Syarahan Inaugural

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Bertajuk

OILS AND FATS ANALYSIS - Recent Advances and Future Prospects

- 27 Mac 2004
- Dewan Taklimat Tingkat Satu,
  Bangunan Pentadbiran Universiti Putra Malaysia

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OILS AND FATS ANALYSIS - RECENT ADVANCES AND FUTURE PROSPECTS

ABSTRACT

Rapid and continued developments in electronics, optics, computing, instrumentation, spectroscopy and other branches of science and technology have led to considerable improvements in various analysis techniques. Due to this revolution in techniques, we are now able to solve problems, which were thought to be extremely difficult to do so a few years ago. These new techniques enable us to better characterize, monitor and authenticate oils and fats and improve our understanding of these important food constituents. Analytical techniques have now moved from the confines of the chemistry laboratory to form an indispensable part of the analytical armory of many researchers involved in the fields of oils and fats. Recent years have also seen a dramatic improvement in the ease with which analytical instruments can be used, and the quality and quantity of the analytical data that they can produce. Non-destructive techniques such as differential scanning calorimetry (DSC), Fourier transform infrared (FTIR), near-infrared (NIR) spectroscopy and electronic nose (EN) were developed and applied to analyse various quality characteristics of oils and fats products. The new techniques offer alternative to the use of time- and work-consuming conventional standard methods and minimize the use of toxic chemicals that are hazardous not only to the analysts but also to the environment. These techniques are rapid, accurate, reliable and safe and potentially suitable for routine analyses in quality control laboratories. These analyses are important tools for analytical chemists, food professionals and regulatory authorities in ensuring compliance and enforcement of approved specifications and legal requirements. Globally, as consumers are becoming more educated, well informed and more demanding on their rights to purchase quality food products, more research and analytical development for food authenticity, especially for halal food, are needed. This lecture highlights recent advances on various analytical techniques and their potential applications for quality assessment and authentication of oils and fats.

INTRODUCTION

What is a recent advance is somewhat open to interpretation. All of the latest advancements in analytical techniques I found were built on previous work. What makes some techniques stand out more than others are their ability to appear to address the needs of the future and at the same time to respond to some of the driving forces in the industry today.

Driving forces in the area of analysis of edible oils and fats include:

- First, environmental and safety concerns that have restricted the availability and use of many chemicals essential for many classical oils and fats analysis. Chemicals such as benzene has been banned in most laboratories in accordance to the Montreal Protocol on Substances that Deplete Ozone Layer 1987 (Giese, 1996). Special permission to work with chemicals such as carbon tetrachloride, methylene chloride, chloroform or carbon disulphide is required. Even hexane has come under scrutiny as a possible neurotoxin. The list of safe solvents is growing smaller and any technique that allow analysis with minimal or no use of solvent will be well received.

- The second driving force is globalization of trade and communications. This has resulted in increased demand for "just in time" analysis in order to meet contract demands. International treaties and organizations such as *Codex Alimentarius* have also placed more emphasis on rapid and accurate quality and compositional measurement in an increasingly complex mixture of oils with more and more emphasis on health and environmental concerns.

- The third driving force is technology itself. The continuing spin off of technological developments from heavily funded research programs, coupled with the development of faster and less expensive computer chips have resulted, not only in increased automation of equipment and analytical methods, but also in expansion of the analytical capabilities of the equipment itself. Many equipment and techniques that were only found in well equipped major research laboratories 25 years ago, now are priced within reach of much smaller laboratories, even to small process laboratories.

- The fourth driving force is biotechnology. This increased the complexity of the types of oils and fats tested in most laboratories today. Biotechnology's fingerprinting capabilities are gaining acceptance as a mean of evaluating the authenticity of food and ingredients and finished products to detect any possible adulteration

- The fifth driving force is halal factor, which is globally accepted as an essential component of food quality system such as Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) systems.

In this lecture, I shall focus on the advances on four new techniques of analysis within my areas of expertise that I think represent the developments of analytical techniques as a result of the driving forces above. They are the differential scanning calorimetry (DSC), infrared (IR) spectroscopy, electronic nose (EN) and a brief note on DNA-based technique relating to my other area of expertise, that is on halal food.

OILS AND FATS INDUSTRY

Oils and fats are important raw materials for food as well as non-food applications. World production of oils and fats has been increasing steadily at 3.35% per annum for the period from 1990 - 2000. For the year ending 2003, worlds production of fats and oils was at 105 million metric tonnes (MMT) with soybean oil as number one at about 32 MMT and palm oil as number two at 28 MMT (Gunstone, 2004).

Export of oils and fats is also growing steadily at 3.38% per annum since 1990. For 2003, palm oil accounted for 19 MMT out of a total world trade of 35 MMT, giving it a 54% share of the worlds oils and fats trade. In terms of oils and fats trade, palm oil is the most traded oil in the world. Malaysia shares of palm oil trade is about 64% (Basiron, 2004). Thus, the oils and fats industry, in particular palm oil industry, is of special importance to Malaysia.

Palm oil and their products are employed in numerous food and non-food applications. More than 80% are used in food product manufacturing. They can be used as frying media and for making margarines, shortenings, soap, oleochemicals and other products. The use of edible oils and fats are a critical economic component for nearly every country as they provide fatty acids, which are essential for good human health. They are also a source of energy, a carrier of fat-soluble vitamins, and provide essential material for nerve cells, membranes, and biliary acids. A number of consumer's health concern, whether valid or perceived, include cis-trans saturation, lower cholesterol levels and the need to determine authenticity and halal, functionality and nutrition labelling, quality and safety.

CHANGING THE ANALYTICAL PARADIGM IN THE OILS AND FATS INDUSTRY

Laboratories in the oils and fats industry, as in so many other industrial laboratories, face a variety of challenges to remain competitive. It is essential that issues such as limited resources, cost containment, and chemical safety be closely monitored and continually improved. Though these are prominent issues throughout organizations, the analysis for quality is perhaps even more sensitive.

The quality assurance/quality control (QA/QC) laboratory typically receives and conducts analysis on a variety of in-process samples. Many standardized analysis employed by industry are specified by organizations such as AOCS, IUPAC and AOAC International.

These methodologies require a significant amount of time to prepare and perform the analysis, use solvents, can be subjective at times, and assume a certain level of operator skill, thus are not very practical for effective quality control. Examples in the oils and fats industry include:

- The current measurement for free fatty acid (FFA), which employs either ethanol or isopropyl alcohol, and relies upon a subjective pink colour end-point titration that must be pertinent for 30 seconds;
- The original method for the measurement of iodine value (IV) by Wijs method, which required the use of carbon tetrachloride. This method was subsequently altered to specify a safer solvent (cyclohexane). Additionally, a reaction time of one hour is specified for oil samples with an IV of less than 150. Though the reaction time can be greatly reduced by utilizing compounds such as a mercury acetate catalyst, the use of such compounds greatly adds to disposal costs;
- Total *trans* determination is described by at least five official test methods that include both gas chromatography (GC) and infrared (IR) methods. The IR method requires a run time of 40 to70 minutes.

In the palm oil industry, the quality of oil feedstocks is a major concern of both the refiners and the oleochemicals industries. The main considerations are the IV, peroxide value (PV), moisture content, FFA, colour, odour and fatty acid composition. These parameters determine the quality and specification of the oils and are analyzed at various stages of the refining processes usually by conventional chemical methods which are laborious and greatly inefficient.

These issues have clear implications for process control. Whether the system has to shut down or wait due to recycle, the end result is lost production. There is constant a pressure to turn around analysis quickly and to shorten analysis times, thus the increase need to change the analytical paradigm in the oils and fats industry.

