# PRELIMINARY STUDY ON CLASSIFICATION OF RAW BOVINE'S MILK USING ATR-FTIR COUPLED WITH PCA FROM PENINSULAR MALAYSIA

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**ABSTRACT.** A classification of raw bovine's milk samples according to their geographical origin in Peninsular Malaysia by Makmal Kesihatan Awam Veterinar, Department of Veterinary Services Malaysia (DVS). Six hundred bovine milk samples were collected from Perlis, Kedah, Perak, Selangor, Pahang, Negeri Sembilan, Melaka and Johor states by 26 milk collecting centres under DVS. This study was carried out directly using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy method coupled with a multivariate principal component analysis (PCA). The spectra generated by ATR-FTIR were analysed and regions of interest were found in between 3851.651 $cm^{-1}$ until 2700.819 $cm^{-1}$ and 2419.173 $cm^{-1}$ until 977.368 $cm^{-1}$. The absorbance and wavenumber data of the regions were then analysed using PCA and the results show presence of clustering towards their geographical origin. ATR-FTIR coupled with multivariate PCA has potential for classifying the geographical origin of raw milk produced within Peninsular Malaysia. This method provides a rapid and non-destructive secondary methodology in milk classification without further sample preparation.

*Keywords*: raw, milk, geographical origin, ATR-FTIR, PCA, Peninsular Malaysia

## INTRODUCTION

Traceability of food products is one of the main concerns for stakeholders in food quality and safety. These stakeholders include government, farmers, food processing plants, global regulations and standards as well as consumers. If an outbreak of food poisoning should occur, the food products can be trace back to its origin by the authority. Traceability is also very important to protect farmer product names from misuse and imitation (Scampicchio *et al.*, 2015). Therefore, a potential method to trace food products based on classifying their geographical origin is very crucial.

Analytical tools which have been developed to classify raw milk according to its geographical origin include gas chromatography (GC), mass spectroscopy (MS) and infrared spectroscopy (IRS) (Scampicchio *et al.*, 2015). Among these methods, mid-infrared spectroscopy (MIR) which is based on the mid-infrared region (ranging from 4,000 $cm^{-1}$ to 400 $cm^{-1}$) has been widely used as secondary methodology for predicting geographical origin of food products such as butter, milk and olives (Bassbasi *et al.*, 2014; Scampicchio *et al.,* 2015; Karoui R. and Baerdemaeker J. De, 2007; Bittante G. and Cecchinato A., 2013; Elbassbasi M. *et al.*, 2010). In comparison, GC and MS methods are expensive, time consuming, requiring experience technicians and are not cost effective. Multi-method approaches have been used in combination with these methods and a chemometric model to discriminate the geographical origin of milk samples (Cheng H. *et al.*, 2013; Scampicchio *et al.*, 2015). One example of MIR is the Fourier transform infrared (FTIR). FTIR spectroscopy is an appealing technology for the food industry because of its simple, rapid and non-destructive measurements of chemical and physical components requiring little or no sample preparation (Scampicchio *et al.*, 2015; Rodriguez-saona L.E. and Allendorf M.E., 2011, Bittante G. and Cecchinato A., 2013). FTIR is commonly used for structural identification (finger-printing) of organic compounds because the absorption bands caused by fundamental vibrations of a specific functional group (Rodriguez-saona L.E. and Allendorf M.E., 2011; Bittante G. and Cecchinato A., 2013).

The infrared spectroscopy technique coupled with chemometric models is a powerful tool in quality control and in fast analytical determination of different attributes in numerous food samples (Anjos O. *et al.*, 2015). Chemometric analysis such as principle component analysis (PCA) is the most basic workhorse of all multivariate data analyses. Chemometric analysis applied to the data from the FTIR in determining components in a sample, can be useful when establishing links to food origins or geographical origin of the sample (Bassbasi *et al.*, 2014).

