

Quantification of Lard in the Mixture with Olive Oil in Cream Cosmetics Based on FTIR Spectra and Chemometrics for Halal Authentication

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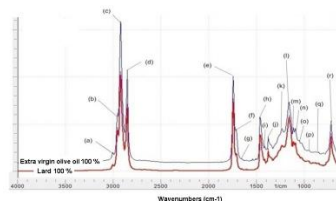
Article history

Received :5 October 2013

Received in revised form :
29 April 2014

Accepted :15 June 2014

Graphical abstract



Abstract

The presence of lard (LD) in cosmetics products is a serious matter for certain religion, like Islam. The Muslim community is not allowed to use cosmetics products containing pig derivatives such as LD. Therefore, analysis of LD in cosmetics products is highly needed. The present study highlighted the employment of Fourier transform infrared (FTIR) spectroscopy in combination with chemometrics of multivariate calibration and principle component analysis (PCA) for quantitative analysis and classification of LD in the binary mixture with extra virgin olive oil (EVOO) as oil base in cream formulations for halal authentication. The lipid component in cream was extracted using liquid-liquid extraction using hexane as extracting solvent, and the lipid obtained was subjected to FTIR spectra measurement, using horizontal attenuated total reflectance as sampling technique. The result showed that FTIR spectroscopy in combination with partial least squares can be used to quantify the levels of LD in the mixture with EVOO in cosmetics creams using the combined frequency regions of 1785-702 cm^{-1} and 3020-2808 cm^{-1} . PCA using absorbance intensities at 1200 – 1000 cm^{-1} as variables has been successfully used for the classification of cream with and without LD in the formulation. The developed method is rapid and not involving the excessive sample preparation.

Keywords: Lard; extra virgin olive oil; cream; FTIR spectroscopy; chemometrics

Abstrak

Kewujudan lemak babi (LD) dalam produk kosmetik adalah satu perkara yang serius untuk agama tertentu, seperti Islam. Masyarakat Islam tidak dibenarkan untuk menggunakan produk kosmetik yang mengandungi derivatif babi seperti LD. Oleh itu, analisis LD dalam produk kosmetik adalah sangat diperlukan. Kajian ini menekankan penglibatan *Fourier Transform Infrared* (FTIR) spektroskopi dengan kombinasi dengan *chemometrics* kalibrasi multivariasi dan analisis prinsip komponen (PCA) untuk analisis kuantitatif dan klasifikasi LD dalam campuran binari dengan tambahan minyak zaitun dara (EVOO) sebagai asas minyak dalam formulasi krim untuk pengesahan halal. Komponen lipid dalam krim telah diekstrak menggunakan pengekstrakan cecair-cecair dan juga menggunakan heksana sebagai ekstrak pelarut, dan lipid yang diperolehi adalah tertakluk kepada FTIR pengukuran spektrum, dengan menggunakan jumlah pantulan mendatar yang dikesilkan sebagai teknik persampelan. Hasilnya menunjukkan bahawa spektroskopi FTIR digabungkan dengan separa dua terkecil boleh digunakan untuk menilai tahap LD dengan campuran EVOO dalam krim kosmetik menggunakan gabungan frekuensi kawasan 1785-702 cm^{-1} dan 3020-2808 cm^{-1} . PCA menggunakan keamatan kuantiti di 1200 - 1000 cm^{-1} sebagai pembolehubah telah berjaya digunakan untuk klasifikasi krim dengan LD atau tanpa LD dalam formulasi. Kaedah yang dibangunkan ini adalah cepat dan tidak melibatkan penyediaan sampel yang berlebihan.

Kata kunci: Lemak babi; minyak zaitun dara tambahan; krim; Spektroskopi FTIR; chemometrics

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1.0 INTRODUCTION

The fats and oils such as lard (LD) and extra virgin olive oil (EVOO) have been used over the centuries in cosmetics and

pharmaceutical products [1]. As one of the cosmetics components, LD is widely used in topical preparation such as cream and lotions. In certain countries, LD is favorite components to be introduced as emulsifying system. Food and

Drug Administration (FDA) has categorized LD as “generally recognized as safe substances (GRAS)” to be used in food, pharmaceutical, and cosmetics products. In cream cosmetics, LD can function as increasing viscosity agent, emollient and occlusive, as well as emulsifying agent [2].

