Middle-East Journal of Scientific Research 7 (5): 726-732, 2011
ISSN 1990-9233

Analysis of Lard in Cream Cosmetics Formulations Using FT-IR Spectroscopy and Chemometrics

[1,2]*Abdul Rohman and* [3]*Yaakob B. Che Man*

[1]Department of Pharmaceutical Chemistry, Gadjah Mada University, Yogyakarta, 55281 Indonesia
[2]Halal Research Group, Integrated Research and Testing Laboratory,
Gadjah Mada University, Yogyakarta, 55281 Indonesia
[3]Halal Products Research Institute, Universiti Putra Malaysia,
43400 UPM, Serdang, Selangor, Malaysia

Abstract: Typically, cream preparation is composed from complex mixtures; therefore its analysis is rather difficult. The presence of lard (LD) in any personal care products is prohibited to be used by the Followers of Islam. In this study, Fourier transform infrared (FT-IR) spectroscopy combined with partial least square (PLS) and discriminant analysis (DA) was developed for the quantification and classification of LD in cream formulations. PLS and DA were performed at two frequency regions of 3,020 – 2,995 and 1,200 – 1,000 cm^{-1}. The PLS calibration model obtained for the relationship between actual (*x-axis*) and FT-IR predicted (*y-axis*) values of LD was $y = 0.997x + 0.065$, with coefficient of determination (R^2) and root mean square error of calibration (RMSEC) of 0.997 and 0.808% (v/v), respectively. In addition, DA can successfully classify creams containing LD in its formulation using 9 principal components. FT-IR can be used as a potential analytical technique to quantify and to classify LD in cream preparations with total analysis time at about 3 min/one sample measurement.

Key words: Lard · FT-IR spectroscopy · Cream cosmetics · Partial least square · Discriminant analysis

INTRODUCTION

Today, the use of cream cosmetics products has increased tremendously [1]. Human exposure to cosmetics formulations and their ingredients occurs primarily via the topical route such as cream and lotion [2]. Several considerations are necessary regarding the suitability and safety of cosmetics, because cosmetics preparations are used nearly constantly and in direct contact with the skin [3].

Lard (LD) obtained from the rendering process of fatty porcine tissue is commonly used as viscosity-increasing agents in several cosmetic products. Food and Drug Administration (FDA) has listed LD as one of the generally recognized safe substances [4]. However, cosmetics products containing LD are prohibited to be used for the followers of some religions, like Islam [5]. For the restriction for exposure to LD, the development of analytical method offering fast and reliable results is highly required. One of the developed methods is Fourier transform infrared (FT-IR) spectroscopy.

In the analytical field, FT-IR spectroscopy provides a highly effective choice. It is fast and non-destructive technique, sensitive and free or simple in sample preparation and can be considered as green analytical technique due to the little use of chemical reagents and solvents in its operational [6]. Due to its simplicity in sample handle technique, an accessory of horizontal attenuated total reflectance (HATR) is used for analysis of fats and oils [7]. Previous research has shown the potential of FT-IR spectroscopy as a method for analysis of LD, mainly in food systems such as in cake and chocolate products [8,9] or LD in the mixture with other fats and oils [10]. Guillen and Cabo [11] as well as Che Man *et al.* [12] used FT-IR spectroscopy to characterize lard and other edible fats and oils.

Corresponding Author: Abdul Rohman, Department of Pharmaceutical Chemistry, Gadjah Mada University, Yogyakarta, 55281 Indonesia, Tel: +603-89430405, E-mail: abdulkimfar@gmail.com.

Almost all studies performed previously were based on food or food products. In cosmetics science, FTIR spectroscopy has been used for measure the diffusional parameters in membranes [13] and to study the surface chemistry of hair fibres [14]. Using literature study, there is no report available related to the use of FT-IR spectroscopy for analysis of LD in cream formulations. Therefore, the objective of this research was to develop FT-IR spectroscopy combined with chemometrics of partial least square (PLS) and discriminant analysis (DA) for quantification and classification of LD in cream formulation.

