SHORT COMMUNICATION

QUANTITATIVE ANALYSIS OF VIRGIN COCONUT OIL IN CREAM COSMETICS PREPARATIONS USING FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

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ABSTRACT

Today, virgin coconut oil (VCO) is becoming valuable oil and is receiving an attractive topic for researchers because of its several biological activities. In cosmetics industry, VCO is excellent material which functions as a skin moisturizer and softener. Therefore, it is important to develop a quantitative analytical method offering a fast and reliable technique. Fourier transform infrared (FTIR) spectroscopy with sample handling technique of attenuated total reflectance (ATR) can be successfully used to analyze VCO quantitatively in cream cosmetic preparations. A multivariate analysis using calibration of partial least square (PLS) model revealed the good relationship between actual value and FTIR-predicted value of VCO with coefficient of determination (R^2) of 0.998.

Keywords: Virgin coconut oil (VCO), FTIR spectroscopy, partial least square, cosmetics.

INTRODUCTION

VCO is a vegetable oil which originates from fresh coconuts. It can be extracted straightforwardly from coconut under ambient temperature, therefore the loss of minor components, such as pro-vitamin A, tocopherol, and phenolics compounds due to solar UV irradiation during coconut drying, can be avoided. VCO may have more beneficial effects than copra oil, since it retains most of the unsaponifiable components (Nevin and Rajamohan, 2008).

VCO has shown the biological effects such as antiviral and antibacterial activities. Those effects may be attributed from large amount of short-chain fatty acids, like capric acid (C-10: 0), caproic acid (C-6: 0), and caprylic acid (C-8: 0) (Villarino *et al*., 2007). Nevin and Rajamohan (2004) has reported that VCO can lower the levels of triglycerides, phospholipids, total cholesterol, low density level (LDL), and very low density lipoprotein (VLDL) cholesterol. VCO also increased high density level (HDL) of cholesterol in tissues and serum. Phenolics contents of VCO were able to prevent LDL oxidation *in vitro* with formation of reduced carbonyl.

Cosmetics preparations typically consist of mixture of several components of natural origin or their derivatives. Their analysis is made rather difficult by their usually high complexity. In cosmetics preparations, VCO is excellent material used as softener and skin moisturizer. An investigation has also revealed that VCO can be used effectively and safely like mineral oil with no adverse effect reactions (Agero and Verallo-Rowell, 2004).

Infrared spectroscopy is a technique that can be easily adapted for use by untrained personnel in laboratories or on factory floor. A particular attraction is the easy of sample presentation (Marigheto *et al*., 1998). Fourier transform infrared (FTIR) spectroscopy is also a fast and non-destructive technique, sensitive, and free of chemical preparation (Wang and Paliwal, 2007; Xing *et al*., 2007).

Currently, FTIR spectroscopy has gained a special attention as a reliable technique for fat and oil analysis, due to its finger print technique. For example, FTIR can be successfully used for peroxide value (PV) determination in edible oils based on the reaction between hydroperoxides and triphenylphosphine stoichiometrically resulting of triphenylphosphine oxide (Ma *et al*., 1997; van de Voort *et al*., 1994) and using disposable polyethylene infrared cards (3M IRcards) (Ma *et al*., 1998); investigation for the simultaneous monitoring of aldehyde formation and the determination of anisidine value (Dubois *et al*., 1996); determining of iodine and saponification values of neat fats and oils (Li *et al*., 1999; Sedman *et al*., 2000).

FTIR combined with chemometrics of principal

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component analysis has been used for analysis of adulteration of VCO with palm kernel olein (Manaf *et al*., 2007). However, there are no research reports which are available to analyze VCO quantitatively in cosmetics preparations. Therefore, in this study, we attempt to develop FTIR to be used as a fast analytical technique and friendly for environment for quantitative analysis of VCO in cream cosmetics preparations.

MATERIALS AND METHODS

VCO, stearic acid, lanolin, cetyl alcohol, and triethanolamin were obtained from local market in Jogjakarta. Lard was prepared according to Che Man *et al*. (2005).

