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Determination of Adulterated Ghee Butter Compositions by FTIR Spectroscopy and Multivariate Regression Analysis

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ABSTRACT: The authentication of luxury oils is an important issue. Ghee is a famous traditional dairy product that likely to be adulterated with the other oils and fats specially the hydrogenated vegetable oils. Recently, Fourier transforminfrared (FT-IR) spectroscopy accompanying with chemometric techniques (partial least square) were introduced for validation of different oil quality. In the present study, IR spectra of pure ghee and three partially hydrogenated vegetable oils (soya bean oil, canola and sunflower oils) along with mixtures of different amounts of vegetable oils with ghee (10, 20 and 30%) were investigated. Partial Least Squares (PLS) model correlates the actual and FT-IR predicted values of oil adulterants where coefficients of determination (R^2) were 0.986, 0.997 and 0.991 for soya bean oil, canola oil and sunflower, respectively. The resulted RMS%RE values of soya bean oil, canola and sunflower oils are 0.94% and 1.192 and 2.54%, respectively. The method could be a rapid and precise alternative for identification of adulterated ghee.

Keywords: *Adulteration, Fourier Transformed Infrared, Ghee, Vegetable Oils*.

Introduction

The adulteration of foods is one of the most important issue concerned with the products, industries and consumers and is the significant from the view point of economic and human health (European Parliament, 2014). Regulatory agencies, including the United States Food and Drug Administration (FDA) and the World Health Organization (WHO) have focused on quality and identity assessment of cosmetics, pharmaceuticals, agrochemicals, consumable food products and food supplements to lessen adulteration as far as possible (European Parliament, 2014; FDA, 2009). Some effective methods proposed for validation of food product quality were reported including gas-chromatography, (Kim *et al.*, 2015), high-performance liquid chromatography (Domingues *et al.*, 2014), nuclear magnetic resonance (Fadzillah *et al.*, 2015), electroanalytical techniques (Apetrei & Apetrei, 2014). However, high cost, required skilled operator, require precise interpretation and long time for analysis have limited the application of these methods. Alternative methods that proposed for authenticity of adulterated foodstuffs comprise Raman (de Sa Oliveira *et al.*, 2016), fluorescence (Tan *et al.*, 2016), near infrared (Ding, Ni, & Kokot, 2015), and Fourier transformed infrared (FTIR) (Kurniawati, Rohman, & Triyana, 2014) accompanying with chemometric

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multivariate regression analysis has been widely explored recently for the precise analyses of food products (Rohman & Che Man 2010, 2012). Oils and fats particularly luxury ones might be subjected to adulteration and several researchers were interested in finding approaches for oil quality validation. In this respect, Fourier transformed infrared along with multivariate analysis is appealing due to small sample requirements and fast method of analysis and this method is capable to analyze complex mixtures, simultaneously and there is no need for sample preparation and this method has shown good performance in detection of adulteration in different oils and fats. (Elzey *et al.*, 2016; Liang *et al.*, 2013; Che Man & Rohman, 2013; Rohman *et al.*, 2014).

Ghee butter is one of the traditional dairy products obtained from either sheep or cow butter with different quality. (Suwarat & Tungjaroenchai, 2013). The adulteration in ghee butter is quite common due to high price of ghee. The addition of hydrogenated vegetable oil, is the most common type of adulteration. The aim of this study is to develop a rapid and accurate and low cost method for detection and simultaneous determination of the percent of added partially hydrogenated vegetable oils such as soya bean, canola and sunflower) that has been adulterated with natural sheep ghee butter.

Materials and Methods

- *Chemicals and supplies*

Pure sheep ghee was purchased from local store. Partially hydrogenated vegetable oil (sunflower, canola and soya bean oil) were obtained from local producers.

- *Fatty acid composition*

Fatty acid compositions of tested oils, were determined by Gas Chromatography (GC). Oils and fats were esterified before GC analysis using the method described by Mirrezaie Roodaki *et al*. (2016). A Hewlett Packard (Avondale, PA) 5890 Series II Gas Chromatograph equipped with flame ionization detector and 30 m Carbowax Capillary column was used to analyze the samples. The HP3396A integrator automatically gave individual peak retention times, peak areas and area percentages of each peak, at the end of each runs.

As presented in Table 1, oleic acid and palmitic acid are the major fatty acids of ghee. In the case of three tested vegetable oils, linoleic acid and oleic acid are the two predominant fatty acids. Accordingly, there is a substantial difference between fatty acid composition of ghee and vegetable oils (Table 1).

Table 1. Percent fatty acid composition of selected oils

Fatty acid composition (%)	Ghee	Partially hydrogenated Canola oil	Partially hydrogenated Soya bean oil	Partially hydrogenated Sunflower oil
Butyric acid	5.0	-	-	-
Caproic acid	2.5	-	-	-
Caprylic acid	1.5	-	-	-
Capric acid	3.0	-	-	-
Lauric acid	1.0	-	-	-
Myristic acid	10	-	-	-
Pentadecanoic acid	3.0	-	-	-
Palmitic acid	25.0	5.0	11.0	8.0
Palmitoleic acid	3.0	0.5		
Stearic acid	13.0	11.5	12.0	8.0
Oleic acid	22.5	58.0	22.5	23.0
Linoleic acid	5.0	18.0	45.0	58.0
α-Linolenic acid	2.0	5.0	6.0	1.0
Arachidic acid	2.0	1.0	2.0	1.0
Eicosenoic acid & Eicosadienoic acid	1.5	1.0	1.5	1.0

Sulieman *et al.*, (2013) investigated different brands of ghee and reported capric, lauric, myristic, palmitic, oleic, linoleic, arachidic acids are the main fatty acids present in different ghee samples. Mehta (2013) investigated different brands of ghee and found wide range of saturated fatty acids including small chains (C_4-C_8), medium chain (C_{10}-C_{14}) and long chain (C_{15}-C_{20}) present. Orsavova *et al*. (2015) pointed out that linoleic acid (62.2%), oleic acid (28%) were the main fatty acid of sunflower oil. In the case of canola oil, oleic acid (63.3%) and linoleic acid (19.6%) were the predominant acids. These reported results are quite similar to Belitz *et al.* (2010).