Hence, this study aims to evaluate the potential usage of ATR-FTIR spectroscopy coupled with multivariate PCA to classify raw bovine's milk according to their geographical origin in Peninsular Malaysia. These methods provide a rapid and non-destructive secondary methodology in milk classification using ATR-FTIR without further sample preparation.

## MATERIAL AND METHOD

### Sample and Collection

A total of 600 samples of raw bovine milk were tested between March and November 2015 at the Makmal Kesihatan Awam Veterinar, DVS. Twenty-six milk collecting centres under the DVS were involved in collecting the milk samples from 160 farmers. Samples were collected from eight states (Perlis, Kedah, Perak, Selangor, Pahang, Negeri Sembilan, Melaka and Johor states) of Peninsular Malaysia.

### ATR-FTIR Spectra Acquisition

A 4100 ExoScan FTIR Spectroscopy with ATR sampling interface (Agilent Technologies, USA) was used in the MIR region. This spectroscopy was equipped with deutered triglycine sulfate (DTGS) detector and zinc selenite (ZnSe) beam splitter. The MIR spectra were collected from 4000 $cm^{-1}$ to 650 $cm^{-1}$ at a resolution of 4 $cm^{-1}$ with 64 scanning. By direct contact of the samples with the single reflection diamond ATR sampling probe, FTIR spectra was generated via MicroLab PC software (Agilent, USA). Each raw milk was sampled at at least 5 replicates to give better results. The spectra were background corrected, averaged, smoothed and data were transformed into MSExcel form using Spekwin32-spectrophotometer software Version 1.71.6.1, 2012. Binning process was conducted by selecting wavenumber regions with prominent peaks.

### Chemometric Analysis

The treated absorbance and wavenumber data of all 600 samples were analysed using multivariate PCA. A PCA model of the raw milk was developed from the classification by using Minitab 16 Software, USA.

## RESULTS AND DISCUSSION

### ATR-FTIR Spectra Analysis in Raw Bovine's Milk Samples

Representative ATR-FTIR spectra of raw milk in the region 4,000 $cm^{-1}$ to 650 $cm^{-1}$ are shown in Figure 1. Wavenumber region between 3851.651 $cm^{-1}$ to 2700.819 $cm^{-1}$ and 2419.173 $cm^{-1}$ to 977.368 $cm^{-1}$ were selected due to prominent peaks indicating presence of functional groups. Variations between samples depends on the respective functional groups, therefore it was possible to build a PCA model. Kishor K. and Thakur R., 2015, had stated that the importance of IR spectroscopy for the qualitative analysis originates from its properties especially as a finger-print technique. They stated 3100 $cm^{-1}$ until 910 $cm^{-1}$ regions was considered to be the finger-print region. According to Rodriguez-Saona L.E. and Allendorf M.E., 2011, the finger-print region, is located within the MIR region between 1,200 $cm^{-1}$ and 700 $cm^{-1}$ as it contains bands from lipids, proteins, carotenoids, and polysaccharides, and therefore, rich in structural information. Bittante G. and Cecchinato A., 2013, had mentioned that the International Committee for Animal Recording has approved FTIR spectrometry as the standardized rountine method for analyzing fat, protein and lactose contents of milk. Therefore, finger-print regions for the raw milk in this study are in between wavenumber region 3851.651 $cm^{-1}$ to 2700.819 $cm^{-1}$ and 2419.173 $cm^{-1}$ to 977.368 $cm^{-1}$.

