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Detection of Butter Adulteration with Lard by Employing ^{1}H-NMR Spectroscopy and Multivariate Data Analysis

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Abstract: The authentication of food products from the presence of non-allowed components for certain religion like lard is very important. In this study, we used proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy for the analysis of butter adulterated with lard by simultaneously quantification of all proton bearing compounds, and consequently all relevant sample classes. Since the spectra obtained were too complex to be analyzed visually by the naked eyes, the classification of spectra was carried out.The multivariate calibration of partial least square (PLS) regression was used for modelling the relationship between actual value of lard and predicted value. The model yielded a highest regression coefficient (R^2) of 0.998 and the lowest root mean square error calibration (RMSEC) of 0.0091% and root mean square error prediction (RMSEP) of 0.0090, respectively. Cross validation testing evaluates the predictive power of the model. PLS model was shown as good models as the intercept of R^2Y and Q^2Y were 0.0853 and −0.309, respectively.

Key words: butter, lard, ^{1}H-NMR, multivariate data analysis, adulteration, chemometric

1 Introduction

Food authentication has received much attention from consumers, producers and policy makers. It is of utmost importance concern in food industry due to its relation to social growth and health. Authenticity of food mainly focused on two domains: food safety and quality. However, this field becomes more complex due to fraud practices in manufacturing such as adulteration and mislabeling. Protection against mislabeling is an important part of food control carried out by the authorities. Legislation would be effective, only when the authenticity of food is enforced and monitored. Development of new analytical methods for determination of food adulteration is very difficult because ways of adulterating food are highly sophisticated[1)].

One of the main foods that play role in feeding the population is dairy products. This dairy product is essential for everyone especially for certain groups of consumers (women, children, and the elderly)[2)]. The most expensive commodities derived from dairy products is milk fat. For this economically reason, detection of foreign fat in milk fat is scrutinized issues. There is several ways adulteration of milk fat such as homogenization of skimmed milk with less expensive foreign fat and by direct incorporation of foreign fat in butter or butter fat. These frauds were developed in the eighties[3−8)] and were standardized[9)]. Butter is one of the milk fat products that are important economically to dairy industry and high in nutritionally and functionally to customers. Due to high standard of quality of the products, the authenticity of butter is great importance for commercial and health reason.

Butter is the only one original product that exclusively from cow milk. It made by churning fresh or fermented cream or milk. It has high in lipid cholesterols and saturated fat which consist of phospholipids, tocopherols and ca-

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rotenoids[10]. Butter is characterized by an extraordinarily rich fatty acids composition. Butter has to be considered as a natural dairy product that must contain at least 82% of milk fat, 16% water and 2% non fat dry extract. It must not contain extraneous fats of those coming from milk[11]. Butter is one of edible animal fats used as a finished product for cooking and as an ingredient in various alimentary preparations.

Recently lard is commonly used as an edible animal fats raw material in food products. The presence of lard in food products can be viewed from two perspectives, economy and religion. On an economic perspective, some food products make good blends with lard to produce cost-effective products such as margarines, shortenings, and other oil-based food[12]. Therefore, the food industry likes to mix lard with butter to lessen the production cost[13]. As from a religious point of view, religions such as Islam and Judaism forbid their followers in consuming and using food that contains lard or any pig-derived ingredient[14]. For this reasons, analytical methods are urgently needed for rapid and reliable techniques to detect lard in food products.

Although adulteration does not necessarily cause harm to consumers, it does abuse their consumer right by selling product that is not as what it been claimed. This drives to the development of methods for accessing adulteration in butter in order to maintain the quality of the products. On the basis of reducing the cost in butter production, manufacturers alternatively altered the ingredients by adding non-milk fats into butter. Several research group has developed the analytical methods to detect the presence of this fat has been reported and reviewed, however most of them are time and resource consuming[15-20]. This disadvantages has creates an urge to find simpler and effective method to identify and quantify adulterated butter. In this study we proposed proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy conjugation with multivariate statistical for analysis butter adulterated with lard.