MATERIALS AND METHODS

Lard Preparation: Lard (LD) was prepared according to Rohman and Che Man [15]. LD sample was extracted by rendering the adipose tissue of pig (*Sus sacrofa)* obtained from various slaughtered houses around Jogjakarta, Indonesia. The rendering process was done at 90–100°C for 2 h in the conventional oven. The melted fat was strained through triple-folded muslim cloth, dried with anhydrous Na_2SO_4 and centrifuged at 3000 rpm for 20 min. The fat layer was decanted, shaken vigorously and centrifuged again before being filtered via Whatman filter paper. The filtered fats were stored in tightly closed containers in the refrigerator until being used for preparation of cream cosmetics. VCO was bought from Jogjakarta, Indonesia (POVCO®) and was prepared from the fresh mature kernel of coconut. The kernel was grated and made into viscous slurry and squeezed through a layer of cheese cloth to obtain coconut milk by adding boiling water (1:1). The coconut milk was left to settle for 1 hr in order to separate the cream from the skim. The cream was taken, stirred and left for 5 hr in order to form a layer of oil between the dregs and the water. To separate the gum from the oil (VCO), the oil was subsequently removed, left to settle for 24 hrs and filtered.

Determination of Iodine Value and Fatty Acid Composition: Iodine value was determined according to the American Oil Chemist' Society (AOCS) method Cd 1d-92 [16]. The composition of fatty acids in LD and VCO was determined according to procedure as reported by Rohman and Che Man [17].

Preparation of Calibration Model: The calibration model was constructed using PLS algorithm. In order to make a calibration curve, 10 samples of LD was blended with VCO at concentration range of 0.0 – 50.0% v/v in chloroform. The frequency of spectral regions where the variations were observed between LD and VCO was chosen for developing PLS model.

Preparation of Cream Cosmetics Formulation for Validation Samples: For prediction or validation samples, ten laboratory prepared cream samples with certain LD concentrations were prepared. The cream formulation studied consisted of stearic acid (3.0% w/w); LD or VCO as well as their blends (50.0 w/w); lanolin (4.0% w/w); distilled water (39.5% w/w); cetyl alcohol (1.0% w/w); and triethanolamine (2.5% w/w). Cream formulation was prepared by weighing each ingredient on an analytical balance (sensitivity 0.1 mg). Triethanolamine together with water was heated at about 70°C (water phase). Cetyl alcohol, stearic acid, lanolin and LD-VCO blends were heated at about 70°C (oil phase). Oil phase was poured into water phase and stirred with magnetic stirrer until reached ambient temperature (30 min). The cream obtained was further subjected to liquid-liquid extraction in order to extract the fat from cream formulation.

Fat Extraction: Representative samples of cream (20 g) were added with 2 ml of concentrated HCl and 18 ml of water and shaken vigorously. The filtrate was transferred to a separatory funnel and extracted using 2 x 15 ml of chloroform. The chloroform extracts were combined and drained into a 250 ml round-bottom flask and concentrated with a rotary evaporator at 40°C. The concentrated lipid extract was quantitatively transferred to a vial and made up to a final volume of 25.0 ml with chloroform. The contents of LD were further determined using FT-IR spectrometer.

Discriminant Analysis: Each of ten laboratory prepared cream cosmetics with and without LD in their formulations is used as training set or standard in DA. The creams containing LD were assigned as "with lard", while a series of creams which are absent from LD are marked "without lard". Furthermore, the developed DA model was used to classify the commercial cream samples and those spiked with certain amount of LD.

Instrumental Analysis: FT-IR spectrometer Nicolet 6700 (Thermo Nicolet Corp., Madison, WI) equipped with a detector of deuterated triglycine sulphate (DTGS) and connected to software of OMNIC operating system (Version 7.0 Thermo Nicolet) was used during FT-IR spectra acquisition. The oil samples were placed in direct contact with horizontal attenuated total reflectance (ZnSe crystal, with an aperture angle of 45° and refractive index of 2.4 at 1000 cm^{-1}) at controlled ambient temperature (Smart ARK, with dimension of 1.0 x 6.0 cm). FT-IR spectra were collected at frequency regions of 4000 – 650 cm^{-1} by co-adding 32 scans and at resolution of 4 cm^{-1}. All spectra were substracted against a background of air spectrum. After every scan, a new reference air background spectrum was taken. These spectra were recorded as absorbance values at each data point in triplicate. The normal FT-IR spectra of samples were subjected to "mean centered" before being used for PLS and DA.