Iñón F.A. *et al.*, 2004, described the absorption band located at region between 3,650 cm$^{-1}$ to 3,000 cm$^{-1}$ and 1,680 cm$^{-1}$ to 1,600 cm$^{-1}$ as indicative of water. Absorption band at region 2,360 cm$^{-1}$ was reported to be caused by atmospheric carbon dioxide. Whereas, absorption band for amide group (CONH) of milk proteins was at 1,546.8 cm$^{-1}$. Jaiswal P. *et al.*, 2015, reported that the peaks of the amide group of milk proteins show up in the wavenumber range 1,472 cm$^{-1}$ to 1,239 cm$^{-1}$ correspond to N-H bending and C-N stretching vibrations in amide III, wavenumber range 1,560 cm$^{-1}$ to 1,553 cm$^{-1}$ correspond to amide II and wavenumber range 1,680 cm$^{-1}$ to 1,631 cm$^{-1}$ corresponds to amide I. According to Iñón F.A. *et al.*, 2004, carbon hydrogen groups (C-H) and carbonyl groups (C=O) of milk fat absorb at 2,873.60 cm$^{-1}$ and 1,747.3 cm$^{-1}$ which is the chemical structure of fat molecules. Hydroxyl group (OH) of lactose absorbed at 1,039.60 cm$^{-1}$. These findings correspond with those in this study.

## ATR-FTIR Spectrum Data and Multivariate Statistical Analysis in Milk

In order to know whether there is classification present from the ATR-FTIR

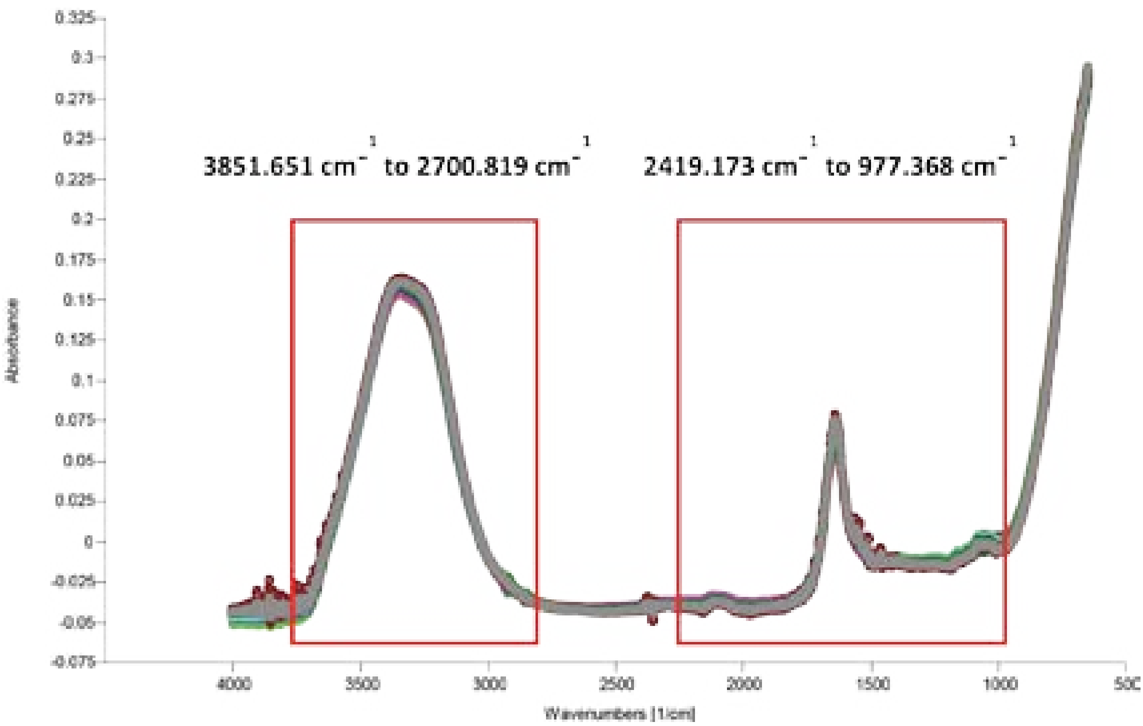


**Figure 1.** Representative ATR-FTIR Spectral from Raw Bovine's Milk. The two red box indicating milk spectrum regions selected for chemometric analysis.

