CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Over the few decades, chemometrics on lard profiles has gained interest in authentication research. Lard is the fats rendered from slaughtered pigs, stated as non-*halal* or prohibitive substances under Islamic guidelines, Kosher food regulations, and dietary rules (Ali & Naquiah, 2018). Therefore, chemometrics application on lard profiles is the most studied in multivariate data of the Fatty Acids (FAs) and Triacylglycerols (TAGs), utilising spectroscopy and chromatography techniques. In addition, various studies have been combined with chemometrics to differentiate other products from pigs or porks, such as pork meat (Leng et al., 2020) and gelatin (Cebi et al., 2019).

FAs of lard have been reported to have variations depending on different pig food sources (Franco et al., 2014; Joven et al., 2014; Tartrakoon et al., 2016; Tian et al., 2015). Furthermore, the composition of FAs in lard is also different in parts of the pigs' bodies (Jiang et al., 2018; Overholt et al., 2018). Unfortunately, while the lard was extracted or self-rendered from fat tissues in the laboratories for authentication tasks, there is still insufficient data on lard profiles from various sources as references.

Fats-based food involves heat convection; heating and its duration become the main parameter. The FAs alteration that occurs during the heating-process is inevitable due to the degradation of TAG into secondary oxidation products such as ketone and aldehyde. The degradation of FAs and

TAG has led to one significant problem, which is difficult to recognise in identifying fats and oils from their origin species (Barriuso et al., 2013; Brühl 2014; Vaskova & Buckova, 2015; Zhang, et al., 2012). Thus, many researchers have opted for the univariate analysis rather than multivariate analysis on FAs data from fats-based food products (Afaneh et al., 2017; Alzaa et al., 2018). As the growing authentication of fats-based food products, the detection of lard is not only a matter of establishing its difference from other edible fats but, more importantly, to access its dissimilar deterioration upon TAG degradation after heating-process at certain temperatures and times.

Fourier Transform Infrared (FTIR) spectroscopy analysis on complex FAs produces continuous data that often requires the use its multivariate analysis if the data among a large number of samples need to be performed. Mousa et al., (2022) reviewed that FTIR spectroscopy has several advantages over the other techniques for authenticity and adulteration detectors when combined with chemometric methods. FTIR analysis was successfully represented for rapid detection in edible oils, namely linear discriminant analysis (LDA) and partial least squares discriminant analysis (PLS-DA). However, there has been little discussion on the profiling of lard analysis and other techniques, such as Mahalanobis discriminant analysis (MDA) and quadratic discriminant analysis (QDA), which have better performances as classifier tools. The additional algorithm, linear support vector machines discriminant (SVMDA), likewise exhibited to consider the possibility of their robustness in the classification (Siddiqui et al., 2021).

Rohman and Windarsih, (2020) looked at how the FTIR has been used in the analysis of oils and fats, and various chemometrics methods in the selected spectral regions (the wavenumbers) were applied for classification, i.e., principal component analysis (PCA), and soft independent modelling of class analogy (SIMCA) and multivariate calibration, i.e., as partial least squares regression (PLSR). PLSR is widely used in multivariate analysis and adulteration analysis of oils using FTIR data. It was found that multivariate regression, such as principal component regression (PCR), and orthogonal signal correction partial least squares regression (OSC-PLSR), could provide robust recognition of lard that could have been unexplored by other researchers. Noted that the OSC-PLSR is a modification of the PLSR algorithm and has proven useful for the interpretation of important "omics" data that has been used in ^{1}H-NMR spectroscopy (Rivera-Pérez et al., 2022), liquid chromatography (LC) and gas chromatography (GC) with mass spectrometry (Brigante et al., 2022; Feizi et al., 2021). Furthermore, there is still little attention in the authentication application by OSC-PLSR to find the chemical features of lard spectra.

In routine analysis, the determination of FAs of lard still requires separation by chromatographic methods, such as gas chromatography with flame ionization detection (GC-FID), which is known by their selectivity and sensitivity. On the other hand, the most advanced liquid chromatography with tandem mass spectrometry (LC-MS/MS) with electrospray instruments and high sensitivity has been concerned with *halal* analysis (Salamah et al., 2019). However, the output of this instrument has also increased the complexity, such as lipidomics (Nagai et al., 2020; Wu et al., 2021).

Therefore, to date, too little attention has been paid to the profile of lard using LC-MS/MS, which certainly is worth exploring.