Preparation of cream cosmetics formulation

The cosmetic mixture studied consisted of stearic acid (3.0 % wt/wt); VCO or lard or their blends (50.0 wt/wt); lanolin (4.0 % wt/wt); distilled water (39.5 % wt/wt); cetyl alcohol (1.0 % wt/wt); and triethanolamine (2.5 % w/w).

Cream formulation was prepared by weighing each ingredient on an analytical balance. Triethanolamine together with water was heated at about 70°C (water phase). Cetyl alcohol, stearic acid, lanolin, lard/VCO/or their 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).

Calibration standard and validation sets

To make a calibration curve, VCO was mixed with the other components used in cream cosmetics preparation at concentration range of 0.0-50.0 % (wt/wt). A set number of laboratory prepared cosmetic samples containing all components in cosmetics mixture studied with certain VCO concentrations were also prepared for validation sets.

Fat extraction

Representative samples of cream formulation (25 g) were added with 2 ml of concentrated HCl and 18 ml of water and shaked 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 grade of solvents used is pro analysis (p.a).

Instrumental analysis

FTIR spectra of all samples were acquired on the software Nicolet Omnic (version 7.0, Thermo Nicolet). A Thermo Nicolet 6700 instrument (Thermo Nicolet Corp., Madison, WI), equipped with a multibounce plate ZnSe attenuated total reflectance (ATR) crystal, detector of deuterated triglycine sulfate (DTGS), and a germanium on potassium bromide substrate beam splitter, was used to take measurement of oil sample spectra. Samples were located on a crystal of Zink Selenium (ZnSe) ATR at controlled ambient temperature. Analyses were made at resolution of 4 cm^{-1} and 64 scanning. Subsequently, after being used for spectra measurement, the plate was cautiously cleaned with hexane twice and acetone until there is no oil sample residue and dried with tissue before measurement of the next sample. To avoid any spectral variation from day to day to a minimum, an air background spectrum was collected before each sample spectrum. The instruments were maintained at constant humidity to minimize water vapor interference. Sample spectra were recorded at mid infra red region, i.e. from 4,000 cm^{-1} to 650 cm^{-1}. All measurements were performed triplicate.

Statistical Analysis

Chemometrics analysis of partial least square was carried out using software TQ Analyst version 6.0 obtained from Thermo Nicolet, Madison, WI. PLS was used to develop a calibration model as well as a validation model. Frequencies which are specific for VCO spectrum was chosen to construct PLS calibration. The correlation between actual value and FTIR-predicted value was done with aid of software of Microsoft Excel 2007.

Validation

The "leave-one-out" cross-validation technique was used to verify the calibration model. The values of root mean standard error of cross validation (RMSECV) and R^2 were used for assessment of method accuracy. The predicted residual error sum of squares (PRESS) values were calculated from the errors in prediction from the standards and plotted as a function of the factor numbers used in PLS calibration. Further verification was obtained by calculating the mean difference (MD) and standard deviation of difference (SDD) between the actual data and FTIR-predicted values for method accuracy and precision, respectively (Mirghani *et al*., 2002).

RESULTS AND DISCUSSION

Spectra Analysis of VCO

The spectrum of VCO and other components (mainly lard) used as a cosmetics preparation model was shown in fig. 1. Both of spectra were recorded at frequency 4000 – 650 cm^{-1}. VCO spectrum showed a typical characteristic of absorption bands for common oils (Safar *et al*., 1994). The assigned peaks with **a** at frequency 962 cm^{-1} and **b** at 721 cm^{-1} were used as a marker to differentiate VCO from other components used in cosmetics samples.