The presence of LD in cosmetics and pharmaceutical products is serious problems in view of religious concerns. Some religion like Islam, Hindus, and Jews prohibited their followers to use any cosmetics products containing LD in its formulation [3-4]. Islam classified lard as one of “non halal components”. Indeed, analytical methods capable of detecting lard in cosmetics products is highly required in order to assure the halalness of cosmetics products. Rohman and Che Man [5] have reviewed some physico-chemical techniques for analysis of lard in food-based systems. One of the highlighted techniques is Fourier transform infrared (FTIR) spectroscopy. However, the use of FTIR spectroscopy in cosmetics field especially for quantitative analysis of lipid components (fats and oils) is limited.

FTIR spectroscopy, especially in combination with powerful chemometrics software and horizontal attenuated total reflectance as sampling technique, offers rapid and relative sensitive techniques, minimum sample preparation, non destructive and can be used for qualitative and quantitative analysis [6-7]. Besides, FTIR spectroscopy allows analysis of fats and oils as a whole matter (whole spectra) rather than analyzing the specific class of fats and oils components [8]. FTIR spectroscopy was also ideal technique due to the minimum or absence of solvents and reagents used during analysis; therefore, this technique can be considered as green analytical method [9]. FTIR spectra are known as fingerprint profile due to its capability to differentiate all samples evaluated [10]. However, FTIR spectroscopy has the main drawback, i.e. the developed model using FTIR spectra data can only be applied to analyze lard in the certain mixture and certain formulation. If the fats/oils used as lard mixture are different, the model should be newly developed [11].

In cosmetics analysis, our group have used FTIR spectroscopy in combination with certain chemometrics of multivariate calibration, principle component analysis and discriminant analysis for quantification, discrimination and classification of virgin coconut oil in cream [12], lard in binary mixture with virgin coconut oil in cream cosmetics [13] and lard in lotion cosmetics [14]. Based on the limitation of FTIR spectroscopy as mentioned earlier, and the fact that there is no previous research regarding the application of FTIR spectroscopy for analysis of lard in the binary mixture with EVOO, the present study was directed to apply FTIR spectroscopy coupled with multivariate calibration and principle component analysis for analysis of lard in the mixture with EVOO in cream cosmetics.

2.0 MATERIALS AND METHODS

The preparation of lard (LD) was performed by rendering the adipose tissue of pig (*Sus scrofa*) obtained from various slaughter houses in Jogjakarta, Indonesia, as described in Rohman and Che Man [15]. Into Beaker glass, the adipose tissue was cut into small pieces using commercial cutter, and Beaker containing adipose tissue was introduced into conventional oven at 100 – 105°C for 3 h. The melted fat was strained through triple-folded muslin cloth, dried with anhydrous sodium sulphate, and centrifuged at 3,000 rpm for 20 min. The fat layer was decanted, shaken vigorously, and centrifuged again before being filtered using Whatman filter paper. The filtered fats were stored in tightly closed containers in the refrigerator until being used for

preparation of cream cosmetics. Extra virgin olive oil and other materials used were bought in Jogjakarta.

2.1 Preparation of Cream Formulation

The cream formulation was based on our previous paper [13-14] with slight modification. The prepared cosmetic creams (for each 50 g) consisted of lard (LD) or extra virgin olive oil (EVOO) as well as their mixtures (12.5 g); stearic acid (2.5 g); cetyl alcohol (2.2 g); propyl paraben (0.1 g), butylated hydroxytoluene (0.1 g); vitamin E (0.1 g); distilled water (30.0 g); disodium EDTA (0.5 g); glycerin (0.75 g); TiO₂ (0.1 g), perfume (0.25 g) and triethanolamine (0.9).

The cream was prepared by weighing each ingredient on the analytical balance having sensitivity of 0.1 mg. Cetyl alcohol, stearic acid, propyl paraben, lanolin, vitamin E and oils used (LD and or EVOO) were heated at about 70°C (oil phase). Triethanolamine together with water was heated at about 70°C (water phase). The water phase was poured into the oil phase and stirred with a magnetic stirrer until it reached ambient temperature (30 min). Perfume was added to the cream obtained and was further subjected to liquid-liquid extraction in order to extract the fat/oil from the cream formulation.