Chemometrics: Chemometrics analysis, including quantification using PLS and classification using DA of LD in cream cosmetics was carried out using the software TQ Analyst^{TM} (Thermo electron Corporation). PLS calibration was assessed using root mean standard error of calibration (RMSEC) and coefficient of determination (R^2). The PLS model was subsequently cross-validated using "leave-one-out" technique. In this technique, one of the calibration samples is left out from PLS calibration model and the remaining samples are used to make PLS model. Furthermore, the removed sample is calculated using the new developed PLS model. This procedure was repeated; leaving each calibration sample out in turn one sample [18]. Furthermore, root mean standard error of prediction (RMSEP) and R^2 values were used as the assessment criteria of PLS model to predict the level of LD in validation/prediction data sets.

RESULTS AND DISCUSSION

Spectra Analysis: Figure 1 shows FT-IR spectra of LD and VCO at mid infrared (IR) region (4,000 – 650 cm^{-1}). In this study, the selection of VCO was relied on its popularity as one of new components used in cream cosmetics, especially in Indonesia and Malaysia. Both spectra look very similar and appear typical characteristic of absorption bands for common edible oils. Each peak in FT-IR spectra corresponds to functional groups responsible for IR absorption [19]. The peak assignment together with functional groups responsible for peak absorption in Fig.1 is shown in Table 1.

Table 1: Functional groups responsible for peak absorption in FTIR spectra of LD and VCO[17,19]

Frequency	Functional groups and mode the vibration
(a) 3007 cm^{-1}	C-H vinylic (stretching)
(b) 2922	methylene (-CH$_2$-) group (stretching)
(c) 2852 cm^{-1}	methyl (-CH$_3$) group (stretching)
(d) 1740 cm^{-1}	carbonyl (C=O) of the triacylglycerol ester linkage with strong intensity due to the great difference in electronegativity properties between carbon and hydrogen atoms (stretching)
(e) 1465 cm^{-1}	methylene (-CH$_2$-) group (bending)
(f) 1375 cm^{-1}	methyl (-CH$_3$) group (bending)
(g)1235, (h) 1160, (i)1117, (j) 1098 and (l) 721 cm^{-1}	The overlapping of the methylene (rocking vibrations) and cis-disubstituted olefins (out of plane bending vibration)

Table 2: Fatty acid composition (%) and iodine value of LD and VCO

Fatty acid (%)	LD	VCO
C8:0	nd	7.81±0.36
C10:0	nd	6.08±0.31
C12:0	0.01±0.00	47.01±0.67
C14:0	1.30±0.03	18.45±0.47
C16:0	20.66±0.24	8.99±0.37
C16:1	1.98±0.01	0.02±0.00
C17:0	0.48±0.02	nd
C18:0	10.91±0.12	3.19±0.23
C18:1	39.13±0.09	6.23±0.28
C18:2	19.56±0.04	1.37±0.05
C18:3	1.21±0.06	0.10±0.00
C20:0	0.91±0.01	0.01±0.00
C20:1	0.96±0.04	nd
Iodine value(g iodine per 100 g oil)	6.90±0.12	53.09±0.67

The big differences of two FT-IR spectra are assigned with arrows in Fig. 1 at two frequencies, 3007 and 1098 cm^{-1}. A peak at 3007 cm^{-1} (a) is only present in LD. This is not surprising because LD has a higher iodine value (IV) than that of VCO (Table 2). The high IV indicates the higher content of unsaturated fatty acids, which in turn contributes to a high absorbance value in the region of *cis* C=CH stretching [19]. Compared with VCO, LD has more unsaturated fatty acid contents, especially oleic and linoleic acids (Table 1). Another peak used to distinguish LD from VCO can be observed at frequency region of 1117 – 1097 cm^{-1}, in which LD showed overlapping of two peaks of 1117 cm^{-1} (i) and 1098 cm^{-1} (j), while VCO shows only one absorption peak at 1117 cm^{-1}. The spectrum of LD obtained was in agreement with that reported by Syahariza *et al*. [8] and Jaswir *et al*. [20].