^{1}H-NMR spectroscopy is a versatile spectroscopic technique for studying opaque heterogeneous samples, which has already proof to have a number of useful applications in dairy research[21]. Unique information that can be applied to research or to quality control of dairy samples can be obtained through this technique too. In addition, it is a non-destructive and very versatile technique, providing data on the same sample under different parameters and also has the ability to provide the global concentration of the sample and not just the surface concentration. ^{1}H-NMR is an important tool in the study of food, lipids, oils, and fats, which has gained growing applications, particularly because of the great amount of information. It allows discrimination between oils of different composition in a very short time[22].

^{1}H-NMR spectroscopy is a very wide-ranging analytical technique and can detect quantitatively and simultaneously all proton-bearing compound and consequently all relevance sample classes. Since the spectra obtained are too complex to be analyzed visually, the classification of spectra in this study was carried out by coupled with chemometric analysis. Chemometrics is an approach based on the measurements related to the chemical composition of a substance are taken, the value of a property of interest is inferred from them through some mathematical relation[23].

Qualitative investigations based on the combination of NMR and principal component analysis have been commonly applied in the fields of metabolic profiling[24], food technology[25, 26] and recently for the classification of feverfew preparations[27]. The present study highlights the application of ^{1}H-NMR spectra combined with multivariate data analysis for classification and quantification of butter adulterated with lard. These spectra are subjected to several spectral treatments.

2 Materials and methods

2.1 Lard samples

Pure lard sample was extracted by rendering the adipose tissue from various parts of slaughtered pigs, which were obtained from several local markets in Selangor, Malaysia. Sampling was done triplicates and consists of three pig samples. Adipose tissues were heated using microwave according to the method proposed by De Pedro[28]. Melted fat was collected and filtered with glass wool. Fat was dried over anhydrous sodium sulphate and kept in a freezer at (−20℃) before further analysis.

2.2 Butter sample preparation

Fresh milk from Universiti Putra Malaysia (UPM), Serdang dairy farm field was used as a raw material. The heavy cream was taken out from the milk by using cream separator. The heavy cream then was shaken in the mixer with a medium-high speed till the colour of cream change to pale yellow. The pale yellow buttermilk was collected and kept at −20℃ before further usage.

2.3 Calibration and Validation standard

The calibration sets was prepared by spiking lard to butter in concentration range of 0.5%-80%, using 13 different levels of lard, namely 1,0%; 5,0%; 7,5%; 10,0%; 12,5%; 22,5%; 27,5%; 35,0%; 40,0%; 45,0%; 55,0%; 60,0% and 80%. Butter containing of 100% concentration and 100% pure lard was also made to observe the spectral differentiation. All blends were prepared on a weight basis to calculate the exact actual percentage of the added lard. The pure and the blends samples were analyzed by means ^{1}H-NMR spectroscopy.

2.4 NMR measurement

A 10 mg sample of each sample was dissolved in 700 μl of deuterated methanol containing 0.5% TMS and mixed using a vortex mixer. The sample was then sonicated for 1 hour in a sonicator bath and left to solubilise for a further 12 hour at room temperature. This was followed by centrifuging for 5 min to remove insoluble components, after that the clear supernatant was transferred into NMR tubes. ^{1}H NMR spectra were recorded at 26℃ on a 500 MHz Varian INOVA NMR spectrometer operating at a proton NMR frequency of 499.887 MHz. Each ^{1}H NMR spectrum acquired over a spectrum width of 20 ppm, consisted of 64 scans requiring 3:15 min acquisition time. The resulting spectra were manually phased and base-line-corrected, using Chenomx software (v. 5.1, Alberta, Canada). The spectra were referenced to TMS at δ 0.00ppm.

2.5 Bucketing of ^{1}H NMR spectra

^{1}H NMR spectra were automatically reduced to ASCII files using Chenomx software (v. 5.1, Alberta, Canada). Spectral intensities were binned by equal width (δ 0.04) corresponding to the region of δ 0.50–10.00. The region of δ 4.70–4.96 (water) and δ 3.28–3.33 (residual methanol) were excised from the analysis.

