*, 2018
NIR	Frying oil	Degradation detection	Ng *et al.*, 2007