2.2 Preparation of Calibration and Validation Samples

Nine cream samples with certain concentrations of LD and EVOO were prepared in the laboratory as calibration samples. The weight percentage ratios of LD and EVOO used were as follows: F.1 (LD 0 g, EVOO 12.5 g); F.2 (LD 1.5 g, EVOO 11.0 g); F.3 (LD 3 g, EVOO 9.5 g); F.4 (LD 4.5 g, EVOO 8.0 g); F.5 (LD 6.0 g, EVOO 6.5 g); F.6 (LD 7.5 g, EVOO 5.0 g); F.7 (LD 9.0 g, EVOO 3.5 g); F.8 (LD 11.0 g, EVOO 1.5 g); F.9 (LD 12.5 g, EVOO 0 g). F = formula.

Another series of nine cream cosmetics, which were different from the calibration samples, was also made independently in our laboratory as prediction or validation samples. LD, EVOO as well as their mixtures in cream formulations were extracted, evaporated, and analyzed using an FTIR spectrophotometer. The spectral regions where the variations among them were observed were more preferred for developing partial least square (PLS) and principal component analysis (PCA) models.

2.3 The Extraction of Lipid Component in Cream Samples

The lipid components (LD, EVOO and other non-polar components) was extracted from cream samples using liquid-liquid extraction with hexane as extracting solvents, according to our previous paper [14]. Briefly, to an approximately of 10 g cream samples were added 5 mL HCl 37% and 20 mL of water, and then shaken vigorously. The filtrate was transferred to a separatory funnel and extracted using 2 x 15 mL of hexane. The hexane extracts were combined and drained into a 250-mL round-bottom flask and evaporated using a vacuum rotary evaporator at 40°C, until hexane was completely removed. The FTIR spectra of lipid extracts obtained were further measured using an FTIR spectrophotometer.

2.4 FTIR Spectra Acquisition

FTIR spectra of extracted lipid component from cream samples were scanned using a FTIR spectrophotometer ABB MB3000 (Clairet Scientific, Northampton, UK) equipped with a DTGS detector and beam splitter composed of KBr using a resolution of

4 cm^{-1} , number of scans 32 co-adding in the frequency region of 400–4,000 cm^{-1} . Spectra were acquired using Horizon MB FTIR software version 3.0.13.1 (ABB, Canada). The samples were placed in good contact with horizontal attenuated total reflectance (HATR) element (ZnSe crystal) at a controlled ambient temperature (20°C). All spectra were ratioed against a background of an 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 3 replicates.

2.5 Data Analysis

Quantitative analysis of lard in cream cosmetics was performed with partial least square (PLS) model, with the aid of Horizon MB FTIR software version 3.0.13.1 (ABB, Canada). The values of the root mean square error of calibration (RMSEC) and coefficient of determination (R^2) were used as the validity criteria for the calibration model. The predictive ability of PLS calibration model was further used to calculate the validation or prediction samples. The parameters used for prediction model are root mean square error of prediction (RMSEP) and R^2 for the relationship between actual and FTIR predicted value of LD in cream cosmetics. Furthermore, principal component analysis was evaluated with the aid of Minitab software version 16 (State College PA, USA).

3.0 RESULTS AND DISCUSSION

3.1 FTIR Spectra Analysis

Mid infrared (MIR) spectroscopy is rapid and non destructive technique. This technique was employed to analyze LD as one of

lipid components in cream cosmetics. The importance of IR spectroscopy in the elucidation of molecular structures comes from the much information content obtained, and the possibility to assign certain absorption bands related to functional groups [16]. Figure 1 is FTIR spectra of lard (LD) and extra virgin olive oil (EVOO) obtained from lipid extraction of cream containing 100 % LD or 100 % EVOO. Each peaks and shoulder indicated functional groups responsible for infrared absorption at wavenumbers of 4000 – 400 cm^{-1} , corresponding to stretching and bending vibrations of functional groups. Peak assigned with (a) at 3007 cm^{-1} attributed from the stretching vibration of *cis* C=H [14]. The assignment of other peaks was compiled in Table 1.