NEEDS FOR RAPID TECHNIQUES

Most of the standard conventional analytical techniques are time- and labor-consuming methods and use toxic chemicals hazardous to environment as well as to the analysts. There is a strong driving force in the industry to replace traditional methods of analysis by instrumental methods, in order to improve efficiency and to address increasing environmental concerns about the use of large volumes of solvents and reagents in quality control laboratories.

Technology has advanced tremendously producing some high resolution scientific instruments which are more efficient and yet affordable by most of the oils and fats industries. It is high time to use some instrumental techniques to substitute the classical analysis in order to strive for better efficiency, accuracy, reproducibility and perhaps not so problematic to the environment and safety to the analyst.

Today sheer knowledge of analytical chemistry in the laboratory is becoming rare. As a result of this lost of knowledge, dedicated instruments or analysers have to fill the gap. The 'scientist' is no longer in the laboratory but in the instrument itself. As the knowledge of analytical chemistry diminishes in the quality control laboratories, the instrument must be equipped with user-friendly, 'point and click' analyser that will replace the traditional laboratory instruments for rapid analysis.

DEVELOPMENT OF NEW TECHNIQUES FOR FATS AND OILS ANALYSIS

In our laboratory, we focus our research on the development of new techniques of analysis including differential scanning calorimetry (DSC), infrared (FTIR and NIR) spectroscopy, electronic nose (EN) and also DNA-based technique related to halal authentication of oils and fats.

Differential Scanning Calorimetry (DSC)

DSC is one of the most versatile thermal analysis techniques with a range of applications. The calorimeter measures heat flow into (endothermic) or out of (exothermic) a sample undergoing a phase change, as well as heat capacity of a sample. The principle of DSC is to keep, for a given temperature program, sample and reference, placed in separate micro ovens, at equal temperature. The electrical power needed for the compensation is equivalent to the calorimetric effect. Examples of thermal transitions are: melting, crystallization, decomposition, out-gassing, or a change in heat capacity. DSC also measures a transition onset temperature, the temperature at maximum reaction, and the ending temperature. It is also useful to monitor different samples of the same material to assess their similarities or differences (Wendlandt, 1986).

- **Thermal behaviour and quantatitive analysis**

Our research group studied thermal profiles of palm oil and its products.The importance of thermal behaviour of various palm oil products were outlined and we concluded that the DSC thermal profiles can be used as guidelines for fractionation of crude palm oil or RBD palm oil (Che Man et al., 1999; Swe and Che Man, 1994; Che Man and Swe, 1995). We have also analysed thermal profiles of 17 edible oils from different plants. The melting and crystallization curves of the oil samples were reported (Figure 1 and Figure 2). The contrasting DSC thermal curves provide a way of distinguishing among these oil samples. Generally, oils with a high degree of saturation (IV<65) showed DSC melting and crystallization profiles at higher temperature regions than the oil samples with high degree of unsaturation (IV>65). Each thermal curve was used to determine three DSC parameters, namely, onset temperature (T_o), offset temperature (T_f) and temperature range (difference between T_o and T_f). This DSC technique promises to offer a sensitive, rapid and reproducible fingerprint method for quality control purpose (Tan and Che Man, 2000; Che Man et al., 1999).

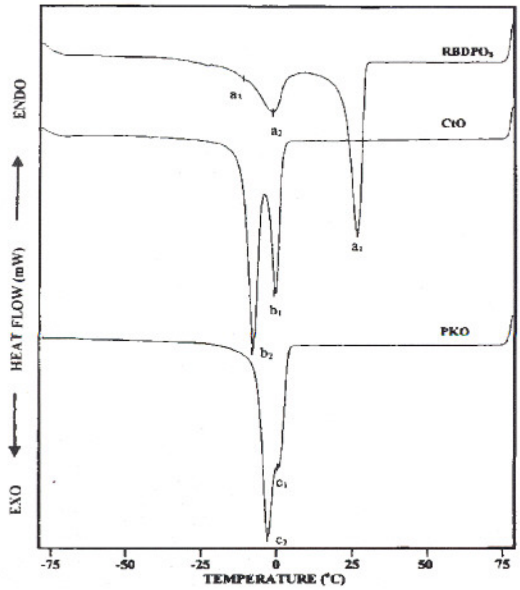


Figure 1: DSC crystallization thermogram of RBDPOo coconut oil (CO), and palm kernel oil (PKO). (Source: Che Man et al., 1999)

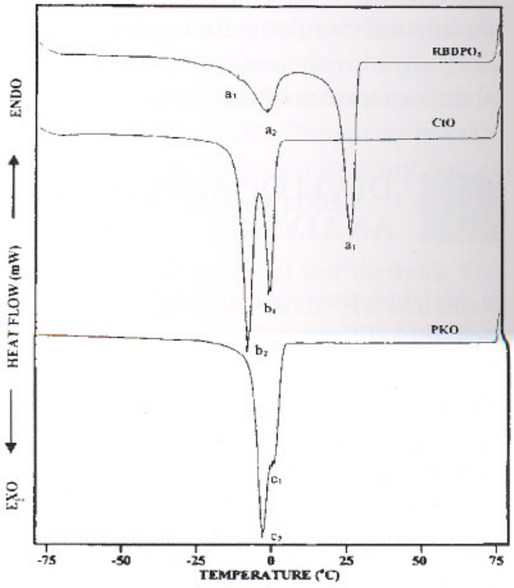


Figure 2: DSC melting thermogram of RBDPO, coconut oil (CO), and palm kernel oil (PKO). (Source: Che Man et al., 1999)

New DSC techniques for concurrently determining three important quality parameters in deep-fat frying industry, total polar components (TPC), FFA and IV of heated coconut oil (CO), refined, bleached and deodorised palm olein (RBDPO$_o$) and soybean oil (SO) using the crystallisation thermogram of DSC was developed in our laboratory. The DSC variables were used as independent variables while values from standard chemical methods were used as dependent variables. This study revealed that a single DSC cooling curve could predict the TPC, FFA and IV of heated oils using stepwise regression analysis (Tan and Che Man, 1999a; 1999b; 2002a; 2002b).

- **Monitoring oxidation**

Recently, we have developed a simple and reliable DSC method for monitoring the oxidation in heated oils (Tan and Che Man, 2002a; 1999a; 2003a; 2003b). Three different oils, namely corn oil (CnO), RBDPO$_o$, and SO were used. The DSC cooling curve of oil showed a well-defined single crystallization peak (Fig. 3). Two DSC parameters of this single crystallization peak, namely peak temperature and enthalpy were determined. In addition to DSC method, deterioration of heated oils was also quantified by means of seven chemical methods. A statistical comparative study was carried out among DSC method and chemical methods. The results revealed that there are good correlation between the DSC technique and other standard chemical methods.

