

## Authentication analysis of butter from beef fat using Fourier Transform Infrared (FTIR) spectroscopy coupled with chemometrics

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### Abstract

The use of Fourier transform infrared (FTIR) spectroscopy coupled with chemometric techniques to differentiate butter from beef fat (BF) was investigated. The spectral bands associated with butter, BF, and their mixtures were scanned, interpreted, and identified by relating them to those spectroscopically representative to pure butter and BF. For quantitative analysis, partial least square (PLS) regression was used to develop a calibration model at the selected fingerprint regions of 1500–1000  $\text{cm}^{-1}$ , with the values of coefficient of determination ( $R^2$ ) and root mean square error of calibration (RMSEC) are 0.999 and 0.89% (v/v), respectively. The PLS calibration model was subsequently used for the prediction of independent samples containing butter in the binary mixtures with BF. Using 6 principal components, root mean square error of prediction (RMSEP) is 2.42% (v/v). These results proved that FTIR spectroscopy in combination with multivariate calibration can be used for the detection and quantification of BF in butter formulation for authentication use.

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### Keywords

Authenticity

chemometrics

FTIR spectroscopy

butter

beef fat

### Introduction

One of the important dietary source of nutrients and energy is milk fat (Kaylegian and Lindsay, 1995; Hillbrick and Augustin, 2002). Typically, milk fat contains 66% saturated fatty acids, 30% monounsaturated fatty acids, and 4% polyunsaturated fatty acids (Aigster *et al.*, 2000). Due to its taste, milk fat is considered by the consumer as better in quality compared to other fats. Therefore, its adulteration has always been a serious problem, either for consumer or milk producers. The adulteration practice was motivated by the profitable advantages, taken by partly replacing the high-priced milk fat with low priced animal fat (eg: beef fat) without labeling the product accordingly. Compared to other milk fat products, butter is the most expensive milk fats; as a consequence, butter is subjected to adulteration practices.

The composition of butter is influenced by genetic factors (Gibson, 1991) and feeding conditions (Guyot,

1977a,b). European Union (EU) has developed strict standard of identity for butter (European Union, 1990). EU has established that butter should be obtained at least 82% from exclusively milk fat. In spite of this, butter has always been subjected to adulteration by the addition of less expensive vegetable or animal fats. As a result, the detection of non milk fat in butter is far more important today than ever before, and a number of investigations have been carried out by several research groups to develop analytical methods for this purpose. In recent years, different reviews have also appeared with updated information on the detection of non milk fats in butter (Lipp 1995; Contarini *et al.*, 1999; De la Fuente and Juarez, 1999; Ulberth and Buchgraber, 2000; Kamm *et al.*, 2001; Jee, 2002).

The improvement of new and increasingly sophisticated techniques for the authentication of food products grows rapidly due to the increased consumer awareness of food safety and food quality issues (Reid *et al.*, 2006). Adulteration and

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misrepresentation is often difficult to detect because of sophisticated techniques used by unscrupulous food processors. Increased consumer awareness has led to the development of new and increasingly sophisticated techniques for dairy products authentication such as isotopic analysis (Rossmann *et al.*, 2000), chromatography (Fernandez *et al.*, 2003), electronic nose (Navratil *et al.*, 2004), polymerase chain reaction (Lipkin *et al.*, 1993), enzyme linked immunosorbent assay and thermal analysis (Reid *et al.*, 2006; Luykx and Van ruth, 2008). However, most of these techniques are time consuming, and require extensive sample preparation and hazardous chemicals as well as skilled and experienced operators. These disadvantages have prompted for the adoption of new and simpler methods such as Fourier Transform Infrared (FTIR) spectroscopy method.