2.6 Statistical validation of the model

Partial Least Square (PLS) was performed with the SIMCA-P software (v. 12.0, Umetrics, Umeå, Sweden) using Pareto scaling and different spectral filters were used for multivariate calibration. Permutation test was used to validate the model. It provides statistical significance of the estimated predicted power. In order to obtain an estimate of the significance of a Q^2Y value, a number of parallel models based on fit to randomly re-ordered Y data were developed. For the training set in the permutation test, the X data are left intact, while the Y data are permuted to appear in a different order. The Y data remain numerically the same, but their positions are shifted by random shuffling. PLS model is then fitted to compute for the derived model. These "permuted" values may then be compared with the estimates of R^2Y and Q^2Y of the "real" model to get a first indication of the significance of the latter values. The permutation procedure was repeated 100 times and by establishing an equivalent number of parallel PLS models, it is possible to achieve reference distributions of R^2Y and Q^2Y based on random data. Such reference distributions are useful for appraising the statistical significance of the R^2Y- and Q^2Y- parameters of the parent PLS model.

3 Results and Discussion

3.1 Visual inspection of ^{1}H-NMR spectra of pure samples

Figure 1 shows the typical ^{1}H-NMR spectra of pure butter, pure lard and adulterated butter with lard. The range of chemical shifts at 0-4 ppm cover most the free fatty acids and fatty acids that attached to the glycerol while chemical shift range 4-6 ppm is assigned to the signals of proton at glycerol backbone in butter and lard. Comparing the spectra from samples, many similarities and dissimilarities can be seen, however due to the huge amount of combined data, no obvious relation between both samples makes a visual analysis set of data impossible. Therefore, by employing ^{1}H-NMR with multivariate data analysis, this analysis data set would be possible to analyze. This method measurement of butter and lard seems to be a

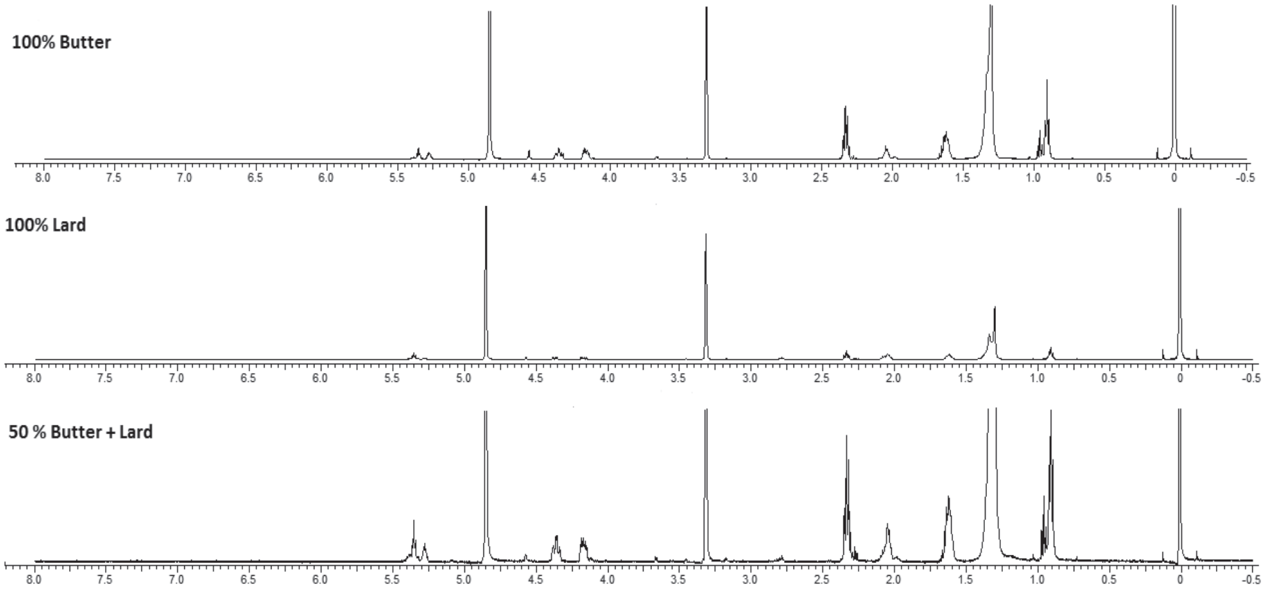

Fig. 1 ^{1}H-NMR spectra of pure butter, pure lard and adulterated butter with lard from 0-8 ppm.

rapid and informative analytical tool compared to other conventional methods as it provides a clear and characteristic profile.