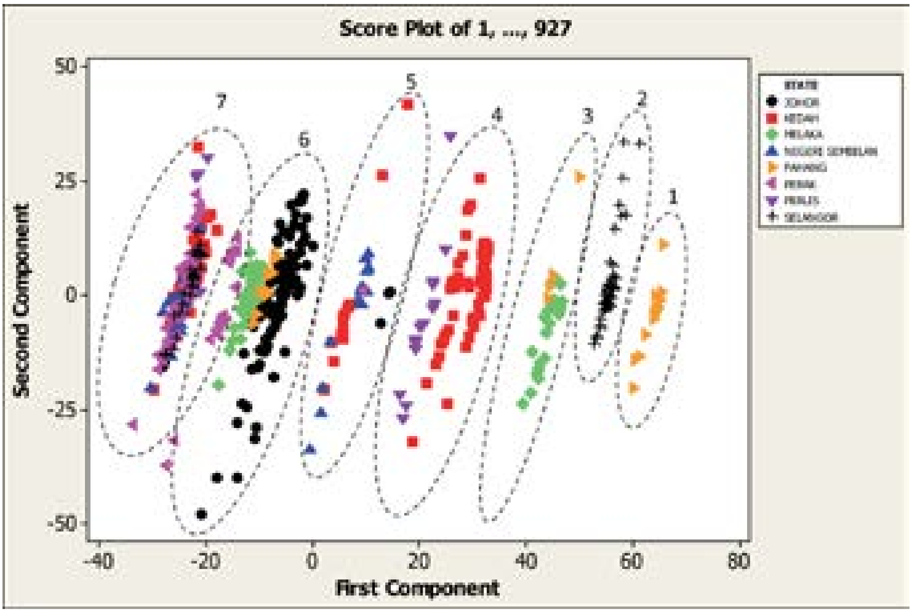


**Figure 2.** PCA Score Plot of first principal components (PC 1) and second principal components (PC 2) of raw bovine's milk ATR-FTIR Data from Peninsular Malaysia shows presence of 7 groups of cluster.

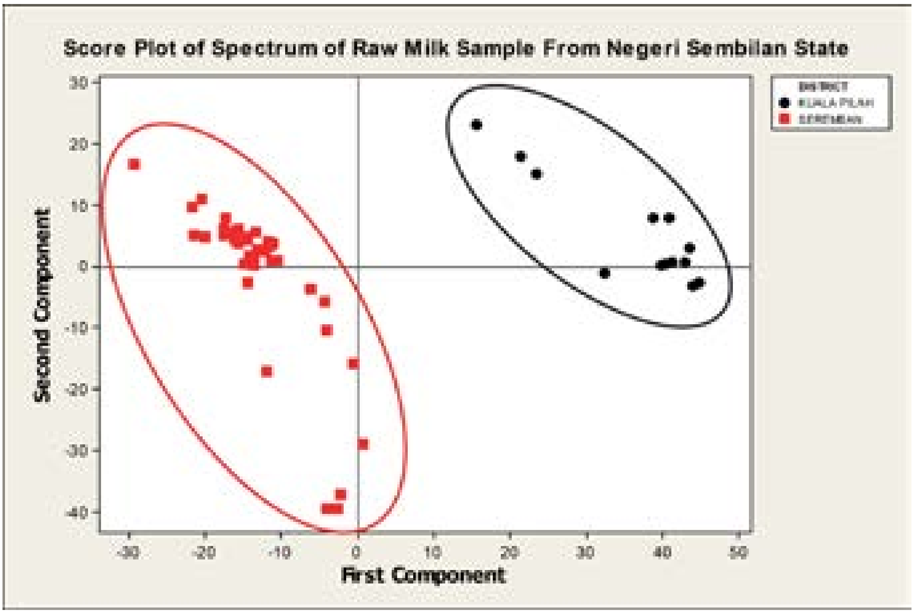


**Figure 3.** PCA Score Plot of first principal components (PC 1) and second principal components (PC 2) of raw bovine's milk ATR-FTIR Data from Negeri Sembilan state. Oval shape lines represent the clustering boundary of milk samples from 2 different districts in Negeri Sembilan state.

