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# FOURIER TRANSFORM INFRARED SPECTROSCOPY AS A TECHNIQUE FOR MULTIVARIATE ANALYSIS OF LARD ADULTERATION IN FOOD PRODUCTS: A REVIEW

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**Abstract**

*The presence of lard in food products is prohibited (haram) for Muslim and a few other religions i.e. Judaism and Hinduism. Due to the advantages of lard such as easily available, cheap and able to produce better quality of food products, the adulteration of food products with lard has become a serious issue. The complex composition of food products and similar properties of lard as an adulterant make identification of food adulterations a big challenge. Chemical analysis is one way to authenticate the halal status of the food products which is based on the specific markers present or absent in the products they contain. This article highlights on the use of Fourier transform infrared (FTIR) spectroscopy to determine the presence of lard combined with multivariate analysis. This technique also able to quantify the amount of lard in the food products that are claimed to be halal.*

*Keywords: FTIR spectroscopy, Multivariate, Lard, Adulterant, Halal*

## INTRODUCTION

The adulteration of food products with pig fat or known as lard is a major and serious issue. In food industry, lard is an excellent substitute for the more expensive fats and oils. Its special properties also make lard suitable for use in many kinds of food products. Gillies (1974) mentioned that fats and oils are not totally replaced by lard, but mixture of lard with vegetables oils are being used by certain manufacturers especially in making food products such as margarines, shortening, and other foods that based on fats and oils. From a religious point of view, Islam, Judaism and Hinduism prohibit their followers from consuming any foods containing porcine ingredients. In Islamic perspective, even though the presence of the unlawful (*haram*) ingredients is less, it is still prohibited to be consumed or eaten. This is clearly mentioned by Allah S.A.W. in Surah An-Nahl:

*"Then eat of what Allah has provided for you, which is lawful and good. And be grateful for the favor of Allah, if it is indeed Him that you worship. He has only forbidden to you dead animals, blood, the flesh of swine, and that which has been dedicated to other than Allah. But whoever is forced by necessity, neither desiring it nor transgressing its limit – then indeed, Allah is Forgiving and Merciful. And do not say about what your tongues assert of untruth. "This is lawful and this is unlawful" as to invent falsehood about Allah. Indeed, those who invent falsehood about Allah will not succeed."*

(An-Nahl: 114-116)

The prohibition was also mentioned in Surah Al-Maidah:

***"Forbidden to you is anything that dies by itself, and blood and pork, as well as whatever has been consecrated to something besides Allah, and whatever has been strangled, beaten to death, trapped in a pit, gored, and what some beast of prey has begun to eat, unless you give it the final blow; and what has been slaughtered before some idol, or what you divide up in a raffle; (all) that is immoral!"***

**(Al-Maidah: 3)**

The consequences of taking *haram* source such as from pork or its derivatives is also supported by Jabir R.A. who reported that, Allah's Messenger PBUH said,

*"That flesh will not enter Paradise which has grown from Haram, and all that flesh which has grown from Haram, the fire (of hell) is more worthy of it."*

(Hadith narrated by Ahmed, Darimi, Baihaqi)

The prohibition of consuming *haram* foods has been clearly explained and thus, any contamination of *haram* source in food products even though in a very small quantity is considered *haram*. Therefore, the verification of the unlawful components and from sources acceptable to the consumers is required. This is supported by the statement from Muslim scholar, Syeikh Al-Qaradawi (1995); knowing the status of raw materials and production process of the consumer items is essential due to the Syari'ah obligation stating that any Muslim must consume only the *halal* and wholesome products. However, the identification of food adulteration is not an easy task especially when it presence in a small quantity.

*Halal* authentication cannot be done by physical inspection. Therefore, the use of latest technology in analytical instrumentation is necessary. Analytical techniques which are appropriate and specific have been developed to deal with particular issues. The most suitable technique for any particular sample is often determined by the nature of the sample itself. This review focuses on the application of Fourier transform infrared (FTIR) spectroscopy combined with chemometrics techniques such as discriminative analysis (DA), partial least square (PLS) and principal component analysis (PCA) as technique to detect and quantify the content of lard in food products. These techniques require minimal sample preparation and the combination allows rapid and simultaneous determination of lard in the mixtures of food components.