3.2 Quantification of lard in butter using ^{1}H-NMR spectroscopy and PLS

Partial Least Square (PLS)[29] is a versatile tool with many desirable properties, (i) it is able to cope with highly collinear and low rank data, which is not the case of multiple linear regression[30]; PLS allows analysing data with more variables than observations; (ii) PLS provides models with high stability of predictions because the risk of overfitting is minimised; (iii) PLS is very efficient in handling missing data and therefore it provides inferential models extremely robust to sensor failure and (iv) with the aid of careful data analysis and easy-to-use charts, PLS is able to detect outliers, which improves the quality of the fitted models and reduces the risk of extrapolation when new observations are projected over the model.

Quantification of lard in butter was performed using multivariate calibration of PLS model. PLS is based on data compression and inverse calibration[31]. A PLS calibration model was developed for quantifying those lard in concentration range of 0.5%-80% (v/v). The model obtained can be used for the prediction of concentration an unknown samples. The theory of the method as well as some applications in food science has been described in a number of publications[32-36].

Figure 2 display the PLS calibration model which correlates the actual and estimated values of butter and lard (% v/v) obtained from ^{1}H-NMR spectra. The difference between the actual and the observed concentration of adulterant is relatively small with coefficient of determination (R^2) value is 0.998 for lard. RMSEC was used to evaluate the error in calibration model.

The term "actual" refers to the known or true concentration of selected standards. Meanwhile the "calculated" or "predicted" refers to a value computed by the model using spectral data; where the number of samples used in the calibration sets; and number of factors used in the calibration model. The low value RMSEC indicates the good performance of PLS model. The RMSEC values of lard in butter obtained was 0.0091.

The evaluation of the goodness of fit in the validation is performed by calculating the root mean square error of prediction (RMSEP) and R^2. The value of RMSEP was 0.0090. The high values of R^2 and low values of RMSEP indicate the success of PLS regression model. PLS models were optimized in terms of ^{1}H-NMR spectral regions and spectral filters. Table 1 compiled PLS performance in terms of R^2, RMSEC and RMSEP, either for normal and other spectra filter such as derivatives, multiplicative signal correction (MSC) and standard normal variate (SNV). As shown in Table 1 and Fig. 2, PLS calibration in the MSC spectra revealed the highest of R^2 (0.998) and the lowest of RMSEC (0.0091) and RMSEP (0.0090) values compared with other spectra filter (Table 1).

The aim of data pre-processing is to remove unwanted systematic variation such as baseline shifts, scatter effects and effects from uncontrolled external factors. Methods generally applied can be divided into two categories. The first category consists of classical methods for spectral normalization and smoothing and differentiation, e.g., multiplicative signal correction (MSC), standard normal variate