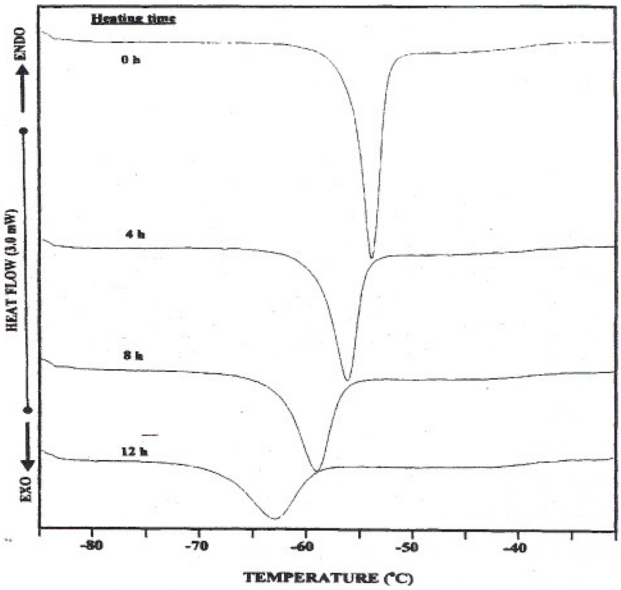


Figure 3: Cooling thermogram of oil samples with different heating times (0, 4, 8, and 12 h). (Source: Tan and Che Man, 1999)

The effects of microwave heating on the changes in DSC cooling and/or melting profiles of three vegetable oils (CO, SO, and RBDPO$_o$) are reported in two separate scientific papers (Tan et al., 2002a; 2002b). The influence of microwave power (low-, medium-, and high-power settings) and heating time on lipid deterioration produced during the microwave heating of vegetable oils was evaluated. A statistical comparative study was carried out on the DSC and chemical parameters. In general, there were good correlations between these parameters. Likewise, the experimental data showed that for a given microwave-power setting, a good correlation between DSC curve parameters and heating periods was found. This is an indication that DSC can be used as an objective non-chemical and instrumental technique to monitor lipid oxidation in microwave heated oils.

- **Oxidative stability**

Our research group has conducted a comparative study to determine the oxidative stability of twelve different edible oils by DSC and oxidative stability instrument (Che Man and Tan, 2002a; Tan and Che Man, 2002b; 2002d; 2000). The DSC technique was based on an isothermal condition with purified oxygen as purge gas. The isothermal DSC technique for direct determination of the oxidative stability of vegetable oils has been built and a comparative study to oxidative stability instrument (OSI) was demonstrated. The DSC cell temperature was set at four different isothermal temperatures: 110, 120, 130, and 140°C. A dramatic increase for evolved heat was observed with the appearance of sharp exothermic curve during initiation of the oxidation reaction. The oxidative induction time (T_o) was automatically determined by extrapolation of the downward portion of the DSC oxidation curve to the time axis. The results indicated that there is good correlation between the DSC T_o and OSI values.

- **Kinetics of lipid oxidation**

In our laboratory, the isothermal DSC method was applied to obtain the kinetic data for lipid oxidation of ten different edible oils (Tan et. al, 2001; Tan and Che Man, 2002e). The temperature dependence of the rates of lipid oxidation gave highly significant correlations when analyzed by the DSC method. In addition, based on the Arrhenius equation and activated complex theory, reaction rate constants (k), activation energies (E_a), activation enthalpies (ΔH^A) and activation entropies (ΔS^A) for oxidative stability of vegetable oils were calculated. The E_a, ΔH^A, and ΔS^A for all vegetable oils ranged from 79-104 kJ mol^{-1}, 76-101 kJ mol^{-1}, and -99 to -20 J K^{-1} mol^{-1}, respectively. In general, isothermal or dynamic DSC techniques can be applied to obtain the kinetic data of lipid oxidation in vegetable oils. However, many of the complicated nature oxidation phenomena during dynamic DSC technique has not been well explained by many researchers. These phenomena include the variation in reaction rate, oxygen concentration (different in solubility) in vegetable oils during linear programmed heating and differences in oxidation pathways.

- **Evaluation of antioxidant activity**

A simple DSC method for measuring the antioxidant activity in RBDPO$_O$ is developed in our laboratory (Che Man and Tan, 1999: Che Man and Tan, 2002; Tan et al., 2001). The oxidation temperature was 150°C and the oxygen flowed at a rate of 50 ml/min. In this method, the thermal changes occurring during oxidation of the oil are recorded. Generally, the results show that the antioxidants act mainly by increasing the induction period (T_{on}) of lipid oxidation. Figure 4 illustrates the T_{on} values of three added natural antioxidants with different concentrations in RBDPO$_o$. The increase in induction time with increasing antioxidant concentrations is best fitted by linear or polynomial equations. These calorimetric results indicate that DSC is a valuable technique in the development and optimization of an antioxidant system for various vegetable oils. This work could contribute to the selection of an appropriate antioxidant (or combination of antioxidants) at optimum level in various vegetable oils.

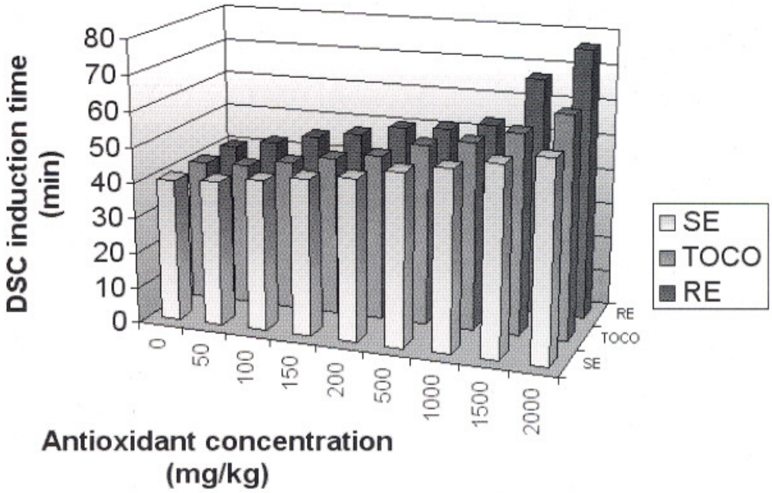


Figure 4: Scatter plot showing DSC onset time versus antioxidant concentration. Abbreviations: SE, sage extract; TOCO, tocopherol; RE, rosemary extract. (Source: Che Man and Tan, 2002)

- **Detection of adulteration by DSC**

Food adulteration is one of the major problems in food trade and industry. Adulteration of food has taken place for centuries and has ranged from the simple addition of natural compounds to the much more serious case of contamination with harmful substances. Adulteration is sometimes deliberate and sometimes accidental. Mixing of animal fats especially lard and tallow in any form in food products is a cause of concern for certain religions. Food products containing pork and lard are of great concern for Islam and Orthodox Jewish religion while those mixed with tallow are prohibited in the Hinduism. Therefore, monitoring of adulteration practices has become essential in order to protect consumers and food industries. Recently, our laboratory has developed a method for detecting the presence of lard and randomized lard as adulterants in RBD palm oil based on DSC (Marikkar et al. 2001; 2002a; 2002b). In general, DSC cooling curves of adulterated RBD palm oil revealed an adulteration peak corresponding to lard/randomized lard in the low-temperature region (Fig.5 and Fig.6). Using this method, a detection limit of 1% lard/randomized lard was reached.

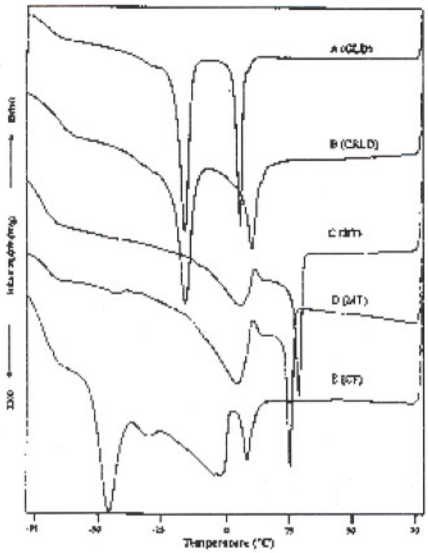


Figure 5: DSC cooling thermograms of (A) genuine lard (GLD), and (B) chemically randomized lard (c) beef tallow (D) mutton tallow (E) chicken fat. (Source: Marikkar et al., 2001).