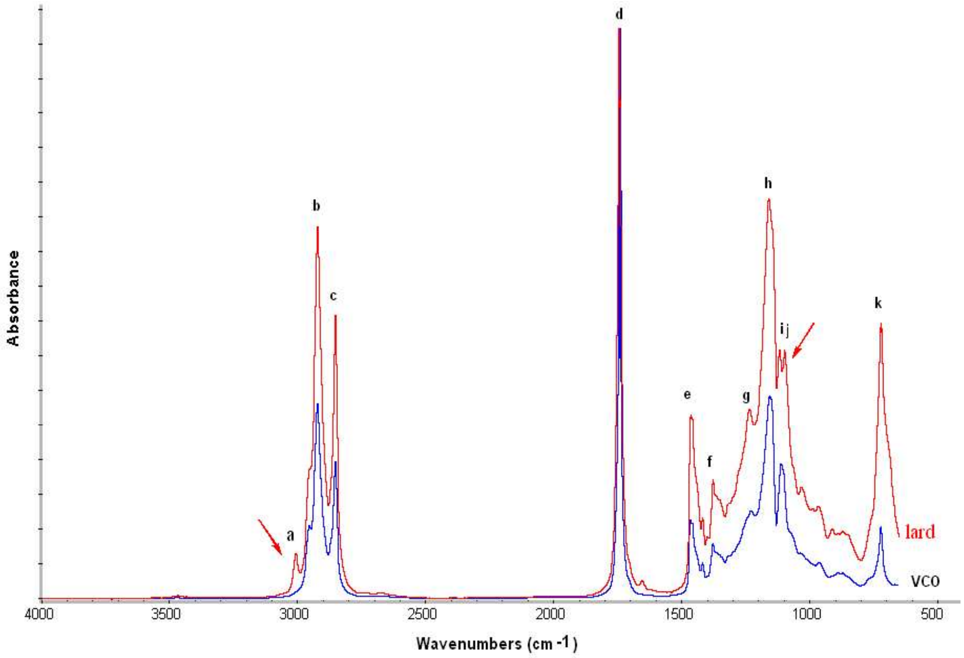


Fig. 1: FTIR spectra of pure lard and pure VCO. The assigned peak with arrow is absorption bands which are significant in differentiation.

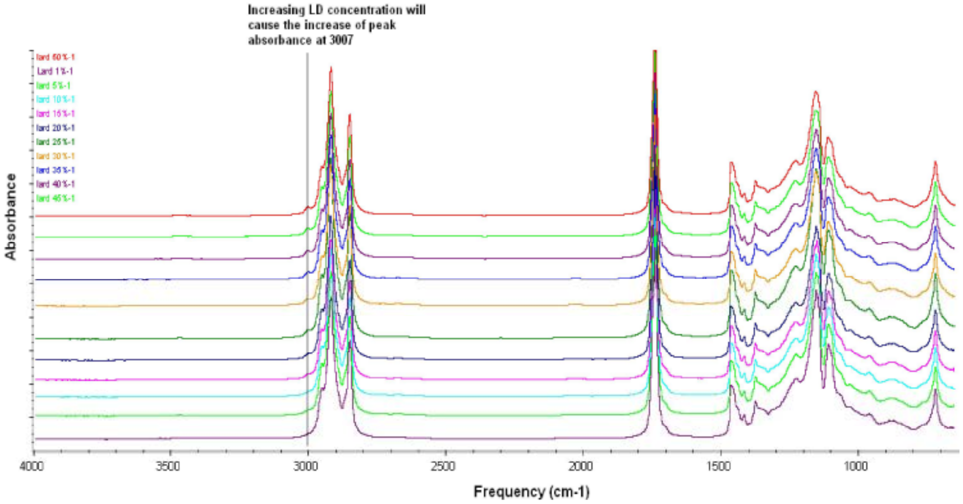


Fig. 2: The FTIR spectra with different concentration of LD and VCO used for making calibration model

Quantification of Lard in Cream Cosmetics: Quantification of LD in the mixture with VCO as oil base in cream cosmetics was performed using multivariate calibration of PLS at the combined frequency regions of 3020 – 2995 cm^{-1} and 1200 – 1000 cm^{-1}. These frequencies were chosen due to its ability to offer the higher value of R^2 and the lower value of RMSEC, compared with other frequency regions. The main advantage of PLS is due to its ability to develop the correlation between FT-IR spectra and an analyte of interest, even when no differences are visually observable in FT-IR spectral data [9]. PLS calibration model was developed based on the calibration standard that included the different weighted amounts of LD blended with VCO. The FT-IR spectra with different concentration of LD and VCO used for making calibration model was shown in Figure 2. Using 3 PCs, the relationship between actual value (*x-axis*) against FT-IR predicted value (*y-axis*) of LD is shown in Figure 3, with R^2 and RMSEC values of 0.997 and 0.808% (v/v), respectively.