## LARD IN FOOD INDUSTRIES

Lard contains trans fat that has some advantages to the food processing industry because it is able to solidify and stabilize vegetable oil. It is also normally used as substitutes for saturated fats in food products for longer shelf life. However, as part of a healthy diet, the intake of food products containing saturated fats and trans fats should be reduced. In the Malaysia Dietary Guidelines 1999, the minimum amount of dietary fat recommended was 15% kcal (Malaysia Dietary Guidelines, 2010). From medicinal point of view, diets rich in lard are known to lead to hypertension (Khan et al., 2003), colon cancer (Reddy, 1992), breast cancer (Di Pietro et al., 2007) and prostate cancer (Cho et al., 2015).

Food product that contains lard has slightly different in physical appearance such as color, texture, taste and smell. Food made from ingredient that contains lard is said to be more flavorful, crispy and do not become soggy as they are cooled. The use of lard in food products is very popular as it has been used as a flavor enhancer for pie crust, softens the cake and makes it suppler and in some countries lard is also used as a frying medium. In fact, lard is also used as a mixing ingredient in meat products because of the stickiness properties found in it. In certain countries, there are some manufacturers who mixed vegetable oils with lard as a reason for reducing cost of the products (Aida et al., 2005). Lard could also be combined with other vegetable oils effectively to manufacture specialty food oils such as shortenings and margarines (Marikkar et al., 2002a).

Lard is solid state in room temperature and melts clearly in about 40°C. It was proven that lard could melt at a very low temperatures compared with its melting which is sharply at a single temperature, and its shelf life is not particularly long due to the rancidity phenomenon (Dahimi, 2013). Therefore, these are largely responsible for the creaming abilities and grainy mouth feel of lard. Moreover, the use of lard in making food products also helped in developing emulsion. It was found that if the lard is used in meat emulsions, it changed the properties of meat emulsions and resulted into more stable emulsions (Miklos et al., 2011). Examples of emulsions are vinaigrettes, milk, mayonnaise, and some cutting fluids for metal working. Less greasy food products will be obtained if they are fried in lard due to its smoking point higher than other fats. It also has higher probability to produce ultra-flaky pastry crust.

On the other side, many studies have been conducted to replace lard with other fats sources to reduce the rate of saturation and calories to produce healthier food products. An emulsified meatball was produced by substituting the lard with 11 different types of vegetable oil which are from corn oil, coconut oil, soybean oil, sunflower oil, palm oil, olive oil, peanut oil, and tea seed oil and hydrogenated oil from coconut oil, palm oil, and soybean oil (Hsu and Yu, 2002). The results showed that replacing 25% lard with plants-oil products did not change the textural properties of meatball. Margarine containing free lard fat was also produced by using formulations of rice bran oil, canola oil, palm stearin and palm kernel oil by enzymatic interesterification process (Kim et al., 2008; Adhikari et al., 2010). The partial replacement of pork fat by vegetable oil in burger patties had caused a significant reduction of saturated fatty acids and a concomitant enrichment in unsaturated fatty acids (Rodriguez–Carpena et al., 2011). This could improve the nutritional properties of patties and thus produce healthier food products.

## FATTY ACIDS CONTENT IN LARD

Lard is normally produced from adipose tissue of pig fats. It composed of fats which are known as triglycerides. These fats are formed from three molecules of fatty acids and the distribution of fatty acid in lard is varied from saturated and unsaturated acid. Even though most animal fats and vegetable oils composed of the same chemical components of fats and oils but their composition is different and unique from one to another as shown in Table 1.

Table 1. Comparison of Fatty Acid Composition (Weight Percent) of Fats and Oils

| **Origin** | **Fatty acid composition by weight (%) (approximately)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Total saturated** | | | | **Total unsaturated** | | | |
| | **Lauric (12:0)** | **Myristic (14:0)** | **Palmitic (16:0)** | **Stearic (18:0)** | **Palmitoleic (16:1)** | **Oleic (18:1)** | **Linoleic (18:2)** | **Linolenic (18:3)** |
| Lard | - | 2 | 27 | 11 | 4 | 44 | 11 | - |
| Beef | - | 3 | 27 | 7 | 11 | 48 | 2 | - |
| Chicken | - | 1 | 22 | 6 | 6 | 37 | 20 | 1 |
| Soybe | 0.1 | 0.1 | 10.2 | 3.7 | - | 22.8 | 53.7 | 8.6 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| an | | | | | | | | |
| Palm | 0.1 | 1.0 | 42.8 | 4.5 | - | 40.5 | 10.1 | 0.2 |
| Coconut | 46.5 | 19.2 | 9.8 | 3 | - | 6.9 | 2.2 | - |