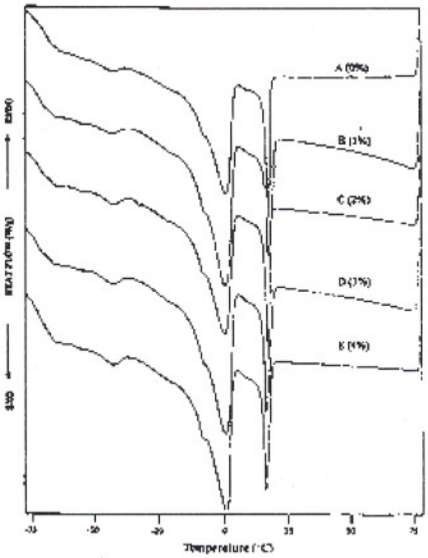


Figure 6: DSC cooling thermograms of (A) RBD palm olein, and RBD palm olein adulterated with (B) 1 % GLD lard (C) 2 % GLD lard (D) 3 % GLD lard (E) 4 % GLD lard. (Source: Marikkar et al., 2001).

Infrared Spectroscopy (IR)

IR spectroscopy has played a major role in fundamental research and qualitative analysis of lipid systems over the past five decades, owing to the large amount of structural information that can be extracted from the IR spectra of lipids. Historically, IR spectroscopy has rarely been used for quantitative analysis of fats and oils with the exception of the official AOCS method (1996) for the determination of isolated *trans* isomers, developed in the 1940s. The amount of information potentially available from an IR spectrum of an oil is substantial (Figure 7), as every peak and shoulder represents, in some way or other, structural or functional group information, either about the lipid or about impurities associated with it (Sedman and van de Voort, 2000).

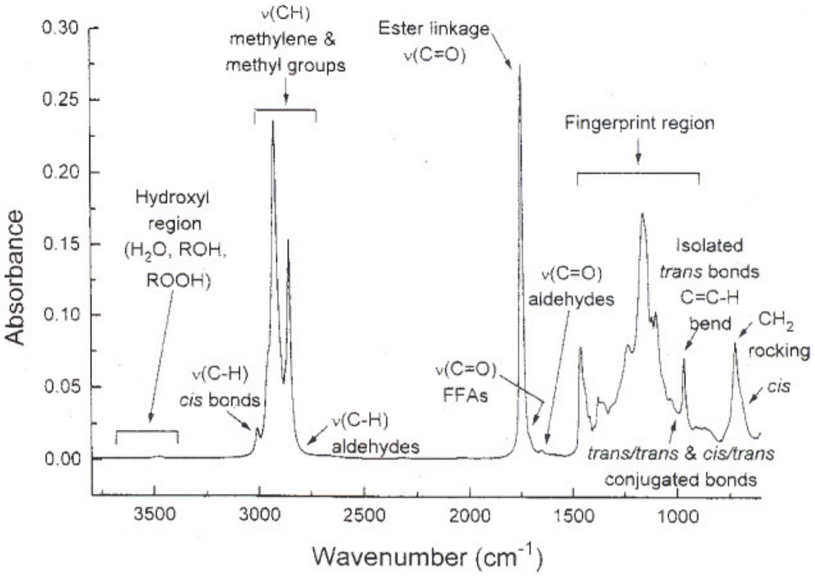


Figure 7: Spectrum of edible oil showing some of the information potentially available from an IR spectrum of edible oil sample. (Source: Sedman and van de Voort, 2000).

- **Application of FTIR and NIR Spectroscopy for Quantitative Analysis of Oils and Fats**

Many analytical methods based on international standards such as American Oil Chemists' Society (AOCS) and International Union of Pure and Applied Chemistry (IUPAC) to measure some parameters of quality of fats and oils are not very practical. Simple, rapid and accurate methods, feasible for the oil industry are therefore necessary. The applicability and ability of FTIR and NIR spectroscopy to do so in oils and fats were conducted by our research group, whereby we developed a number of rapid and reliable FTIR spectroscopic techniques, for the analysis of important quality parameters of edible oils and fats.

Iodine value (IV) - We described a rapid (2 minutes) technique for determination of IV in palm oil by FTIR spectroscopy (Che Man *et al.*, 1999a; 1999b). A calibration standard was developed by blending palm stearin and super olein in specific ratios that covered a range of 27.9 to 65.3 IV units. The spectra of these standards was measured in the range between 3050 and 2984 cm, corresponding to the absorption band of =C-H *cis* stretching vibration. A partial least square (PLS) calibration model for the prediction of IV was developed to quantify the IV of palm oil products. A validation approach was used to optimize the calibration with a correlation coefficient of R^2= 0.9995 and a standard error (SE) of prediction of 0.151. (6, 13).

Free fatty acids (FFA) - A rapid method for quantitative determination of FFA contents in palm olein by FTIR and NIR spectroscopy is described (Che Man and Setiowaty,1999a;

Che Man and Moh, 1998; Che Man and Setiowaty, 1999b). PLS calibration model for the prediction of FFA contents in palm olein was based on the spectral range 1728 – 1662 cm^{-1}. R^2 and SE were 0.997 and 0.017, respectively. This model was tested by cross-validation steps to minimize SE of the model. The method was tested for accuracy and repeatability compared to the AOCS titration method (1996). The new FTIR spectroscopic method provides a practical procedure that can be implemented in a straightforward manner for QC applications in the fats and oils industry.

Peroxide value (PV) - A rapid quantitative FTIR and NIR spectroscopic methods to determine PV in thermally oxidized palm olein were developed by our group (Moh et al., 1999; Setiowaty et al., 2000). Calibration was based on PLS and evaluated by cross-validation. We also developed a NIR spectroscopic method that determines PV in crude palm oil based on the spectral region from 1350 – 1480 nm with reference to a single base line at 1514 nm (Moh et al. 1999).

Anisidine Value (AnV) - A FTIR spectroscopy coupled by transmission cell was described to predict AnV of a thermally oxidized palm olein (Che Man and Setiowaty, 1999c). PLS regression was employed to construct a calibration model based on spectral region 2747 – 2619 cm^{-1} and 1715 – 1673 cm^{-1} individually and simultaneously. The precision of this method was shown to be comparable to accuracy of the AOCS method used to measure AnV.

Saponification number (SN) - A unique and rapid FTIR spectroscopy coupled by NaCl transmission cell was described to predict SN of palm oil (Setiowaty and Che Man, 2002). PLS regression and PCR (Principle Component Regression) were employed to construct calibration models based on spectral data of ranges between 3000 – 2800 cm^{-1} and 1800 – 1600 cm^{-1}. The precision of this method was shown to be comparable to accuracy of the AOCS method used for measurement of SN.

Slip melting point (SMP) - A FTIR spectroscopy with PLS and PCR regressions have been examined and evaluated for efficacy in determining SMP directly in palm oil blends. Results showed that orthogonal models using selected wavelength data of 1750 – 1724 cm^{-1} superior predictive performance (Setiowaty and Che Man., 2002b).

Cloud Points (CP) - A rapid FTIR spectroscopic method was developed for quantitative determination of the cloud point in palm oil sample. Both PCR and PLS calibration models for predicting CP were developed using the spectral regions from 3000 to 2800 and 1800 to 1600 cm $^{-1}$ (Setiowaty and Che Man, 2004). The precision of this method was shown to be comparable to the AOCS method.