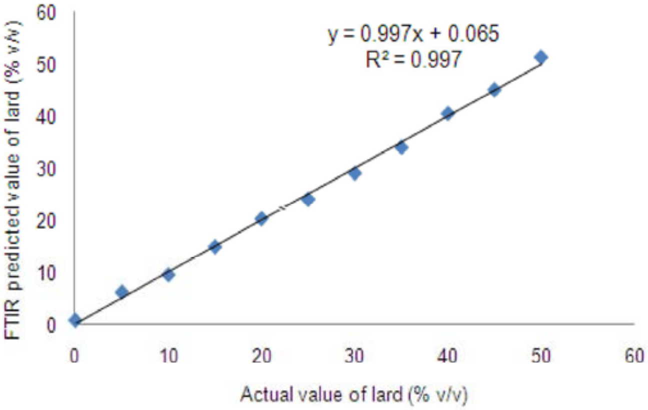


Fig. 3: The scatter plot for relationship between actual value of lard concentrations versus FTIR predicted concentration of lard (% v/v) using PLS calibration at frequency regions of 3020 - 3000 cm^{-1} and 1200 - 1000 cm^{-1}

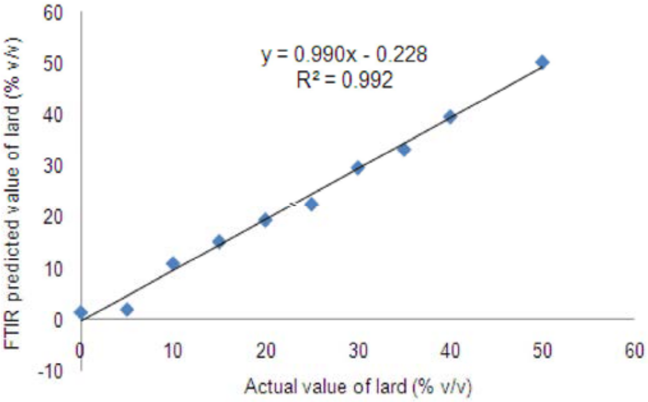


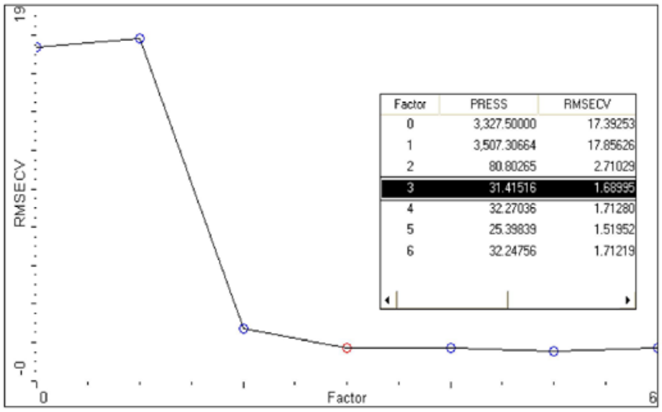
Fig. 4: The scatter plot for relationship between actual value of lard and FTIR predicted concentration of lard (% v/v) in validation model at frequency regions of 3020 - 3000 cm^{-1} and 1200 - 1000 cm^{-1}

PLS calibration model was further subjected to cross validation using "leave-one-out" technique. The root mean standard error of cross validation (RMSECV) obtained is relatively low (1.69% v/v). For the validation/prediction procedure, ten samples prepared in the laboratory were used to minimize the prediction error and to provide an estimate of the overall accuracy of predictions. Using the relationship between actual and FT-IR predicted values of LD content, a linier regression was obtained with R^2 and RMSEP values of 0.992 and 1.43%, as shown in Figure 4.

Verification of spectral regions used for making PLS calibration was carried out by calculating the predicted residual error sum of squares (PRESS) value for different principal components (PCs) or factors. PRESS value was calculated from the prediction errors in calibration standard samples by cross validation and subsequently plotted with the number of factors exploited in calibration model. As shown in Figure 5, the optimal number of factors is three, corresponding to point at which PRESS reaches minimum or begins to level off [21].