Both spectra of lard and EVOO look very similar, however, they revealed some differences in peak intensities and the exact wavenumbers at which the maximum absorbance were observed in LD and EVOO, due to the different nature and composition of both LD and EVOO, especially at wavenumber regions of 3,007 (a), 1160 (l), 1,117 (m) and 1,098 cm^{-1} (n). The wavenumber 3,007 cm^{-1} was coming from *cis*-olefinic C=H, which is can be used as an indicative of unsaturation degree. The more unsaturation degree of fats and oils, the higher the peak intensities in that wavenumber. Meanwhile, wavenumbers of 1160, 1,117 and 1,098 cm^{-1} originate from the stretching vibrations of the ester linkage in triacylglycerols. Fatty acid composition exhibited that EVOO contained more unsaturated fatty acid especially oleic acid than that in LD [17]. As a consequence, EVOO revealed higher peak intensity than LD at 3007 cm^{-1} [18]. In addition, LD has more saturated fatty acid than EVOO.

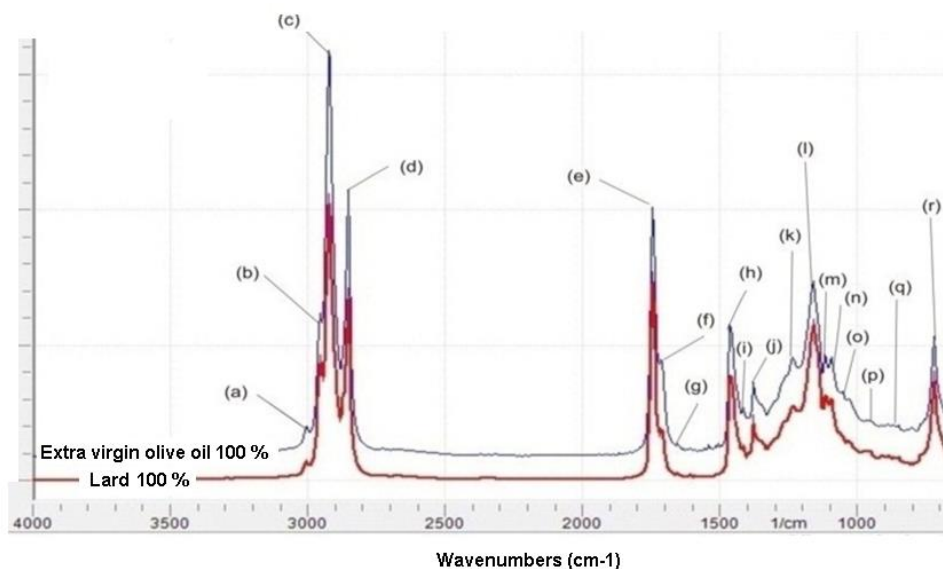


Figure 1 FTIR spectra of lard and extra virgin olive oil obtained from lipid extraction of cream containing 100 % lard or 100 % EVOO

FTIR spectra of EVOO and LD show two overlapping peaks with maximum intensities at 1,117 cm^{-1} (m) and 1,098 cm^{-1} (n), respectively. Both peak intensities were indicated to be inversely related with the proportion of acyl groups and oleic acyl groups, respectively [19]. LD had an approximately equal proportion of stearic and oleic acids which were reflected in LD spectra, in which peaks of 1,117 (m) and 1,098 cm^{-1} (n) appeared, as having

the same intensity. Inversely, EVOO has a higher concentration of oleic groups than saturated group, exhibited unequal heights of peaks at 1,117 (m) and 1,098 cm^{-1} (n). These wavenumbers,

having different intensities for both EVOO and lard, were subsequently optimized for analysis of LD in cream cosmetics.

3.2 Quantitative Analysis of Lard in Cream Cosmetics

LD mixed with EVOO as the oil base in cosmetic creams was quantified with the aid of partial least square (PLS) calibration at the combined frequency region of 1785-702 cm^{-1} and 3020-2808 cm^{-1} . These frequency regions were selected for the reason that those frequencies were capable of providing the higher value of

R^2 and the lower value of RMSEC, compared with other frequency regions (namely 1785-702 cm^{-1} , 3020-2808 cm^{-1} and the combined frequency regions 1785-702 cm^{-1} and 3020-2808 cm^{-1}). Figure 2 exhibited the relationship between actual values (x-axis) against FTIR predicted values (y-axis) of LD in the mixture with EVOO in cream cosmetics with R^2 of 0.9959. Using residual analysis, as shown in Figure 3, it can be stated that there is no systematic error during analysis of LD in cream using PLS calibration.