Moisture Content (MC) - Water strongly absorbs in the region of 3600 - 3000 cm^{-1} and at 1650 cm^{-1} in butters and margarine, allowing one to rapidly differentiate the foods as function of their water content. A simple, rapid and direct FTIR spectroscopic method was developed for the determination of moisture content of crude palm oil (CPO) (Che Man and Mirghani, 2000). A PLS regression technique was employed to construct a calibration model followed by cross-validation step. The precision of this method was shown to be comparable to the accuracy of the AOCS vacuum oven method used for determination of moisture and volatile matters with R^2 of 0.9781.

Thiobarbituric acid (TBA) value - Our research group applied both the PLS and PCR statistical methods to FTIR spectra using different baseline types from a set of palm oil samples to determine their TBARS content without any spectral correction. The methods were then compared with standard method and were shown to be comparable (Mirghani *et al*, 2001a; 2000; Setiowaty and Che Man, 2001a).

- **Detection of Residual Contaminants in oils and fats**

Residual soap in oil - Recently in our laboratory has developed a new analytical method for the determination of soap residues in refined vegetable oils (Mirghani *et al.*, 2001). The vibrational band at 1564 cm^{-1} as well as the weak residual band at 1699 cm^{-1} in the spectra of coal samples soaked in NaOH, were assigned to structures of the type COO^-M^+, although the 1700 cm^{-1} band may be related to COOH. The two vibrational bands of strong and very strong intensity in the glycine alkaline salt ($NH_2CH_2COO^-Na^+$) at 1597 cm^{-1} and 1561 cm^{-1}, respectively, were assigned to COO^-.

Hexane in solvent extracted oil - A new analytical method was developed for the determination of hexane residues in palm and groundnut oils by FTIR spectroscopy (Mirghani *et al.*, 2003). FTIR spectroscopy was based on a horizontal ATR accessory (ZnSe crystal 45° ends) at room temperature and the PLS statistical method was used to derive calibration models for each oil. The accuracy of the method was comparable to that of the AOCS method (1996), with R^2 of 0.9598 and 0.9716 for palm and groundnut oils, respectively.

Aflatoxins - Among the oilseeds, aflatoxin poses the most serious problem in groundnut, but can nevertheless occur in all of them. Thus, at least 60 countries have proposed or established limits for the aflatoxin level in food/feed. Our recent study had introduced a new method for the determination of aflatoxins in groundnut and groundnut cake using the FTIR spectroscopy with ATR (Mirghani *et al.*, 2001). The wavelengths were selected for the four types of aflatoxins (B_1, B_2, G_1, and G_2) and the standards prepared for each by spiking some clean samples with the aflatoxins in concentrations of 0- -1200 parts per billion. The spectra of aflatoxin B1 in solvent, solvent and the difference are shown in Figure 8. PLS regression was used to derive the calibration models for each toxin. The R^2 of the calibration model were computed for the FTIR spectroscopy predicted values vs. actual values of aflatoxins in parts per billion. The R^2 was found to be 0.9911, 0.9859, 0.9986, and 0.9789 for aflatoxins B_1, B_2, G_1, and G_2, respectively. Based on the results obtained, FTIR spectroscopy can be a useful instrumental method for determining aflatoxins in oilseeds and oilseed cakes.

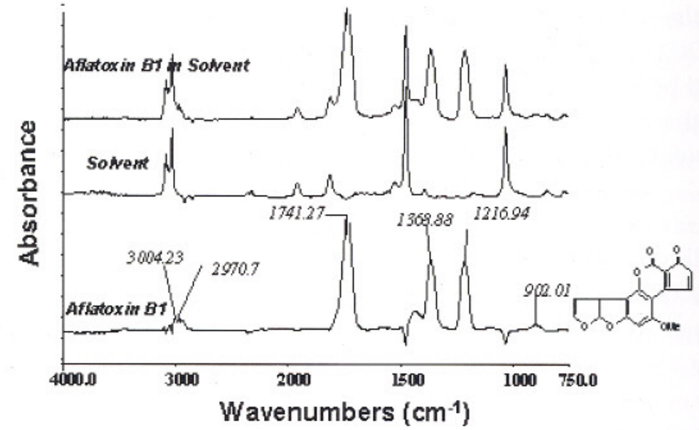


Figure 8: Spectra of Aflatoxin B_1 in solvent, solvent only and hence of aflatoxin B_1 as a result of subtraction of solvent spectrum. (Source: Mirghani et al., 2001).

- **Determination of Minor Components**

α-Tocopherol – A FTIR spectroscopy was developed for the determination of a-tocopherol in RBDPOo (Che Man et al., 2003). The method was based on the NaCl windows and PLS calibration models. The accuracy of the method was comparable to that of the HPLC, with R^2 from calibration samples of 0.9922 at spectral region 3100-2750 cm^{-1}. Figure 9 shows (A) FT-IR spectra of ∝-tocopherol, (B) the difference spectrum of spiked oil by ∝-tocopherol was subtracted from stripped oil. With its speed and ease of data manipulation by the computer software, FTIR spectroscopy is advantageous as a simple and rapid new analytical tool for α-tocopherol determination in RBDPOo.

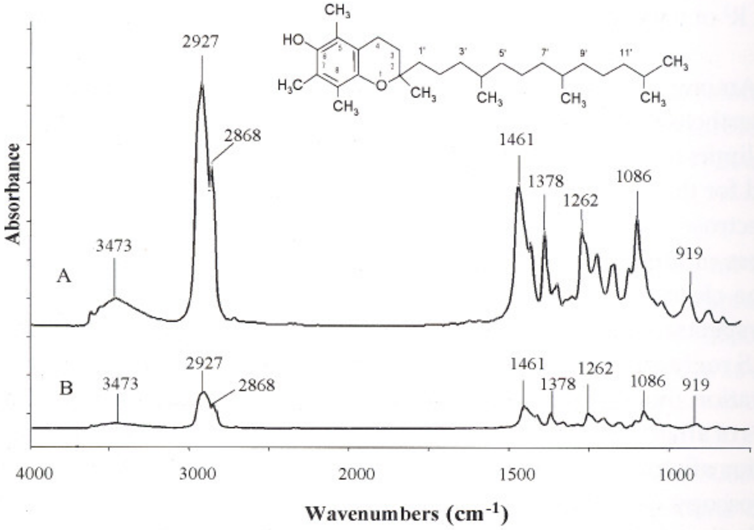


Figure 9: (A) FTIR spectra of α-tocopherol, (B) the difference spectrum of spiked oil by α-tocopherol was subtracted from stripped. (Source: Che Man et al., 2003).

β-Carotene – Both FTIR and NIR spectroscopic techniques have been investigated in our laboratories and compared to UV-visible spectrophotometry to measure the β–carotene content of crude palm oil (Moh et al. 1999). Both techniques provide alternative means to measure β– carotene in crude palm oil. RBD palm olein was pre-analyzed for β-carotene by HPLC method. The calibration and validation samples were prepared by spiking known amount of β-carotene. The PLS calibration statistics models for predicting β-carotene was used. The accuracy of the method was comparable to that of UV visible method with R^2 of 0.9950 at the spectral region of 980-915 cm^{-1} which is associated with trans double bond CH absorption. Both FTIR and NIR methods developed were shown to be efficient, accurate and suitable for routine quality control analysis with results obtainable in about 2 minutes.