This result indicated that FT-IR spectroscopy combined with PLS offers fast and reliable techniques for quantitative measurements of LD in cream formulation; however, it has main disadvantage, i.e FT-IR spectroscopy can only be used for certain formulations. If the composition of the samples to be analyzed is different, the FT-IR spectra will be different; therefore, the calibration model should be developed if the sample matrix which contains LD is different.

Classification of Cream Cosmetics Containing Lard: Classification of cream cosmetics with and without LD in their formulation was performed using DA. DA can be used to determine the class of cream containing LD to that without LD by calculating the distance from each class center evaluated in the Mahalanobis distance units. The class of unknown samples to one of the specific classes can be predicted, after classification model is obtained [18].



Factor	PRESS	RMSECV
0	3,327.50000	17.39253
1	3,507.30664	17.85626
2	80.80265	2.71029
3	31.41516	1.68995
4	32.27036	1.71280
5	25.39839	1.51952
6	32.24756	1.71219

Fig. 5: A plot for the relationship between root mean standard error of cross validation (RMSECV) versus the number of factors for PLS calibration of lard.

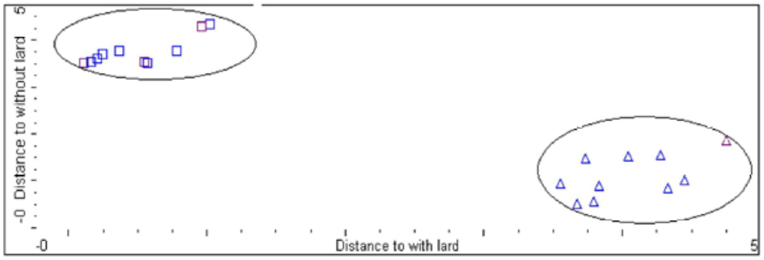


Fig. 6: The Coomans plot of cream cosmetics containing LD and that without lard in its formulations. (Δ): The Mahalanobis distance to cream samples "with LD" in its formulation; (☐) Mahalanobis distance to cream samples "without LD" using 9 PCs.

Frequency regions used for PLS calibration (3020 – 2995 cm^{-1} and 1200 – 1000 cm^{-1}) was exploited for DA. In the first study, a training set or standard cream with LD and that without LD was prepared. The Coomans plot for the classification of both classes is presented in Fig. 6 using 9 PCs. The plot in Fig. 6 shows graphically the Mahalanobis distance between cream with LD (*x*-axis) and cream without LD (*y*-axis) in their formulations. DA can accurately classify 100% of all evaluated samples according to its classes, meaning that there are no samples misclassified from their groups. Secondly, the developed model was further used to predict the classes of unknown (commercial) cream samples. Using this model, the evaluated commercial cream samples are in the class of cream without LD in formulation. However, if these commercial samples are spiked with certain amount of LD, its classes move to those containing LD in formulation.

CONCLUSIONS

It can be concluded that FT-IR spectroscopy using HATR and chemometrics of PLS and DA can be used to analyze the presence of LD in cream cosmetic formulations. The results can be extended to various types of topical cosmetic preparations using oils as a base in their formulations. The tedious time and chemical consuming reagents and solvents can be avoided, therefore this technique can be considered as "green analytical technique".

ACKNOWLEDGEMENT

This research was fully supported by project grant from Gadjah Mada University through Excellent research for National strategic (Riset Unggulan Strategis National, RUSNAS) with No. LPPM-UGM/1309/2009. The authors thanks to Halal Products Research Institute, UPM for FT-IR instrument facility.

REFERENCES

1. Hans, R.K., N. Agrawal, K. Verma, R.B. Misra, R.S. Ray and M. Farooq, 2008. Assessment of the phototoxic potential of cosmetic products. Food Chem. Tox., 46: 1653-1658.