FTIR spectroscopy is a rapid, inexpensive and sensitive technology used for the high throughput analysis of milk based food components that rapidly allows real time measurements at all stages of production without requiring special skills from users. Moreover, it has been acknowledged as a powerful tool for detection of adulteration practice, especially in combination with chemometric techniques. FTIR spectroscopy is also widely utilized for rapid quality control of numerous dairy products, since it provides fingerprint spectra information from complex spectra about the composition of dairy product such as milk (Albanell *et al.*, 1999; Laporte and Paquin, 1999), cheese (Rodriguez *et al.*, 1995; Sorensen and Jepsen 1998a,b; Witrup and Noorgard, 1998) and other dairy products (Rodriguez-otero and Hermida, 1996; Rodriguez *et al.*, 1997; Laporte and Paquin 1998; Karoui and De Baerdemaeker, 2007; Lema Garcia *et al.*, 2010; Nicolaou *et al.*, 2010).

In particular, FTIR coupled with chemometric techniques has been extensively used on butter to differentiate it from other edible fats (Yang *et al.*, 2005), to determine acidity, fat, and moisture of butter for quality controls (van de Voort *et al.*, 1992, 1993; Koczon *et al.*, 2008) as well as to detect the adulteration (Koca *et al.*, 2010). However, no work has been done in differentiation of butter from beef fat using FTIR spectra coupled with chemometric method. Therefore, in this study, we have used FTIR spectroscopy using ATR accessory to analyze the presence of beef fat (BF) in butter.

## Material and Methods

### Sample preparation

Beef fat was obtained from rendering different batches of subcutaneous fat from the back part of cattle

which were obtained from several local markets in Serdang, Selangor, Malaysia. The rendering process was carried out according to Rohman and Che Man (2009). Butter sample was extracted according to AOAC official method 920.118 (2000). The extracted samples were kept in glass vials under refrigerated conditions (-20°C) until being used for analysis. Infrared spectra were collected for each sample to develop a classification model.

### Calibration and Validation

For calibration model, the calibration samples composed a number of standard or training sets consisting of beef fat (BF) in butter at concentration ranges of 1 – 100% v/v were prepared. For validation or prediction samples, 20 independent samples were constructed. Pure butter and BF as well as their blends were analyzed using FTIR spectroscopy. The frequency regions where the variations were observed were chosen for developing PLS model in order to quantify BF in butter.

### FTIR instrumental analysis

Using a Pasteur pipette, fats obtained from the extraction procedure were placed in direct contact with attenuated total reflectance (ATR) crystal on a multi bounce plate at controlled ambient temperature (25°C). A Nicolet 6700 FTIR spectrometer (Thermo Nicolet Corp., Madison, WI) equipped with a detector of deuterated triglycine sulphate (DTGS), a beam splitter of KBr/Germanium, and connected to software of the OMNIC operating system (Version 7.0 Thermo Nicolet), were used during FTIR data collection. To minimize water vapor interference, the instrument was maintained with dehumidifier of silica gel. FTIR spectra were recorded from 32 scans at a resolution of 4 cm<sup>-1</sup> at mid infrared region (4000–650 cm<sup>-1</sup>). These spectra were subtracted against background air spectrum. After every scan, a new reference air background spectrum was taken. The ATR plate was carefully cleaned in situ by wiping it with hexane twice followed by acetone, and dried with soft tissue before filling in with the next sample. The cleanliness of ATR crystal was verified by collecting a background spectrum and comparing it to the previous one. These spectra were recorded as absorbance values at each data point in triplicate.

### Statistical and chemometric analysis

PLS chemometric analysis of was done using the software TQ Analyst™ version 6 (Thermo Electron Corporation, Madison, WI). The “leave-one-out” cross validation procedure was used to verify the calibration model. The PLS performance was assessed

using the values of root mean standard error of calibration (RMSEC) and coefficient of determination ( $R^2$ ). In addition,  $R^2$  and root mean standard error of prediction (RMSEP) were used for the evaluation of validation capability of PLS.