Sesamol in sesame seed oil - FTIR spectroscopy was based on transmission (NaCl) cell accessory at room temperature, and the PLS regression statistical method was used to derive calibration models for determining sesamol spiked in sesame, RBDPOo and groundnut oils, with R^2 of 0.8938, 0.9940 and 0.9662 for the mentioned oils, respectively (Mirghani and Che Man, 2002). The calibration models were validated and the R^2 of validation and the SEP computed. The method was comparable to the actual values of sesamol content spiked in sesame, RBDPOo and groundnut oils. These observations strengthen the premise that FTIR spectroscopy is an efficient and accurate method for determining minor components in edible oils such as sesamol.

Gossypol in cottonseed oil - The rate of darkening in bleached color of cottonseed oil during storage is directly proportional to the gossypol content in oil and time and temperature of storage. Analytical results of laboratories analyzing cottonseed oil for gossypol reported back as at/or below the limits of detection. Currently the best analytical procedure is HPLC. Our research group described a new analytical method to determine gossypol in cottonseed oil using FTIR spectroscopy with NaCl transmission cell (Mirghani *et al.*, 2002c). The wavelength were selected for the gossypol and standards were prepared by spiking some clean cottonseed oil samples with the gossypol for concentrations of 0 – 5%. A PLS regression was used to derive the calibration models. The R^2 of calibration models were computed for the FTIR spectroscopy predicted values versus chemical values of gossypol in percent. Based on the results obtained, the FTIR spectroscopy can be a useful instrumental method for determining gossypol in cottonseed oil.

- **Authentication of oils and fats by FTIR**

The spectroscopic techniques were shown to have the potential to become a tool for rapid determination of adulteration in oils and fats. Our recent study provides a simple and rapid means of monitoring lard when blended with chicken, lamb and cow body fats (Che Man and Mirghani, 2001). A qualitative approach is proposed easily by comparing the pure animal fat with blended ones. A semi quantitative approach is proposed to measure the percent of lard in its blend with lamb body fat according to FTIR spectral frequency shifting within the region 3009 – 3000 cm^{-1}. For blending with chicken body fat the qualitative determination were at the FTIR spectral frequencies of 3008-3000, 1418-1417, 1390-1380 and 1110-1100 cm^{-1}. For the blend with cow body fat frequency regions 3008-3006, 1417.8 and 966 cm^{-1} were used for qualitative detection. The equations, and SE was created for semi quantitative determinations were computed. This technique is suitable and should be able to play a role in determining halal and non-halal oil and fat-based products in a more complex food system. In another study, we have developed a rapid method to determine the lard content in cake formulation using FTIR spectroscopy (Syahariza et al., 2004). FTIR spectra were recorded using ATR cell. PLS was used to derive FTIR spectroscopic calibration model in regions of 1117-1097cm^{-1} and 990-950cm^{-1}. The R^2 obtained was 0.9790 with standard error (SE) of calibration was 1.7520. A validation approach was used to optimize the calibration and the R^2 of validation and standard error of prediction were computed. Our results support the use of FTIR spectroscopy as an efficient and accurate method for detection lard in cake formulation.

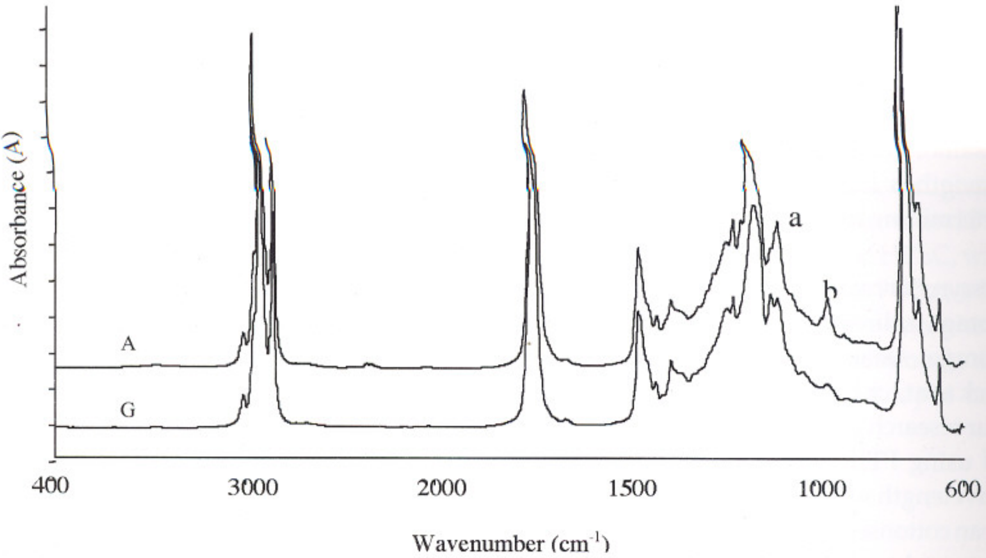


Figure 10: FTIR spectra of FTIR spectra of fat extracted from cakes with (A) 0% lard, and (G) 100% lard in region 4000-600 cm^{-1}. (Source: Syahariza et al., 2004).

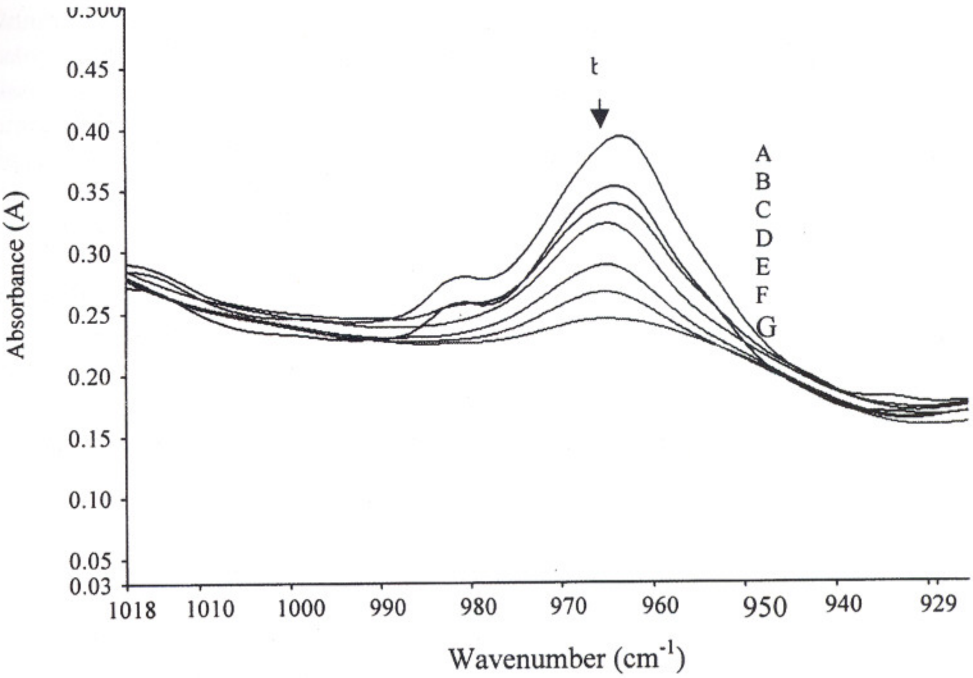


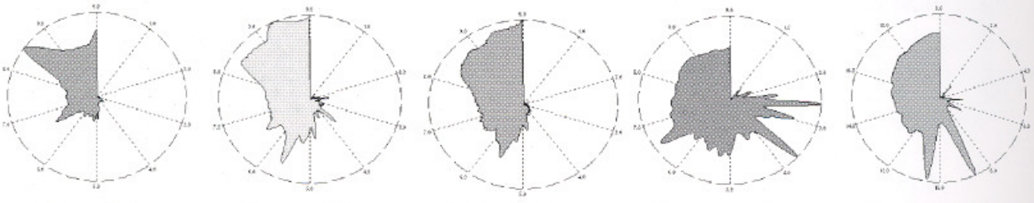
Figure 11: FTIR spectra of fat extracted from cakes with (A) 0% lard, (B)-(F) lard/ shortening mixture, and (G) 100% lard, illustrating changes in absorbance value of the band b in region 990-950 cm^{-1}. The percentage of lard in shortening mixtures are (B) 7%, (C) 33%, (D) 50%, (E) 66%, and (F) 83% lard in shortening. These changes in due to the C=C-H bending vibration of trans double bonds. (Source: Syahariza et al., 2004).