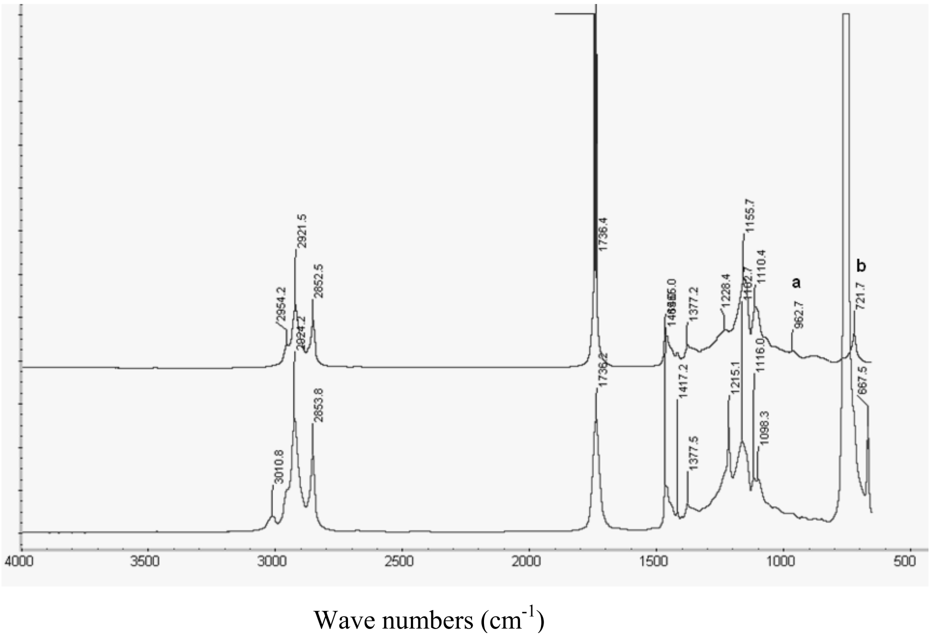

Fig. 1: FTIR spectra of pure VCO and the other components used as cream cosmetics model studied. The assigned peak with **a** and **b** in VCO are absorption bands which are significant in differentiation.

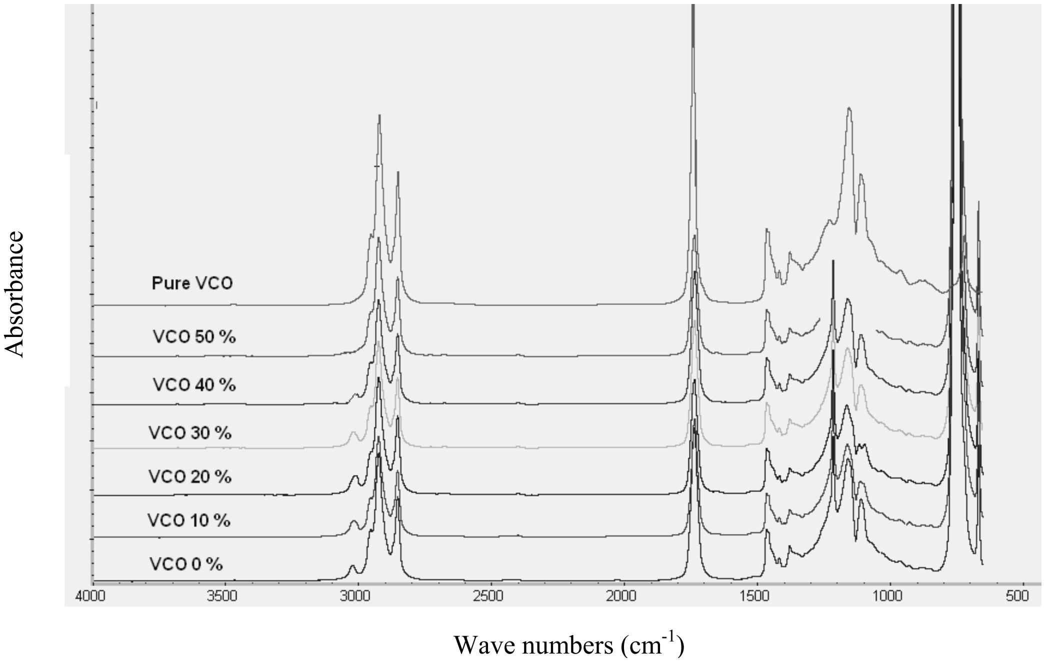

Fig. 2: FTIR spectra of pure VCO and the other components used in cream cosmetics preparation with concentration range from 0-50 % of VCO showing changes in absorbance due to the increase of % VCO in regions 924-930 cm^{-1} and 725-730 cm^{-1}.