1.2 Statement of the Problem

For decades, it has been feasible to distinguish lard from other animal and plant fats by spectroscopy analysis in the *halal* authentication. However, studies show that the FAs profiles of lard varied depending on the pig's feed, causing the other work of profiling lard to be disputable. Another lard profiling issue is that FA deterioration upon heating-process might affect the chemical profiles. Thus, discrimination between other fats species becomes more complex if the fats have been subjected to heating. Modern spectroscopy and chromatography instruments such as FTIR, nucleus magnetic resonance (NMR), and GC-FID produce high multivariate data that complicates interpretation in profiling. Therefore, combining chemometrics and multivariate data generated from multiple analytical platforms can help the researcher better understand lard profiles. Focusing on the chemometrics evaluation of the data can assist the scientist in establishing a more robust strategy for lard determination and possible biomarker discovery for authentication.

This study aims to evaluate the profile of lard collected from different pig parts obtained from different regions of the country and to discriminate lard against other selected edible fats upon heating-process by various instruments and chemometrics. Chemometric approaches were developed based on the selected algorithms reported in the latest publication on oils and

fats profiling studies. In addition, the identification of lard's most significant chemical properties at different analytical platforms is also explored.

1.3 Research Question

1. What are the FTIR profiles of lard collected from different body parts and regions in Peninsular Malaysia?
2. How is the lard classified against other edible fats subjected to the heating-process using FTIR data points with chemometrics analysis?
3. How is the lard classified against other edible fats subjected to the heating-process using ^{1}H-NMR data coupled with chemometrics?
4. Which carbon of TAG structure can discriminate lard from chicken fats subjected to the heating-process?
5. What can possible FA degradation be detected to differ lard against selected fats subjected to the heating-process?
6. What lipid class existed in lard that differs from other selected fats subjected to the heating-process?

1.4 Scope of the Study

This research has focused on the chemometrics evaluation of lard profiles using spectroscopy and chromatography techniques. The initial stage of the study is to profile the collected lard of pigs from different body parts and various locations of Peninsular Malaysia by FTIR as a fundamental technique to ascertain the significant differences in the lard profiles.

The heating treatment is denoted as the heating-process in this study utilised a digital hotplate with controlled temperatures. The temperature selection was according to cooking temperatures at 120 °C, 180 °C, and 240 °C. The heating time employed started at 0.5 hr and reached a maximum duration of 3 hrs where the colour of the fat changed from its original colour.

This research covered the field of multivariate modelling of chemical data or calibration models of lard subjected to the heating-process by spectroscopy and chromatography analysis. The data mining techniques that have been conducted include PCA, multivariate classification, and multivariate regression. FTIR offers fingerprint band and peak assignment at certain wavenumbers, while ^{1}H-NMR provides valuable information for FAs and TAG chemical structures in oil and fats analysis. Both instruments have the advantage that they require minimal sample preparation, and the analyses can be rapid and robust. For further details on FAs studies, chromatography techniques such as GC-FID and LC-MS/MS were utilised. The degradation of FAs upon the heating-process was investigated to evaluate the distribution of FAs of multiple edible fats using GC-FID. More advanced lipidomic identification employing LC-MS/MS data was mainly based on the lipid-class selection of fragmented ions and mass ratio (*m/z*).

Great concern has been focused on pre-processing and feature selection of the spectral data. Thus, classification models could be improved by selecting variables relevant to the FAs of lard containing important wavenumbers on FTIR spectral and chemical shifts on NMR (^{1}H & ^{13}C). Although the GC-FID and LC-MS/MS instrumentation setting are more complicated than FTIR and NMR (^{1}H & ^{13}C), the chromatographic of the

data output are straightforward for FAs identification. In this study, the chemometrics technique was fully utilised by The Unscrambler® X software. However, more complex machines learning, such as ANN, and *k*-NN are unavailable in the software. Thus, SVM was utilised because it is almost similar to machine learning algorithms.

1.5 Objectives of the Study

The objectives of the study are;

1. To evaluate the lard spectral of collected pig samples from Northern, Central, and Southern Peninsular Malaysia using FTIR analysis combined with Principal Component Analysis (PCA).
2. To discriminate fats such as pigs (lard), chicken, beef, mutton, and plant fats after heating-process using FTIR, ^{1}H-NMR, and ^{13}C-NMR coupled with chemometrics technique.
3. To identify the biomarkers of FA by evaluating FAs degradation from lard and selected fats after the heating-process using GC-FID and LC-MS/MS combined with Principal Component Analysis (PCA).

1.6 The Significance of the Study

This study provides information on the profiles of pig fats from different body parts obtained from different regions of the country, thus serving as a snapshot of the lard profiles in Malaysia. The study also provides new insights into data analysis of the multivariate data, especially on fats

subjected to the heating-process, with possible biomarkers deemed useful for lard detection and authentication.