Electronic Nose

One of the more exciting advances in analytical technique has been the development of electronic nose. The instrument uses various sensors to analyse for a range of components using pattern recognition to look for a fingerprint of a complex mixture of volatile components. The units, which are used widely in cosmetic and food industries, are a marriage of modern statistical techniques and recently developed sensor technology. In the oils and fats industry, recent publications on this technology show how it has been used to characterize different type of oils (Guadarrama et al., 2001).

- **Characterization of Oils and Fats**

A novel approach using a surface acoustic wave (SAW) sensing based electronic nose (zNose™) for flavour analysis was explored by our group to characterize sixteen types of vegetable oils. The zNose™ was employed successfully for qualitative distinguish of flavour in different vegetable oils (Figure 12). This is achieved using a visual fragrance pattern, called a VaporPrint™ derived from the frequency of the SAW detector. VaporPrint™ was shown to be particularly useful to assess vegetable oil aroma profile in its entirety. This image is created by transforming the time variable to a radial angle with the beginning and end of the analysis occurring at 0° or vertical. Chemometric method particularly principal component analysis (PCA) was conducted for electronic nose data processing and identification. Analysis of the score plot of the PCA for the zNose™ measurement showed that 97% of the total variance in the data was described by PC 1 and PC 2 (Gan et al., 2004a).



Corn Oil Peanut Oil Palm Olein Sesame Oil Coconut Oil

Figure 12: VaporPrint™ for show large diversity for different types of vegetable oils. (Source: Gan et al., 2004).

- **Monitoring oxidation**

We also conducted a study on monitoring storage stability of RBDPOo using zNose™. A descriptive test was carried out for sensory analysis comprising of ten trained panellists. The results of electronic nose showed significant difference between fresh oil and rancid oil (Figure 13). VaporPrint™ was shown to be particularly useful to assess oil quality in its entirety. A high correlation was observed between electronic nose responses and chemical test data; as well as sensory evaluation score using Pearson's correlation. It can be concluded that the zNose™ may be utilised as an analytical tool to follow the progress of oxidation and breakdown of oils and fats (Gan et al., 2004b).

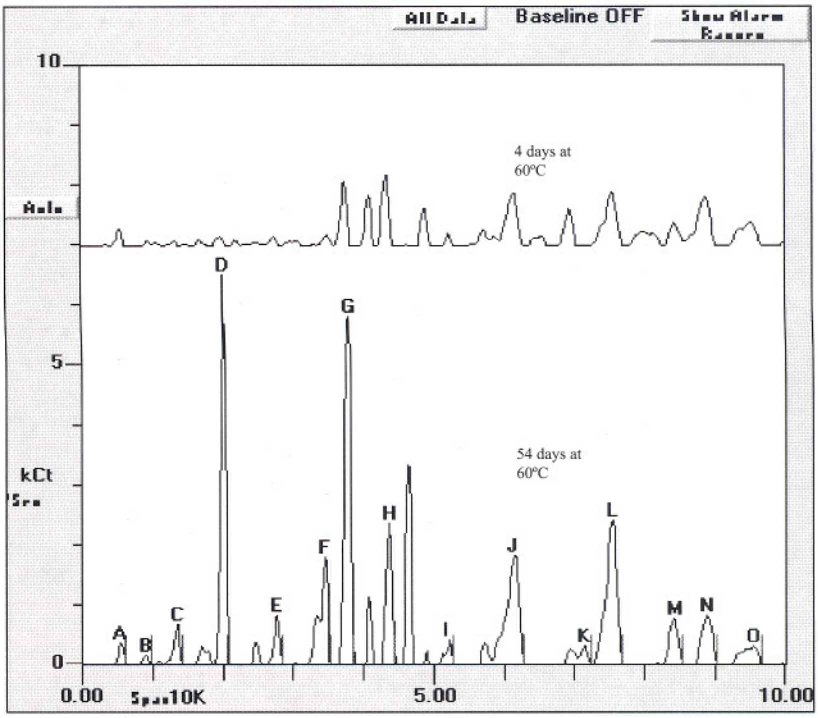
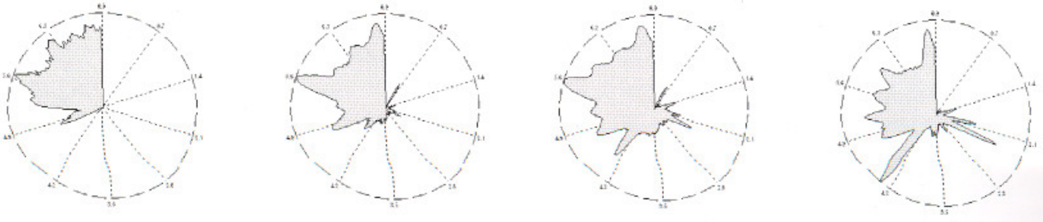


Figure 13: Waterfall chromatogram of SAW detector response for RBDPOo stored at accelerated oven temperature. (Source: Gan et al., 2004).

- **Detection of Lard Adulteration by EN**

The use of zNose™ for monitoring the presence of lard as adulterants in RBD palm olein was also investigated (Che Man et. al, 2004). Mixing of animal fats especially lard in any form in food products is a cause of concern for certain religions. RBDPOo spiked with lard in levels ranging from 1 to 20% (w/w) were analysed. The electronic nose measurement showed that a comparison of chromatogram between RBD palm olein and adulterated RBDPOo revealed that there is a gradual increase in the amounts as the adulterant is increased in concentration. Consequently, lard adulteration could be determined by a few distinct peaks in the electronic nose chromatogram. This finding served as the basis for the investigation of RBDPOo adulterated with lard. It was shown that the detection of RBDPOo samples adulterated with lard as low as 1% using the electronic nose was still possible (Figure 14). An ideal correlation was observed between the electronic nose response and chemical test ($R^2>0.90$).

Another useful advantage of the electronic nose was that it did not require any sample pre-treatment or chemicals for analysis. In addition, accuracy and speed of the electronic nose method for the detection and determination of lard in RBDPOo makes it ideal for quality control purposes.



1% 5% 10% 100%

Figure 14: VaporPrint™ for RBD palm olein adulterated with different percentage of lard. (Source: Che Man et al., 2004).

DNA-based Technology

Most early DNA-based techniques for food authentication employed some from hybridization technology and an appropriate species-specific probe (Chikuni *et al.*, 1990). This required large amounts of undamaged high quality DNA and relatively cumbersome laboratory protocols utilizing radioisotopes. However, with the advent of the polymerase chain reaction (PCR) (Saiki *et al.*, 1988), small amounts of relatively degraded DNA target could be selectively amplified to yield valuable information, while employing a much more straightforward approach. PCR has, therefore, greatly, increased the usefulness of DNA for food authentication purposes (Beneke and Hagen, 1998). DNA detection methods for adulteration foods rely on the complementarities of two strands of DNA double helix that hybridize in a sequence-specific manner.