(Source: Gunstone, 1996)

As can be seen from the Table, it seems to be difficult to detect and to differentiate the contamination of lard in other type of animal or vegetable fats and oils because the differences of fatty acid compositions are too small. Numerous principal techniques and instruments have been developed in analytical field as to analyze lard contained in food products. This includes gas chromatography (GC) (Dahimi et al., 2013; Indrasti et al., 2010), liquid chromatography (LC) (Marikkar et al., 2005a; Marikkar et al., 2005b), differential scanning calorimetry (DSC) (Marikkar et al., 2001; Marikkar et al., 2002a; Marikkar et al., 2005b), DNA-based method (Norrakiah et al., 2015; Aida et al., 2007; Aida et al., 2005) and many others. GC and LC determine the specific components such as fatty acids, triacylglycerols, and tocols components rather than analysis of fats and oils as a whole matter (Rohman et al., 2014a). Meanwhile Chiavaro (2014) stated that differences in fatty acids composition in fats are reflected on the differential scanning colorimetry (DSC) thermograms. DNA method is considered the most reliable method for lard identification but it is difficult to apply in lard derivatives for example glycerol-mononitrate, magnesium stearate, sodium/potassium stearate (Mursyidi, 2013).

## MULTIVARIATE ANALYSIS OF LARD IN FOOD PRODUCTS USING FTIR SPECTROSCOPY

FTIR spectroscopy is one of the vibrational spectroscopy methods that analyze fats and oils as a whole matter. It can be applied as a qualitative and quantitative analysis of specific components in food and has been a focus of study by many researchers especially when it is combined with chemometrics. FTIR spectroscopy works based on the Beer's law where the absorption of the functional groups in the mid infrared (MIR) region (appears as peak intensities in the IR spectrum) are directly proportional to concentration of the analytes. The peak intensities and the frequency at which the maximum absorbance of peaks appear differ according to the nature and composition of the sample. This method becomes more attractive when it is coupled with attenuated total reflectance (ATR) sampling technique. It is considered as "green chemistry" because it requires very minimal or no sample preparation and eliminates the use of chemical reagents which are hazardous to human and environment and thus analysis faster as well as non-destructive (Che Man et al., 2010).

Multivariate analysis is always used with FTIR spectroscopy because the spectra of fats or oils mixtures are very similar to the spectra of individual fats or oils. This combination able to extract subtle information from complex IR spectra that might contain overlapping peaks, interference bands and instrumental artifacts due to the measurement conditions (Gallardo-Velázquez et al., 2008). Discriminative analysis (DA), partial least square (PLS) and principal component analysis (PCA) are among the most frequently used methods for quantitative analysis in the complex mixtures.

Many researchers have focused on using FTIR spectroscopy either to differentiate the spectrum of lard with other types of oils and fats, to authenticate oils and fats or to detect adulteration of oils and fats (Rohman and Che Man, 2009a; Rohman and Che Man, 2010; Rohman et al., 2014b; Ding et al., 2014). For example, Guillén and Cabo (1997) have successfully distinguished lard from 16 different types of edible oils originated from vegetable oils and animal fats using stand-alone FTIR spectroscopy with KBr transmission method. It was proven that this technique able to predict the proportions of saturated, monounsaturated and polyunsaturated acyl groups in oils and lard from the bands in the fingerprint region. Che Man and co-workers (2011a) conducted a similar research in analyzing various types of edible oils and lard using FTIR spectroscopy coupled with ATR. They monitored the differences in the mid-infrared region (4000 – 650 $cm^{-1}$) and the results were analyzed using the chemometrics of PCA and cluster analysis (CA). From these two findings, it can be concluded that lard is distinguishable from other edible oils based on the peak position of fatty acids, saturation level of the chains and the specific minor components present in the fats and oils.