The functional groups responsible for absorption at each frequencies of VCO spectrum are as follows: the asymmetrical stretching vibration of methyl (-CH$_3$) causes a shoulder at wavenumber of 2954 cm^{-1}, whereas the symmetrical vibration of methyl band is not observed. Either asymmetrical or symmetrical stretching vibration of methylene band was observed at approximately 2924 cm^{-1} and near 2852 cm^{-1}, respectively (Guillen and Cabo, 1997).

The absorption of carbonyl (C=O) group from ester linkage of triacylglycerol of VCO can be seen at frequency 1732 cm^{-1} with strong intensity due to the great difference of electronegativity between carbon and hydrogen atoms (Pavia *et al*., 2001).

Absorption with medium intensity at 1465 cm^{-1} is due to the methylene (-CH_2) bending vibration. An absorption band at 1417 cm^{-1} is attributed from rocking vibrations of CH bonds of alkenes with *cis*-disubstituted. The vibration of CH_3-groups on symmetrical bending mode can be seen at wavenumber 1377 cm^{-1}. The bands at 1228, 1155, and 1109 cm^{-1} are attributed from the vibrations of stretching mode from the C-O group in esters (Silverstein and Webster, 1996). An absorption band at frequency of 962 cm^{-1} is related to bending vibration of *cis* disubstituted olefinic groups. Finally, absorption peak at frequency 721 cm^{-1} is resulted from the rocking vibrations of methylene group which overlaps with bending vibration on out of plane mode of alkenes with *cis*-disubstituted (Guillen and Cabo, 1997). The overlay of combined spectra of mixture of VCO and the other componets used in this study ranging from 0 % to 50 % VCO is illustrated in fig. 2.

Quantitative analysis of VCO using FTIR

The development of reliable Instrumentation of FTIR spectroscopy has been continuously developed and enhanced, and equipped with software package having capability to perform processing and analyzing of spectra. This lead to performance of FTIR spectrometer as an analytical instrument for qualitative and quantitative analyses of samples (Sherman, 1997).

Frequency ranges which showed a great difference between VCO and the other components, used as cream cosmetics model studied i.e. 924 – 930 cm^{-1} and 725-730 cm^{-1}, are proposed for making calibration and validation models in quantitative analysis purpose, using chemometrics of partial least square (PLS) calibration. PLS is a form of factor analyis where the spectra and concentration of samples are incorporated into the model in one step process (Smith, 2002). PLS having an ability to use information of sample spectra from wide spectral frequencies and then correlate between spectral absorption change and its concentration of analytes whilst concurrently computing other spectra which may disturb analyte spectra (Che Man *et al*., 2005).

In the calibration stage, the spectra of calibration sets containing VCO in the range 0.0 – 50.0 % (wt/wt) are collected. Fig. 3 showed a plot between actual value of VCO and FTIR predicted value using type of none baseline. An equation of linier regression obtained was $y = 0.998x + 0.047$. The difference values of actual VCO and FTIR predicted VCO produced from the calibration were extremely small with coefficient of determination (R^2) value of 0.998 and the root mean standard error of calibration (RMSEC) of 0.826, indicating that FTIR method is accurate enough for VCO analysis. "Leave-one-out" technique was used for cross validation by removing one spectrum standard at once time. The final value (R^2) of 0.986 and RMSEC value of 1.04 was achieved (fig. 4). Verification and justification of spectra regions used for constructing a PLS calibration model were performed by calculating PRESS value for different principal component (PC) value (Manaf *et al*., 2007). 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 2 as revealed in fig 5, that express RMSEC obtains a stable value, minimally after two-factor.

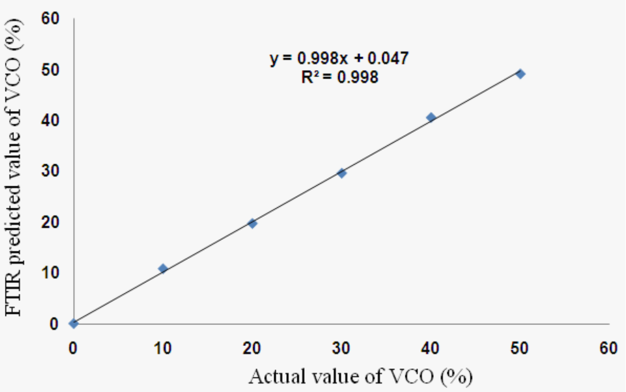

Fig. 3: A plot of actual VCO concentrations versus FTIR predicted concentration of VCO (%) using PLS at frequencies of 924-930 cm^{-1} and 725-730 cm^{-1}.