## Results and Discussion

### FTIR spectral analysis

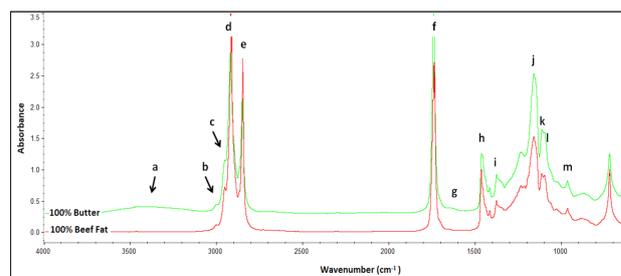
FTIR spectroscopy is one of the advanced techniques that could be used for analysis of fats and oils. Most fats and oils are mainly composed from triglycerides which can be directly applied in its neat form to ATR crystal. This technique has been widely applied, because once the instrument has been calibrated; it can be used for routine analyses. The importance of IR spectroscopy for the qualitative analysis originates from its properties especially as fingerprint technique, meaning that there is no two fats/oils having the same FTIR spectra. In fats and oils, most of the peaks and shoulders of the spectrum are attributable to the specific functional groups (Bendini *et al.*, 2007). The triglycerides (TGs) were the principle components in fats and oils, as a consequence, the FTIR spectra of TGs dominate FTIR spectra of fats and oils. The representative spectra of pure butter and pure beef fat are presented in Figure 1. The analytical evaluation of the butter and beef fat spectra were compiled in Table 1.

**Table 1.** The bands and their vibrational modes arising from the functional groups responsible for infrared absorption of beef fat and butter (Safar *et al.*, 1994; Vlachos *et al.*, 2006; Guillén and Cabo, 1997a)

Assignment	Wavenumber (cm <sup>-1</sup> )	Functional group	Vibration mode
(a)	3700-3000	O-H	stretching (sym.)
(b)	3006	-C-H (cis-)	cis C=CH stretching
(c)	2953	-C-H (CH <sub>3</sub> )	Asymmetric stretching vibration of methyl (-CH <sub>3</sub> ) group
(d)	2924	-C-H (CH <sub>2</sub> )	Asymmetric or symmetric stretching vibration of methylene (-CH <sub>2</sub> ) band
(e)	2854	-C-H (CH <sub>2</sub> )	Asymmetric or symmetric stretching vibration of methylene (-CH <sub>2</sub> ) band
(f)	1745	-C=O (ester)	Carbonyl (C=O) functional group from the ester linkage of triacylglycerol
(g)	1650	-C=C (cis)	cis C=C
(h)	1460	-C-H- (CH <sub>2</sub> , CH <sub>3</sub> )	Bending vibrations of the CH <sub>2</sub> and CH <sub>3</sub> aliphatic groups
(i)	1377	=C-H- (cis)	Symmetric bending vibrations of CH <sub>3</sub> groups
(j)	1161	-C-O-, -CH <sub>2</sub> -	Vibrations of stretching mode from the C-O group in esters
(k)	1097	-C-H- bending	-CH bending vibrations of fatty acids
(l)	1117	-C-H-	-CH deformation deformation vibrations of fatty acids
(m)	966	-HC=CH (trans)	Bending vibration of CH functional groups of isolated trans-olefin

\*(Source: Guillen and Cabo, 1997a).

Absorption bands of water, corresponding to O-H groups, were observed in the region of 3700-3000 cm<sup>-1</sup> and 1600-1500 cm<sup>-1</sup>, which can affect the amide I signal at about 1650 cm<sup>-1</sup> (Rodriguez *et al.*, 2006; Karoui and De Baerdemaker, 2007). In agreement with



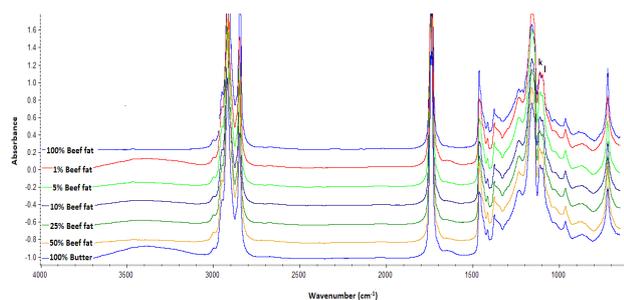
**Figure 1.** FTIR spectra of pure butter and beef fat at mid infrared region (4000-650 cm<sup>-1</sup>)