FTIR coupled with multivariate statistical analysis have been widely used to determine lard adulteration. Table 2 tabulates the application of FTIR in monitoring authentication of foods. As can be seen from the Table, ATR sampling is the most chosen method compared to transmission sampling as it hassle-free and non-destructive technique. PLS is the most widely used statistical analysis because it is a quantitative analysis and can be applied to construct the calibration model. The calibration model is used to deduce a relationship between the spectra and the concentration of samples from a set of reference samples (Rohman and Che Man, 2012). Using this technique, one can be able to quantify the amount of lard present in the sample. Furthermore, it is not only analyzes the specific spectral region but as a whole. The validity criteria for PLS calibration is determined from the values of coefficient of determination ($R^2$) and root mean standard error of calibration (RMSEC). PLS calibration curve is formed by making a mixture of lard in any food samples with serial concentrations series of normally ranging from 0 to 100% and then the intensity of the typical peak of lard is measured.

The typical peaks for lard are identified at peak positions around 3006, 1117 and 1098 $cm^{-1}$. These peaks even though are similar to other types of fats and oils, but their intensities are different as saturation level of the carbon chains and the specific major and minor components present in the fats and oils are also different (Rohman et al., 2012).

Meanwhile, PCA and DA are type of classification analyses. Both are designed to find the mathematical models that able to recognize the membership of each object to its proper class based on the FTIR spectra. Once a classification model has been obtained, the membership of unknown objects to one of the defined classes can be predicted. These methods can be used to determine the class of sample containing lard to that without lard by calculating the distance from each class center evaluated in the Mahalanobis distance units (Rohman and Che Man, 2011). The class of unknown samples to one of the specific classes can be predicted, after classification model is obtained.

Table 2. FTIR Spectroscopy Used for Identification of Pork or Lard in Various Types of Samples

| Sample | Multivariate analysis | Method | Source |
|---|---|---|---|
| **Animal fats** | | | |
| Fresh meats (turkey and chicken) | PLS and PCA | ATR | Al-Jowder et al., 1997 |
| Body fat of lab, cow and chicken | PLS and DA | Transmittance | Che Man and Mirghani, 2001 |
| Cod liver oil | PLS | ATR | Rohman and Che Man, 2009b |
| Body fat of mutton and cow | PLS | Transmittance | Jaswir et al., 2003 |
| **Vegetable oils** | | | |
| French fries pre-fried in palm oil adulterated with lard. | PLS | ATR | Che Man et al., 2014 |
| Palm oil | PLS | ATR | Rohman et al., 2012 |
| Canola oil, corn oil, extra virgin olive oil, soybean oil, sunflower oil | PLS and DA | ATR | Rohman et al., 2011a |

| | | | |
|---|---|---|---|
| Virgin coconut oil | PLS and DA | ATR | Mansor et al., 2011 |
| **Food Products** | | | |
| Chocolate | PLS and PCA | ATR | Suparman et al., 2015 |
| Butter | PLS | ATR | Nurulhidayah et al., 2015 |
| Meatball broth | PLS and PCA | ATR | Kurniawati et al., 2014 |
| Ham sausages | PLS and DA | Transmittance | Xu et al., 2012 |
| Beef meatball | PLS | ATR | Rohman et al., 2011(b) |
| Biscuit | DA | ATR | Che Man et al., 2011(b) |
| Gelatin | PCA | ATR | Hashim et al., 2010 |
| Chocolate formulation | PLS | *ATR* | Che Man et al., 2005 |
| Cake formulation | PLS | *ATR* | Shahariza et al., 2005 |

## CONCLUSION

Adulteration of food with pig sources such as flesh, lard or its derivatives are considered as *haram* in Islamic perspective. This prohibition has been stated in Al-Quran. However, some manufacturers have taken the advantage in the production of food products when they substitute the much higher cost oils or fats with much lower cost of oil such as lard. Therefore, analytical methods have been used in order to identify and quantify the presence of lard. FTIR spectroscopy is recommended to be used for detection of lard in food products because it is the robust technique for food analysis. It is a rapid technique, economic with minimum, ease and no excessive in sample preparation especially with the help of ATR method. Furthermore, it is reliable enough, not involving hazardous solvent and reagent and environmentally friendly. FTIR spectroscopy combined with chemometrics analysis of PLS, DA and PCA were found to be accurate and precise enough for such determination.

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