Table 1 Functional groups responsible for IR absorption of lard and extra virgin olive oil [19]

Assignment	Frequency (cm^{-1})	Functional group vibration
(a)	3005	<i>cis</i> C=CH stretching
(b)	2954	Asymmetric stretching vibration of methyl (-CH ₃) group
(c) and (d)	2924 and 2852	Asymmetric and symmetric stretching vibration of methylene (-CH ₂) group
(e) and (f)	1743 and 1715	Carbonyl (C=O) from the ester linkage of triacylglycerol
(g)	1654	<i>cis</i> C=C
(h)	1465	Bending vibrations of the CH ₂ and CH ₃ aliphatic groups
(i)	1417	Rocking vibrations of CH bonds of <i>cis</i> -disubstituted alkenes
(j)	1377	Symmetric bending vibrations of CH ₃ groups
(k) and (l)	1228, and 1160	vibrations of stretching mode from the C-O group in esters
(m) and (n)	1111 and 1097	-CH bending and -CH deformation vibrations of fatty acids
(o)	1033	C-O stretching
(p)	962	bending vibration of CH functional groups of isolated <i>trans</i> -olefin
(q)	914	Bending vibration of <i>cis</i> -HC=CH-
(r)	721	Overlapping of the methylene (-CH ₂) rocking vibration and to the out of plane vibration of <i>cis</i> -disubstituted olefins

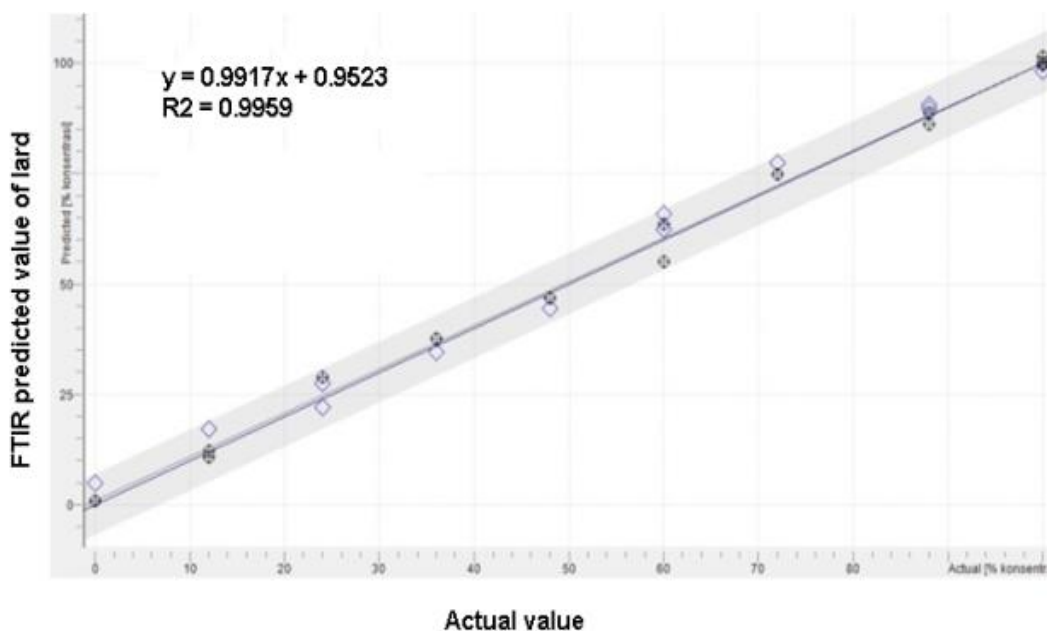


Figure 2 The PLS calibration model for the relationship between actual value (x-axis) against FTIR predicted value (y-axis) of lard in the mixture with EVOO in cream cosmetics

The PLS calibration model was further used to predict the independent samples, prepared separately from calibration samples. To evaluate the predictive capability of PLS model, the values of R^2 and root mean square error of prediction (RMSEP) were used. Figure 4 showed the relationship between actual and FTIR predicted values of LD having R^2 value of 0.991 and RMSEP value of 3.61%. This result indicated that PLS

calibration model was accurate enough for predicting cream samples containing LD and EVOO in its formulation.