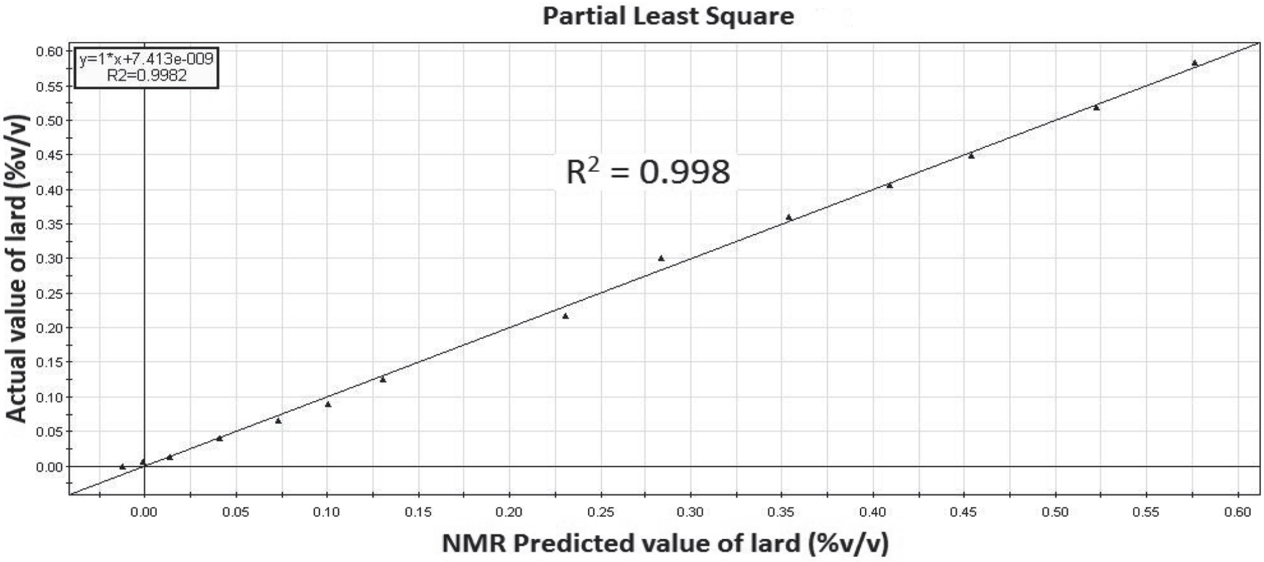

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Fig. 2 Partial least square (PLS) calibration model of actual and predicted value (%v/v) of lard concentration based on ^{1}H-NMR profile of samples.

Table 1 Multivariate calibration for determining butter adulterated with lard using PLS technique.
PLS modelling of the NMR dataset using a number of spectral filters and Pareto scaling, to select the model with overall better properties (SIMCA, 2008).

Spectral filter	R^{2} [a]	RMSEE[b]	RMSEP[c]
No filter	0.9858	0.0443	0.0214
1st derivative	0.9963	0.0313	0.0081
2nd derivative	0.9984	0.0152	0.0173
[d]MSC	0.9982	0.0091	0.0090
[e]SNV	0.9996	0.1219	0.0637
[f]OSC			

[a] R^2, fraction of explained variation by fitting the model; [b]RMSEE, square root of the residual mean square of estimation; [c]RMSEP, square root of the residual mean square of prediction; [d]MSC, Multiplicative Signal Correction; [e]SNV, Standard Normal Variate; [f]OSC, Orthogonal Signal Correction.

(SNV) trans-formation, Savitzky and Golay smoothing and first and second derivatives[37−41)]. The second category consists of methods for variable selection and dimensionality reduction and has received considerable attention over the past decade.

3.3 Statistical validation of the model

PLS is a supervised method and the goodness of fit and the predictability of its result can be subjected to validation to test the possibility of correlation by chance. This statistical validation step is especially important for metabolomics data, because most of the data have a larger number of observations than variables. For the PLS method, we can apply "y-scrambling" validation, where the y variable values are randomly shuffled and the models are rebuilt and analyzed. The validation plot is constructed with the Y-axis representing R^2Y and Q^2Y of the original and permutated models, while the x-axis represents correlation coefficients between permutated and original models. Regression lines were then fitted among R^2Y and Q^2Y points. The PLS models were validated using response of permutation test through 100 permutations. The permutation test assesses the statistical significance of the estimated predictive power previously calculated by cross validation test[40)]. To be a valid model, the original models should have higher R^2Y and Q^2Y value than the permutated models. To achieve this, Erikkson *et al.* mentioned that the intercepts of R^2Y should be less than 0.3-0.4, while Q^2Y should not exceed 0.05[42)]. PLS model was shown as good model as the intercept of R^2Y are 0.08, intercept of Q^2Y are −0.34 (Fig. 3).