2. Kroes, R., A.G. Renwick, V. Feron. C.L. Galli, M. Gibney, H. Greim, R.H. Guy, J.C. Lhuguenot and J.J.M. van de Sandt, 2008. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. Food Chem. Tox., 45: 2533-2562.
3. Kajimura, K., T. Tagami, T. Yamamoto and I. Iwagami, 2008. The release of formaldehyde upon decomposition of 2-bromo-2-nitropropan-1,3-diol (Broponol). J. Health Sci., 54: 488-492.
4. Food and Drug Administration, 2006, *Alphabetical List of SCOGS Substances*, Retrived from www.cfsan.fda.gov/~dms/opascogc. 15th October, 2009.
5. Regenstein, J.M., M.M. Chaudry and C.E. Regenstein, 2003. The kosher and halal food laws. Compr. Rev. Food Sci. Food S., 2: 111-127.
6. Blanco, M., M. Alcalá, J. Planells and M. Mulero, 2007. Quality control of cosmetic mixtures by NIR spectroscopy. Anal. Bioanal. Chem., 389: 1577-1583.
7. Yang, H., J. Irudayaraj and M.M. Paradkar, 2006. Discriminant analysis of edible oils and fats by FTIR, FT-NIR and FT-Raman spectroscopy. Food Chem., 93: 25-32.
8. Syahariza, Z.A., Y.B. Che Man, J. Selamat and J. Bakar, 2005. Detection of lard adulteration in cake formulation by Fourier transform infrared (FTIR) spectroscopy. Food Chem., 92: 365-370.
9. Che Man, Y.B., Z.A. Syahariza, M.E.S. Mirghani, S. Jinap and J. Bakar, 2005. Analysis of potential lard adulteration in chocolate and chocolate products using Fourier transform infrared spectroscopy. Food Chem., 90: 815-819.
10. Rohman, A. and Y.B. Che Man, 2009. FTIR spectroscopy combined with chemometrics for analysis of lard in the mixtures with body fats of lamb, cow and chicken. Int. Food Res. J., 17: 519-527.
11. Guillen, M.D. and N. Cabo, 1997. Characterization of edible oils and lard by fourier transform infrared spectroscopy. Relationships between composition and frequency of concrete bands in the fingerprint region. J. Am Oil Chem Soc., 74: 1281-1286.
12. Che Man, Y.B., A. Rohman and T.S.T. Mansor, 2010. Differentiation of lard from other edible oils by means of Fourier transform infrared spectroscopy and chemometrics. J. Am. Oil Chem. Soc. DOI: 10.1007/s11746-010-1659-x.
13. Watkinson, A.C., J. Hadgraft, K.A. Walters and K.R. Brain, 1994. Measurement of diffusional parameters in membranes using ATR-FTIR spectroscopy. Int. J. Cosmetic Sci., 16: 199-210.
14. Joy, M. and D.M. Lewis, 1991. The use of Fourier transform infra-red spectroscopy in the study of the surface chemistry of hair fibres. Int. J. Cosmetic Sci., 13: 249-261.
15. Rohman, A. and Y.B. Che Man, 2009. Analysis of Cod-liver oil adulteration using Fourier transform infrared (FTIR) spectroscopy. J. Am. Oil Chem. Soc., 86: 1149-1153.
16. AOCS. 1996. *Official and Tentative Methods*. 5th Edition. American Oil Chemists' Society, Champaign, USA.
17. Rohman, A. and Y.B. Che Man, 2009. Monitoring of virgin coconut oil (VCO) adulteration with palm oil using Fourier transform infrared (FTIR) spectroscopy. J. Food Lipids., 16: 618-628.
18. Miller, J.N. and J.C. Miller, 2005. *Statistics and chemometrics for analytical chemistry*. 5th ed. Pearson Education Limited, Edinburgh Gate Harlow, England., pp: 213-239.
19. Safar, M., D. Bertrand, P. Rober, M.F. Devaux and C. Genot, 1994. Characterization of edible oils, butter and margarines by Fourier transform infrared spectroscopy with attenuated total reflectance. J. Am. Oil Chem. Soc., 71: 371-377.
20. Che Man, Y.B. and G. Setiowaty, 1999. Multivariate calibration of Fourier transform infrared spectra in determining iodine value of palm oil products. Food Chem., 92: 365-371.
21. Jaswir, I., M.E.S. Mirghani, T.H. Hassan and M.Z. Mohd Said, 2003. Determination of lard in mixtures of body fats of mutton and cow by Fourier transform-Infra red (FT-IR) spectroscopy. J. Oleo Sci., 52: 633-638.
22. Sedman, J. F.R. Van de Voort and A.A. Ismail, 1997. Application of Fourier transform infrared spectroscopy in *New techniques and application in lipid analysis* edited by McDonald, R.E. and Mossoba, M.M. (pp. 283-324) Champaign, Illinois, USA: AOCS Press.