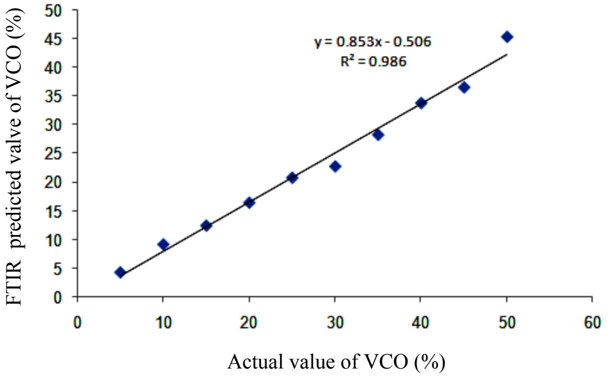

Fig. 4: A plot of actual VCO concentrations in the blend with the other components of cream cosmetics preparations using PLS and spectral data at frequencies of 924-930 cm^{-1} and 725-730 cm^{-1}.

Table 1 compares the values MD and SDD from actual values and the predicted FTIR values of VCO content in cream cosmetics formulations for method accuracy (*a*) and method precision in term of repeatability (*r*), respectively. Accuracy is a measure of how far away a predicted FTIR result of VCO concentration from actual value of VCO. The value MD*a* and SDD*a* is 2.130 and 1.634, respectively; which means that FTIR spectroscopy is an accurate method for quantification of VCO content in modeled cosmetics preparations. Furthermore, precision is a measure of the size of random error. The small values of MD*r* and SDD*r* (2.074, 1.063), respectively, showed that the FTIR method has good method precision. The relative standard deviation (RSD) obtained from the PLS FTIR predicted data and actual data was 6.41%. It can be stated that a RSD lower than 20 % is sufficient for purposes of quantitative analyses.

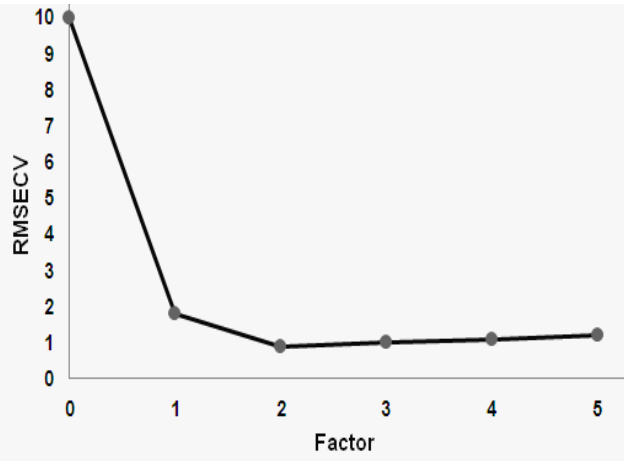

Fig. 5: Root mean standard error of calibration (RMSEC) of cross validation versus principal component (PC) factor.

Table 1: Statistical parameters of FTIR predicted values of VCO content in "leave-one out" cross validation

Statistics	Actual data	PLS FTIR
MD*accuracy*	2.130	
SDD*accuracy*	1.634	
MD*repeatibility*	-	2.074
SDD*repeatibility*	-	1.603

CONCLUSIONS

FTIR spectroscopy combined with chemometrics of PLS regression can be used as an analytical technique for quantification of VCO content in cream cosmetics formulation. It is a possible alternative for fast, reliable, and inexpensive for routine determination at about 3 min.

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REFERENCES

Agero AL and Verallo-Rowell VM (2004). A randomized double-blind controlled trial comparing extra virgin coconut oil with mineral oil as a moisturizer for mild to moderate xerosis. *Dermatitis*, **15**: 109-116.