3.3 Classification of Creams with and Without Lard

Creams with LD and without LD were classified using chemometrics of principal component analysis (PCA). The

frequency regions for PCA were also optimized. Finally, the wavenumbers of $1200 - 1000 \text{ cm}^{-1}$ was chosen due to its capability to provide good separation among evaluated samples. Figure 5 demonstrates the score plot of PCA of LD and EVOO cream cosmetics representing the projection of samples defined by the first component (PC 1), the second component (PC 2) and

the third component. Using this projection, cream containing LD, EVOO and commercial samples are well separated, meaning that PCA can accomplish the classification among them. Based on this profile, it can be stated that commercial samples (region B) do not contain LD in its formulation.

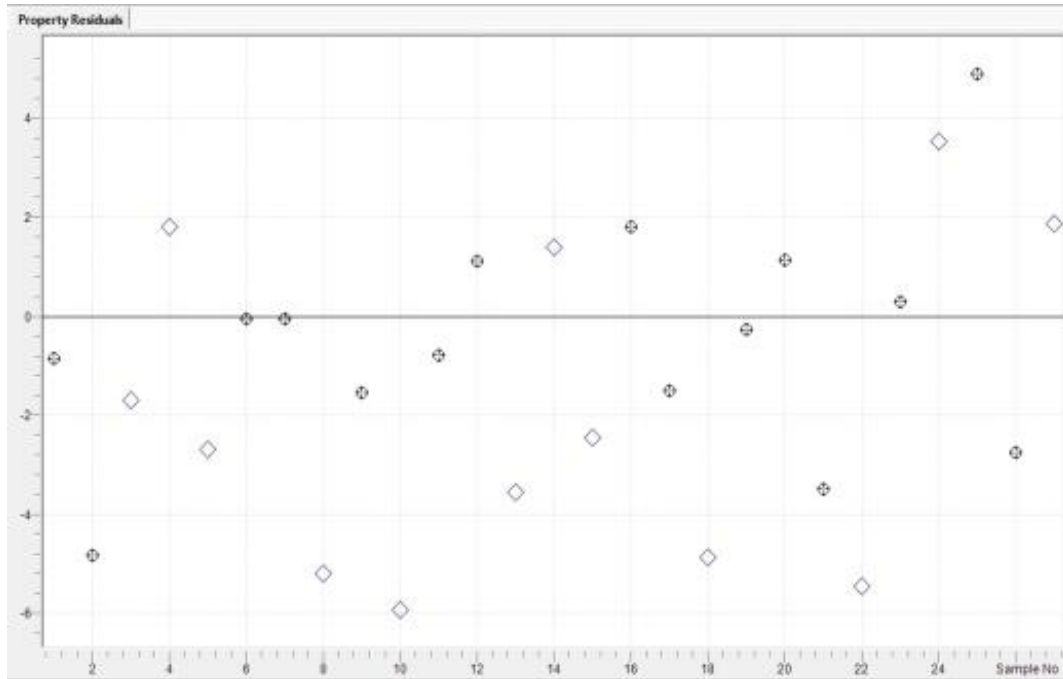


Figure 3 The residual properties for the difference between actual value and FTIR predicted value of lard in the mixture with EVOO in cream cosmetics