Conclusion

In conclusion, we demonstrated the suitability ^{1}H-NMR together with PLS algorithm that provides high performance approach for determination butter adulterated with lard in their entirety of all proton bearing components. 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, the combination of ^{1}H-NMR and chemometrics could be a powerful alternative for authentication of dairy product.

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References

1) Stewart, C. Oil and Fats Authentication. Blackwell Publishing, pp.181-205 (2002).
2) U. S. Department of Agriculture Center for Nutrition Policy and Promotion. ChooseMyPlate.gov. (2011).
3) Timms, R. E. Determination of oxidative deterioration of milk powder and reconstituted milk by measurement of chemiluminescence. *J. Dairy Res.* 47, 295-303 (1982).
4) Precht, D. Detection of foreign fat in milk fat. I Qualitative detection by triacylglycerol formulae. *Z. Leb-*

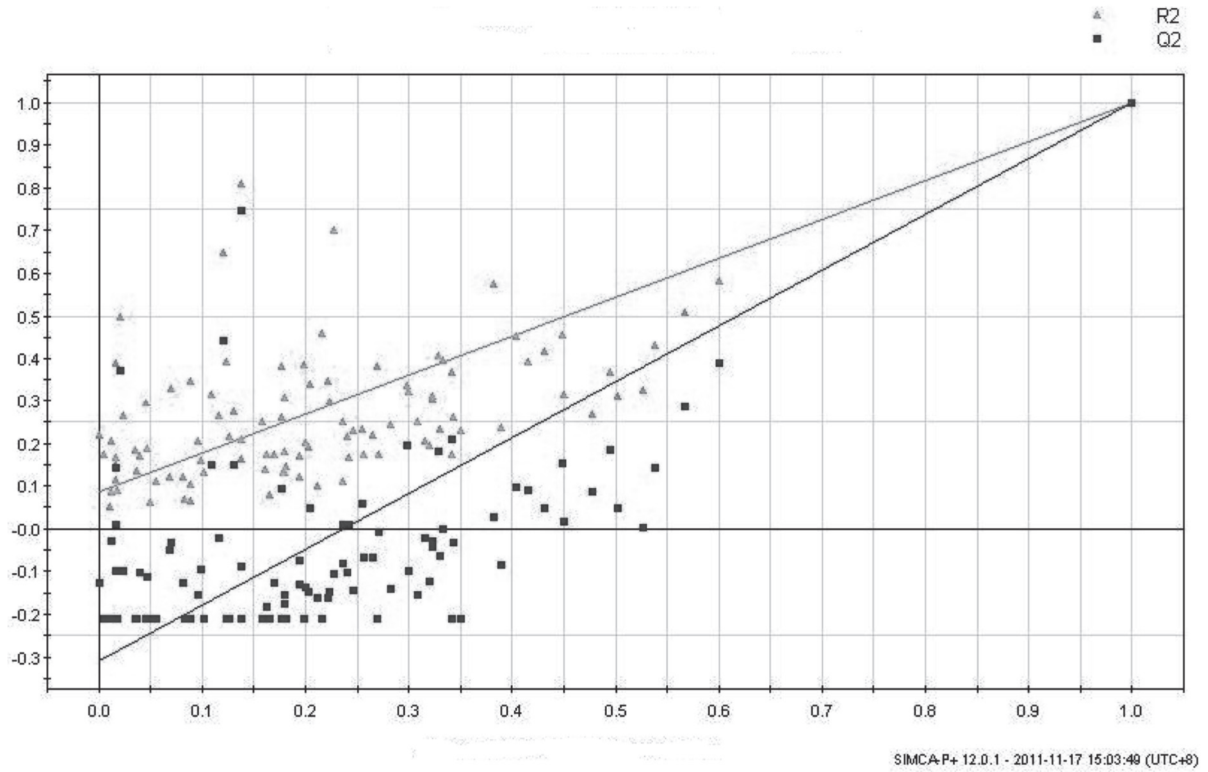

Fig. 3 Results from the response permutation test. The vertical axis gives the R^2Y and Q^2Y values of each test. The horizontal axis represents the correlation coefficient between the 'real' Y and the permuted Y. The PLS model is strongly significant. Intercept of R^2 and Q^2 were 0.0853 and -0.309, respectively.

ensm. Unters. Forsch. **194**, 1-8 (1992).