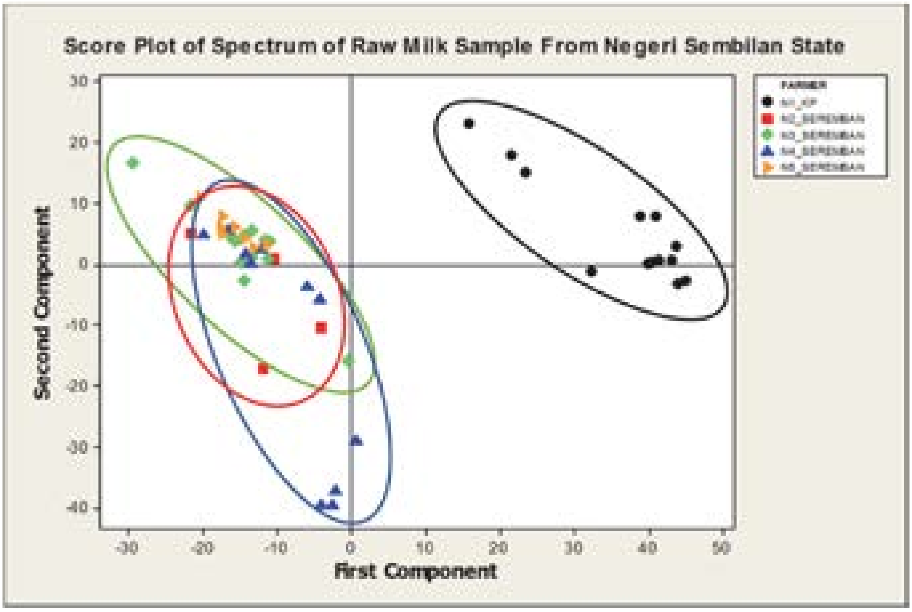


**Figure 4.** PCA Score Plot of first principal components (PC 1) and second principal components (PC 2) of raw bovine's milk ATR-FTIR Data from different farmers originated from Negeri Sembilan state. Oval shape lines represent the 4 clustering boundary of milk samples.

spectra, PCA of FTIR was performed by generating scree plot and score plot from the first two components. The PCA model in Figure 2 demonstrates 7 groupings of raw bovine milk from PCA Score Plot of first principal components (PC 1) and second principal components (PC 2). Group 1: Pahang; group 2: Selangor; group 3: Melaka and Pahang; group 4: Kedah and Perlis; group 5: Kedah and Negeri Sembilan; group 6: Johor, Pahang and Melaka; and group 7: Kedah, Perlis, Perak, Selangor and Negeri Sembilan. Figure 3 shows groupings of 3 different districts of Negeri Sembilan state. The two districts mainly Kuala Pilah and Seremban from Negeri Sembilan shows 2 distinctive groups generated from the ATR-FTIR data. The same data when analysed according to the farmers such as in Figure 4 shows the presence of grouping but some of the groupings may overlapping due to the similar location of the farms. These groupings indicate that there is a classification of raw milk according to their geographical origin. Elbassbasi *et al*., 2010, using PCA model and FTIR, stated that the prediction of geographical origin for unknown raw milk samples can be achieved with great success. PCA modeling is easy to apply, costs relatively little and enables the rapid prediction of the geographical origin for unknown raw bovine milk samples.

## CONCLUSION

In conclusion, the geographical origin of raw milk can be predicted by using ATR-FTIR coupled with multivariate PCA method. By creating a PCA model, raw milk sample was traceable by locating the possible source. The combination of these two methods, are non-destructive, does not require any prior preparation of the sample, requires only a small amount of sample for examination and is not time consuming due to its rapid analysis time as well as eco-friendly and cost-effective as it does not involve any reagent. The usefulness of this information can bring benefits to our country and department. Based on the encouraging results obtained, this study has a potential to be applied to various types of food.

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