Koca *et al.* (2010), strong absorptions were observed at 2900 and 2800 cm<sup>-1</sup>, respectively, corresponding to C-H (CH<sub>2</sub> and CH<sub>3</sub>) stretching vibrations. Moreover, a weak signal at 3000 cm<sup>-1</sup> associated with -C=C-H stretching groups of cis-unsaturation was observed. At 1745 cm<sup>-1</sup>, another strong band was present, which is reported to be originated from -C=O stretching vibrations of acids and esters (Lema Garcia *et al.*, 2010). This band and the next at 1460 cm<sup>-1</sup> arising from N-H bending vibration are likely associated with amide I and amide II of proteins (Karoui and De Baerdemaker, 2007; Rodriguez *et al.*, 2006). In the last part of the spectra (1300-1000 cm<sup>-1</sup>), stretching vibrations of the C-O bond of esters and bending vibrations of methylene group were present (Lema Garcia *et al.*, 2010). The band at 966 cm<sup>-1</sup>, associated with -HC=CH out-of-plane deformation vibrations, has been previously reported as a marker band for the determination of trans-fatty acids in fats and oils. Upon a closer scrutiny, the minor differences (peak heights) can be attainable at 1117 and 1097 cm<sup>-1</sup> (k and l) corresponding to C-H bending vibration and C-H deformation vibrations of fatty acids, respectively. Hence, these frequencies, which FTIR spectra variations were observed, are used as a basis for choosing the spectral regions in the quantification of beef fat in butter samples.

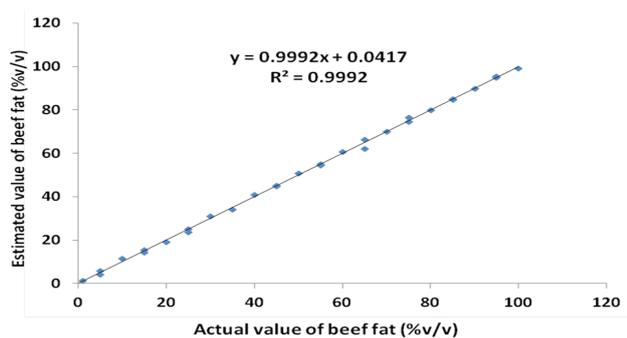
### Calibration and validation

Partial least square (PLS) was used for constructing calibration model. Frequencies at selected fingerprint regions (1500-1000 cm<sup>-1</sup>) were exploited for quantitative analysis of beef fat in butter. The absorbancies at 1117 and 1097 cm<sup>-1</sup> in beef fat (BF) was much higher than that of butter. Consequently, increasing BF concentrations in butter will result in reducing the ratio value, approaching the height ratio of BF at 1117 and 1097 cm<sup>-1</sup> (Figure 2). In PLS model, the samples of BF were divided into two sets of calibration and prediction, respectively. Butter adulterated with 1.0-100.0% of BF was used for quantification purposes.

The relationship between actual value (x-axis) and FTIR predicted value of BF in PLS calibration model



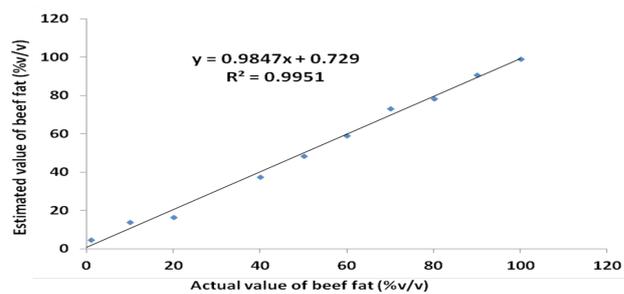
**Figure 2.** FTIR spectra of butter and beef fat as well as mixtures showing changes in the height ratio (R) at 1117 and 1097  $\text{cm}^{-1}$  due to the increase of beef fat percentages at 4000–650  $\text{cm}^{-1}$