5) Precht, D. Detection of foreign fat in milk fat. II Quantitative evaluation of foreign fat mixtures. *Z. Lebensm. Unters. Forsch.* **194**, 107-114 (1992).

6) Precht, D. Control of milk fat purity by gas chromatographic triglyceride analysis. *Kieler Milchw. Forsch.* **43**, 219-242 (1991).

7) Precht, D. Detection of adulterated milk fat by fatty acid and triglyceride analysis. *Fett. Wissens. Tech.* **93**, 538-544 (1991).

8) Precht, D.; Mukherjee, K. D.; Weber, N.; Sherma, J. CRC Handbook of Chromatography, Analysis of Lipids, (Eds) CRC Press, Boca Raton, FL, p.123 (1993).

9) Commission of the European Communities: Detection of foreign fats in milk fat by means of gas chromatographic triglyceride analysis, Doc. No VI/5202/90-EN, VI/2645/91.

10) Jensen, R. G. The composition of bovine milk lipids: January 1995 to December 2000. *J. Dairy Sci.* **85**, 295-350 (2002).

11) Regolamento, C. E. n.2991/94 del 5 dicembre 1994, Gazzetta Ufficiale Comunita` European. L. 316 del 09.12.1994.

12) Marikkar, J. M. N.; Ghazali, H. M.; Che Man, Y. B.; Peiris T. S. G.; Lai O. M. Distinguishing lard from other animal fats in admixtures of some vegetable oils using liquid chromatographic data coupled with multivariate data analysis. *Food Chem.* **91**, 5-14 (2005).

13) Che Man, Y. B.; Rohman, A.; Mansor, T. S. T. Differentiation of lard from other edible oils by means of Fourier transform infrared spectroscopy and chemometrics. *J. Am. Oil Chem. Soc.* **88**, 187-192 (2011).

14) Regenstein, J. M.; Chaudry, M. M.; Regenstein, C. E. The kosher and halal food laws. *Comp. Rev. Food Sci.* **F2**, 111-127 (2003).

15) Lipp, M. Review of methods for the analysis of triglycerides in milk fat: Application for studies of milk quality and adulteration. *Food Chem.* **54**, 213-221 (1995).

16) Contarini, G.; Povolo, M.; Bonfitto, E. The present and future for controlling the authenticity of butter. *Latte* **24**, 60-69 (1999).

17) De la Fuente, M. A.; Júarez, M. Application of chromatographic techniques to the study of triglycerides and sterols of milk fat. *Food Sci. Tech. Int.* **52**, 103-119 (1999).

18) Ulberth, F.; Buchgraber, M. Authenticity of fats and oils. *Eur. J. Lipid Sci. Tech.* **102**, 687-694 (2000).

19) Kamm, W.; Dionisi, F.; Hischenhuber, C.; Engel, K. H. Authenticity of fats and oils. *Food Reviews Int.* **17**, 249-290 (2001).