Che Man YB, Syahariza ZA, Mirghani MES, Jinap S and Bakar J (2005). Analysis of potential lard adulteration in chocolate and chocolate products using Fourier Transform infrared spectroscopy. *Food Chem.*, **90**: 815-819.

Dubois I, van de Voort FR, Sedman I, Ismail AA and Ramaswamy HR (1996). Quantitative Fourier Transform Infrared analysis for anisidine value and aldehydes in thermally stressed oils, *J. Am. Oil Chem. Soc.,* **73**: 787-794.

Guillen MD and Cabo N (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.

Li H, van de Voort FR, Ismail AA and Sedman J (1999). Rapid determination of *cis* and *trans* content, iodine value, and saponification number of edible oils by Fourier Transform Near-Infrared Spectroscopy, *J. Am. Oil Chem. Soc.*, **76**: 491- 497.

Manaf MA, Che Man YB, Hamid NSA, Ismail A and Syahariza ZA (2007). Analysis of adulteration of virgin coconut oil by palm kernel olein using Fourier Transform Infrared spectroscopy. *J. Food Lipids.* **14**: 111-121.

Ma K, van de Voort FR, Sedman J and Ismail AA (1997). Stoichiometric determination of hydroperoxides in fats and oils by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.*, **74**: 897-906.

Ma K, van de Voort FR, Sedman J and Ismail AA (1998). Quantitative determination of hydroperoxides by Fourier Transform Infrared Spectroscopy with a disposable Infrared card, *J. Am. Oil Chem. Soc.*, **75**: 1095-1101.

Marigheto NA, Kemsley EK, Defernez M and Wilson RH (1998). A comparison of mid-infrared and raman spectroscopies for the authentication of edible oils, *J. Am. Oil Chem. Soc.*, **75**: 987-992.

Mirgani MES, Che Man YB, Jinap S, Baharin BS and bakar J (2002) FTIR spectroscopic determination of soap in refined vegetable oils, *J. Am. Oil Chem. Soc.*, **79**: 111-116.

Nevin KG and Rajamohan T (2004). Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. *Clin. Biochem*., **37**: 830-835.

Nevin KG and Rajamohan T (2008). Influence of virgin coconut oil on blood coagulation factors, lipid levels and LDL oxidation in cholesterol fed SpragueeDawley rats. *The European e-J. Clin. Nutr. and Metabolism*, **3**: e1-e8.

Pavia LD, Lampman GM and Kriz GS (2001). Infrared spectroscopy. In *Introduction to Spectroscopy*, 3rd Ed., Thomson Learning, Victoria, Australia, pp.13-101.

Safar M, Bertrand D, Rober P, Devaux MF and Genot C (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.

Sedman I, van de Voort FR and Ismail AA (2000). Simultaneous determination of iodine value and *trans* content of fats and oils by single-bounce horizontal attenuated total reflectance Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.*, **77**: 397-403.

Sherman CP (1997). Infrared spectroscopy *in Handbook of Instrumental Techniques for Analytical Chemistry*, edited by Settle F, Prentice Hall PTR, New Jersey, USA, pp.247-282.

Silverstein RM and Webster FX (1996). Spectrometric Identification of Organic Compounds, 6th Ed., John Wiley & Sons, New York, pp.71-111.

Smith BC (2002). Quantitative spectroscopy: Theory and Practice, Academic Press, Amsterdam, pp.125-179.

van de Voort FR, Ismail AA, Sedman J, Dubois J and Nicodemo T (1994). The determination of peroxide value by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.*, **71**: 921-927.

Villarino BJ, Marsha DL, Concepcion M and Lizada C (2007). Descriptive sensory evaluation of virgin coconut oil and refined, bleached and deodorized coconut oil. *LWT.*, **40**: 193-199.

Wang W and Paliwal J (2007). Near-infrared spectroscopy and imaging in food quality and safety. *Sens and Instrumen. Food Qual.*, **1**: 193-207.

Xing J, Ngadi M, Gunenc A, Prasher S and Gariepy C (2007). Use of visible spectroscopy for quality classification of intact pork meat. *J. Food Eng.*, **82**: 135-141.