For detection of lard, a method for species identification from lard and pork samples using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis of a conserved region in the mitochondrial (mt) cytochrome b (cyt b) gene was developed in our laboratory (Napis, et al. 2003; Aida et al. 2004). Genomic DNA of pork and lard were extracted using Qiagen DNeasy® Tissue Kit and then subjected to PCR amplification targeting for the mt cyt b gene of lard. The genomic DNA have been found to be of good quality and produced clear PCR products on the amplification of the mt cyt b gene of approximately 360 base pairs (bp). To distinguish between samples, the amplified PCR products were cut with restriction enzyme (RE) *BsaJ I* resulting in species-specific restriction fragment length polymorphisms (RFLP). The cyt b PCR-RFLP species identification assay yielded excellent results for identification of pig species (Fig. 15).

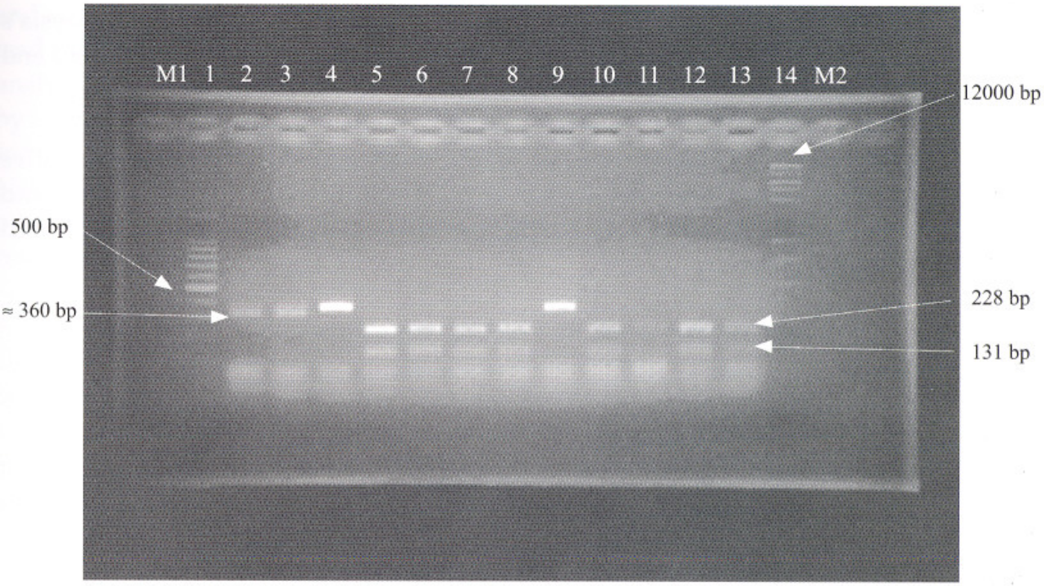


Figure 15: *Bsaj I* restriction profile of cytochrome b PCR products amplified from meat and fat samples. M1- 100 bp DNA ladder; 1- mutton's meat; 2- beef; 3- chicken's meat; 4,5,6 and 7- pork; 10- chicken's fat; 11,12,13 and 14- lard; M2- 1 Kb plus DNA ladder. (Source: Aida et al., 2004).

SIGNIFICANCE OF THE NEW DEVELOPMENTS

Rapid and continued developments in electronics, optics, computing, instrumentation, spectroscopy and other branches of science and technology have led to considerable improvements in various techniques. Due to this revolution in techniques, we are now able to solve problems, which were thought to be extremely difficult to do so a few years ago. The new methods enable us to better characterize, monitor and authenticate oils and fats and improve our understanding of these important food constituents.

Instrumental techniques of analysis have now moved from the confines of the chemistry laboratory to form an indispensable part of the analytical armory of many researchers involved in the fields of oils and fats. Recent years have also seen a dramatic improvement in the ease, with which such instruments can be used, and the quality and quantity of the analytical data that they can produce.

Rapid, accurate and reliable methods for analysis for both the immediate and finished products qualities of edible oils and fats are in great demand. Accurate monitoring of oil quality using rapid techniques can help avoid either risk to public health or cause financial loses to the food industry. The use of DSC, FTIR, NIR and EN developed by our research group offer a quick, accurate, and routine technique to determine various quality parameters for oils and fats industry. This bode well for the wider application of the instruments such as DSC, IR and EN in the development of latest instrumental techniques.

It is our hope that these advances in analytical techniques will contribute to Malaysia's oils and fats industry, halal food authentication program and to the world of science and technology in the following ways:

- The new techniques represent rapid, reliable, cost effective and efficient alternative approaches to conventional standard chemical methods for monitoring and quantitative determination of various parameters in fats and oils, with no need for delicate skills.

- These techniques involve minimal sample preparation.

- These techniques are completely safe and no safety precautions are required.

- These techniques can prevent the use of time- and work-consuming chemical methods and minimize the use of toxic chemicals that are hazardous to the analysts as well as to the environment.

- These techniques could be used for authentication of oils and fats in the food industries, government laboratories and especially halal certification agencies such JAKIM, Chemistry Departments, Ministry of Science, Technology and Environment and Food Quality Control Division of the Ministry of Health.

- The use of various instruments such as DSC, FTIR spectroscope and EN encourages more scientists to innovate new techniques to measure the various physical and chemical properties of oils and fats as well as to monitor quality changes during processing, thus increasing the application of these instruments in academic research and in oils and fats industry.

On the development of DNA-based technique, PCR-RFLP analysis developed by our research group represents a powerful and easy method for differentiating materials of porcine origin for halal authentication.

FUTURE OUTLOOK

It is difficult to predict what the future will hold. In the oils and fats industry, analysis of raw materials or finished products are necessary to determine if one has actually obtained what is called for by specifications and standards. Therefore, the selection of the method of analysis is a vital step in the solution of an analytical problem. Inevitably, in the method selected, a compromise has to be reached among the sensitivity, precision, and accuracy desired of the results and the costs involved. Today, there is constant development and changes in the techniques and methods of DSC, FTIR and NIR spectroscopy, EN and DNA-based technology. Better instrument design and a fuller understanding of the mechanics of analytical processes enable steady improvements to be made in sensitivity, precision, and accuracy. These same changes contribute to a more economic analysis as they frequently lead to the elimination of time-consuming steps. Therefore, the DSC, FTIR and NIR, EN analysis have become increasingly important. Demands for these techniques at 'long range' by instrument packages steadily increase. Generally, this work showed the possibility in increasing the application of DSC, FTIR and NIR and EN in the field of fats and oils. Nevertheless, most of the newly developed techniques for analysing the edible fats and oils quality and authenticity are yet to be tested by other scientists and validated through collaborative studies.

All the equipments used for the development of these techniques are high-cost today. However, if applications were to be more widespread, it could be available for many laboratories in the future. The cost of such a system would rapidly be absorbed through savings on personnel cost, time and chemical reagents required for conventional chemical methods and as such provide a useful advance in quality control methodology for the oils and fats industry.

ACKNOWLEDGEMENTS

I am most grateful to Allah SAW for His blessings and guidance. I sincerely thank to Universiti Putra Malaysia, Fakulti Sains Makanan & Bioteknologi and sponsors of my research grants for the opportunities given to explore and excel in my research interest. Heartfelt gratitude is extended to all colleagues, students and friends who rendered me support and assistance, no matter how much their contribution might be, everyone of you has contributed to my success in one way or another. I am especially indebted to my late father and mother for their doa, support and encouragement in my early upbringing. Most special thank to my beloved wife, Wan Jamilah and our eight children for their doa, sacrifice, endless support and understanding.

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