20) Jee, M. Milk fat and other animal fats. In: Oils and Fat Authentication, Jee M.(eds.), Reading, U. K., Blackwell Publishing, CRC Press, pp.115-142(2002).
21) Duce, S. L.; Amin, M. H. G.; Horsefield, M. A.; Tyszka, M.; Hall, L. D. NMR imaging of dairy products in two or three dimensions. *Int. Dairy J.* **5**, 311-319(1999).
22) Guillen, M. D.; Ruiz, A. High resolution ^{1}H nuclear magnetic resonance in the study of edible oils and fats. *Trends Food Sci. Technol.* **12**, 328-338(2001).
23) Lavine, B. Chemometrics. *Anal. Chem.* **70**, 209R-228R (1998).
24) Nicholson, J.; Lindon, J.; Holmes, E. Metabonomics: Understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica* **29**, 1181-1189(1999).
25) Vogels, J. T. W. E.; Terwell, L.; Tas, A. C.; Dukel, F.; van der Greef, J. Detection of adulteration in orange juices by a new screening method using proton NMR spectroscopy in combination with pattern recognition techniques. *J. Agric. Food Chem.* **44**, 175-180(1996).
26) Belton, P. S.; Colqhoun, I. J.; Kemsley, E. K.; Degadillo, I.; Roma, P.; Dennis, M. K. Application of chemometrics to the ^{1}H spectra of apple juices: Discrimination of apple varieties. *Food Chem.* **61**, 207-213(1998).
27) Bailey, N. J. C.; Sampson, J.; Hylands, P. J.; Nicholson, J. K.; Holmes, E. Multicomponent metabolic classification of commercial feverfew preparations via high-field ^{1}H-NMR spectroscopy and chemometrics. *Planta Medica* **68**, 734-738(2002).
28) De Pedro, E. J.; Casillas, M.; Miranda, C. M. Microwave oven application in the extraction of fat from the subcutaneous tissue of Iberian pig ham. *Meat Sci.* **45**, 45-51(1997).
29) Wold, S.; Wold, H.; Dunn, W. J.; Ruhe, A. The partial least squares(PLS)approach to generalized inverses. *SIAM J. Sci. Stat. Comp.* **5**, 735-743(1984).
30) Paradkar, M. M.; Sivakesava, S.; Irudayaraj, J. Discrimination and classification of adulterants in maple syrup with the use of infared spectroscopic techniques. *J. Sci. Food Technol.* **83**, 714-721(2002).
31) Alam, M. A.; Hamid, S. F. Application of FTIR spectroscopy in the assessment of olive oil adulteration. *J. Appl. Sci. Res.* **73**, 102-108(2007).
32) Nurrulhidayah, A. F.; Che Man, Y. B.; Al-Kahtani, H.; Rohman, A. Application of FTIR spectroscopy coupled with chemometrics for authentication of *Nigella sativa* seed oil. *Spectroscopy–Biomed. Appl.* **25**, 243-250 (2011).
33) Rohman, A.; Che Man, Y. B. Fourier transform infrared (FTIR)spectroscopy for analysis of extra virgin olive oil adulterated with palm oil. *Food Res. Int.* **43**, 886-892(2010).
34) Rohman, A.; Che Man, Y. B. Potential use of FTIR-ATR spectroscopic method for determination of virgin coconut oil and extra virgin olive oil in ternary mixture systems. *Food Anal. Methods* **4**, 155-162(2011).
35) Rohman, A.; Che Man, Y. B. The optimization of FTIR spectroscopy combined with partial least square for analysis of animal fats in quartenary mixtures. *Spectroscopy–Biomed. Appl.* **25**, 169-176(2011).
36) Cadet, F.; De la Guardia, M. in: Meyers R. A.(Ed.), Encyclopedia of Analytical Chemistry, John Wiley & Sons Inc., Chichester, pp.1-26(2001).
37) Barnes, R. J.; Dhanoa, M. S.; Lister, S. J. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Appl. Spectrosc.* **43**, 772-777(1989).
38) Geladi, P.; MacDougall, D.; Martens, H. Linearization and scatter-correction for near-infrared reflectance spectra of meat. *Appl. Spectrosc.* **39**, 491-500(1985).
39) Martens, H.; Stark, E. Extended multiplicative signal correction and spectral interference subtraction: New preprocessing methods for near infrared spectroscopy. *J. Pharm. Biomed. Anal.* **9**, 625-635(1991).
40) Wiklund, S.; Nilsson, D.; Eriksson, L.; Sjostrom, M.; Wold, S.; Faber, K. A randomization test for PLS component selection. *J. Chemom.* **21**, 427-439(2007).
41) Eriksson, L.; Trygg, J.; Wold, S. CV-ANOVA for significance testing of PLS and OPLS models. *J. Chemom.* **22**, 594-600(2008).
42) Eriksson, L.; Johansson, E.; Kettaneh-Wold, N.; Wold, S. Multi and megavariate data analysis. Part 1: Basic Principles and Applications. Umetrics AB: Umeå, Sweden,(2006).