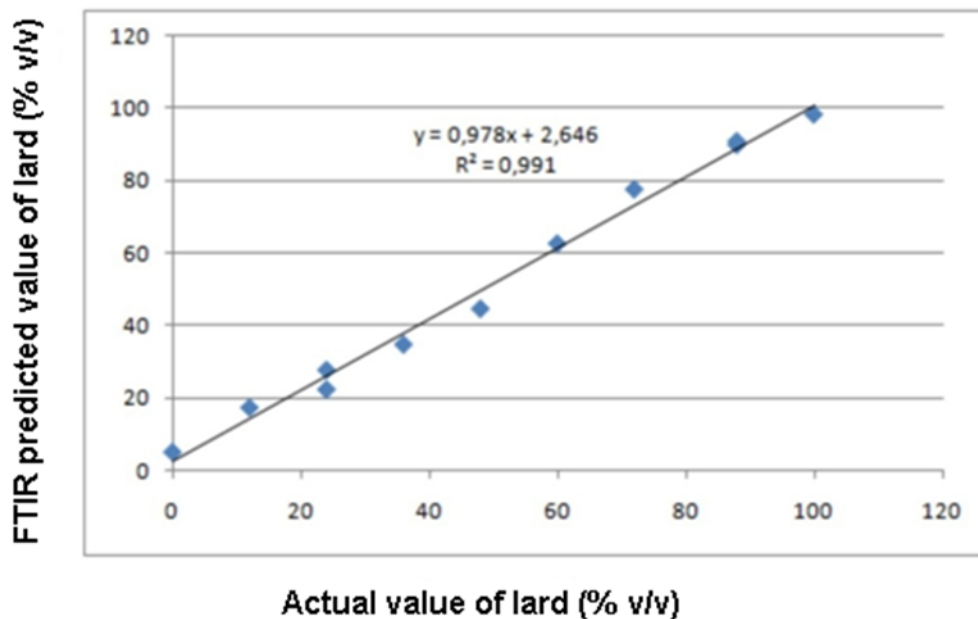


Figure 4 The PLS prediction model for the relationship between actual value (x-axis) against FTIR predicted value (y-axis) of lard in the mixture with EVOO in cream cosmetics

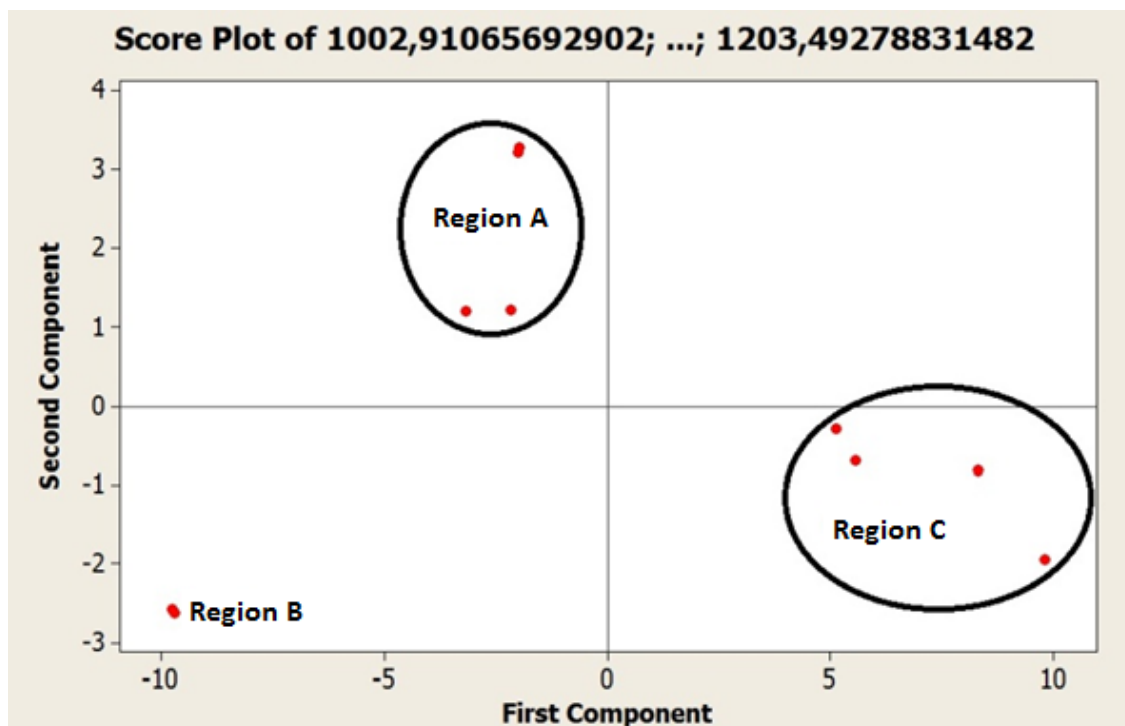


Figure 5 PCA score plot, expressed as first principal component (PC1) and second principal component (PC2) for classification of cream with lard (A), cream with EVOO (C) and commercial samples (B)

4.0 CONCLUSION

FTIR spectroscopy in combination with partial least squares (PLS) can be used to quantify the levels of LD in the mixture with EVOO in cosmetics creams using the combined frequency regions of $1785\text{--}702\text{ cm}^{-1}$ and $3020\text{--}2808\text{ cm}^{-1}$. The pattern of PCA score plot using absorbance intensities at $1200\text{--}1000\text{ cm}^{-1}$ as variables has successfully used for the classification of cream with and without LD in the formulation

Acknowledgements

The Directorate of Higher Education, the Ministry of Education and Culture was acknowledged for the financial support during this research via Riset Unggulan Strategis Nasional.

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