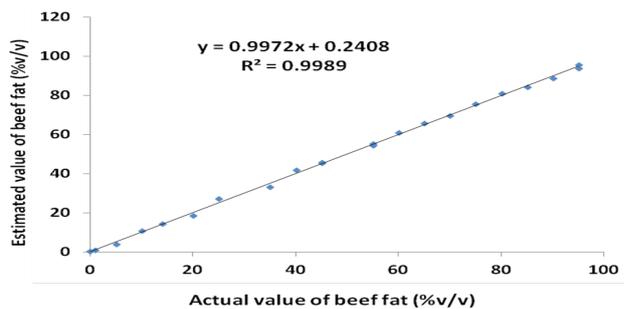
**Figure 3.** Partial least square (PLS) calibration model for the relationship between actual and FTIR estimated values (%v/v) of beef fat as adulterant in butter

was shown in Figure 3. A good linear regression of  $y = 0.999x + 0.0417$ ; was obtained with  $R^2$  and RMSEC values are 0.999 and 0.890% (v/v), respectively. The  $R^2$  value tells how close the relationship between actual and FTIR predicted values of analyte of interest (BF). The closer  $R^2$  value to unity, the better the relationship. According to International Conference on Harmonization (ICH, 1994),  $R^2$  value higher than 0.99 is acceptable for such relationship. Meanwhile, RMSEC refers to the calibration uncertainty. The smaller the RMSEC value, the better the calibration model. PLS calibration model was also used to calculate the BF contents in prediction samples. The validation plot for the relationship between actual and FTIR predicted values of BF in prediction samples was shown in Figure 4, with  $R^2$  and root mean square error of calibration (RMSEP) obtained are 0.996 and 2.42% (v/v), respectively.

The calibration model was further cross validated using “leave-one-out” technique by removing one standard at a time. In cross validation, one of the calibration samples is taken out from PLS calibration model and the residual samples are used to build PLS model. Subsequently, removed sample is calculated using the new PLS regression. This manner was recurred, leaving each sample out in turn (Miller and Miller, 2005). The relationship between actual value (x-axis) and FTIR predicted value of BF in butter during cross validation was shown in Figure 5. The



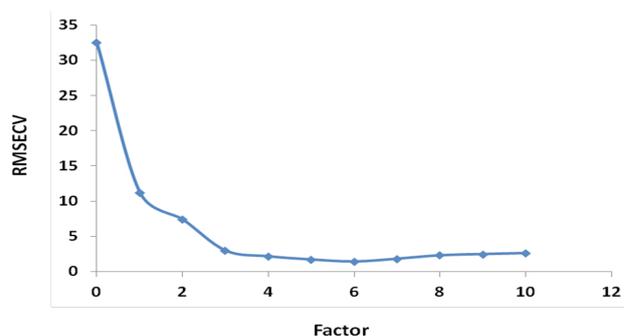
**Figure 4.** Partial least square (PLS) validation model for the relationship between actual and FTIR estimated values (%v/v) of beef fat as adulterant in butter



**Figure 5.** The relationship between actual and FTIR estimated values (%v/v) of beef fat as adulterant in butter during cross validation using “leave-one-out” technique

$R^2$  and root mean square error of cross validation (RMSECV) values of 0.999 and 1.04% v/v were obtained.

The confirmation and validation of the frequency region used for developing the PLS model were performed by computing the predicted residual error sum of squares (PRESS) values for different factors or principal components (PCs). The PRESS value is a direct measure on how well a calibration predict the concentrations left out during a cross validation (Smith, 2002). PRESS informed that the optimal factor number is 6 as revealed in Figure 6, that express RMSEC obtains a stable value, minimally after six-factor. This confirms that the spectral region used for developing the PLS model for quantification of BF exhibits significant correlation with its concentration.



**Figure 6.** Root mean standard error of cross validation (RMSECV) versus principal component (PC) factors

## Conclusion

The suitability FTIR together with PLS algorithm provides high performance approach for determination butter adulterated with beef fat in their entirety components. PLS can be successfully used to quantify the level of adulterant of beef fat at selected fingerprint region (1500-1000  $\text{cm}^{-1}$ ) with the acceptable values of  $R^2$  and errors, either during calibration or prediction. This method is rapid, non-destructive and easy-to-use. Great precision of method is a requirement of quality control in dairy product industry. Thus, a combination between FTIR and chemometrics could be a powerful alternative authentication of dairy product.

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Notes

†Y.B. Che Man deceased 15 July, 2012

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