- ***FTIR measurement***

Different composition of ghee butter is prepared by mixing 0-30% (w/w) of the three vegetable oils with ghee butter. The samples were kept at ambient temperature for 48 hours**.**

The FTIR spectra of the samples were recorded in reflectance mode using FTIR spectrometer (Perkin- Elmer, LR64912C model). The FTIR spectrum of each sample was scanned over the range of 500 cm^{-1} to 4000 cm^{-1}.

- ***Chemometrics multivariate regression analysis***

Chemometrics and PLS multivariate regression data analysis were performed using Software SPSS version 22.

Results and Discussion

- ***Spectra analysis***

IR spectroscopy is an effective method for in the identification of molecular structures of materials and give suitable information about functional groups. In fats and oils, most of the peaks and shoulders of the spectrum are ascending to the particular functional groups (Bendini *et al.*, 2007). Owing to its capability as a fingerprint approach IR spectroscopy could be used for validation of oil quality and differentiate authentic oils and those adulterated with others by the spectra changes (Liang *et al*., 2013).

Figures 1 and 2 show the FTIR spectra of pure examined samples. FTIR spectra of following ghee with different amounts of vegetable oils are exhibited in Figure 3 to 5. Assignment of IR spectra is as following: 3006cm^{-1}(cis double-bond stretching), 2924cm^{-1}(symmetrical and asymmetrical stretching of $–CH_2$), 1742cm^{-1} (–C=O stretch), 1465cm^{-1} ($–CH_2$ bending), 1117cm^{-1} (–C–O stretch) and 722 cm^{-1} (cis–CH=CH– bending out of plane) (Liang, et al, 2013). The spectral regions selected for quantification analysis are 3006, 1456 and 722 cm^{-1} basis of the optimization process in which they comprise highest value of R^2 and the lowest values of error, either in calibration and prediction models.

- ***Quantification of vegetable oils in ghee:***

The quantification of canola, soya bean and sunflower oils in pure ghee was carried out using PLS algorithm. The spectral regions selected for quantification analysis are 3006, 1456 and 722cm^{-1} basis of the optimization process in which they comprise highest value of R^2 and the lowest value of error, either in calibration or prediction models.

Determination of the compositions of the ghee adulterated with soya bean, canola and sunflower oils were performed by multivariate PLS regression analysis: Partial-least-square regression analysis was used to correlate changes in FTIR spectra data of pure ghee adulterated with vegetable oils (Figure3-5) with % w/w composition of different vegetable oils of the adulterated samples. Detailed mathematical descriptions of multivariate regression calibrations in analytical spectroscopy for chemical analysis have been reported by authors (Fakayode *et al*., 2016; Elzey *et al.*, 2016). Briefly, a multivariate regression equation can be simplified and represented by the following equation:

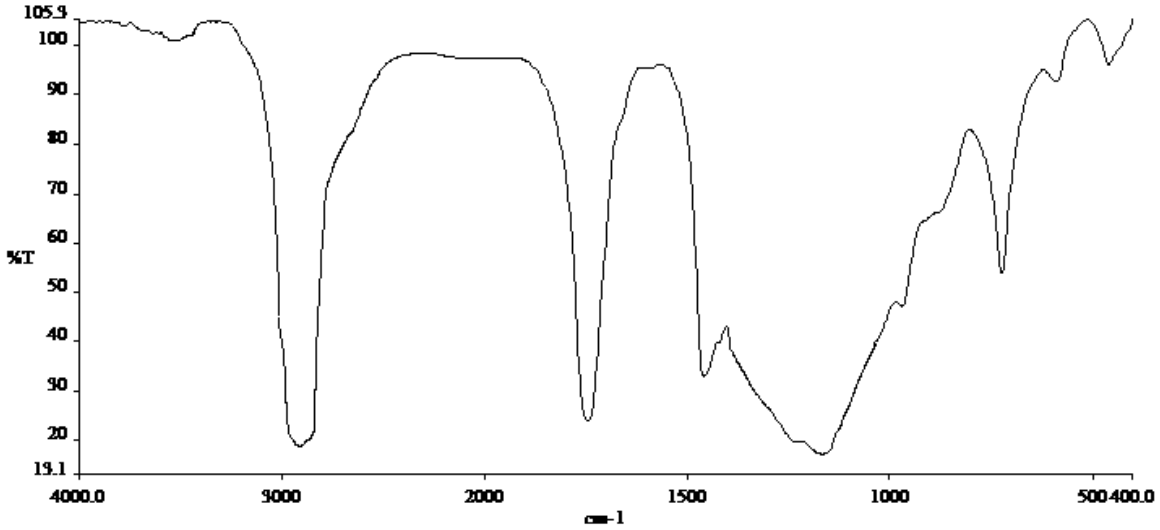

Fig. 1. FTIR spectra of pure ghee.

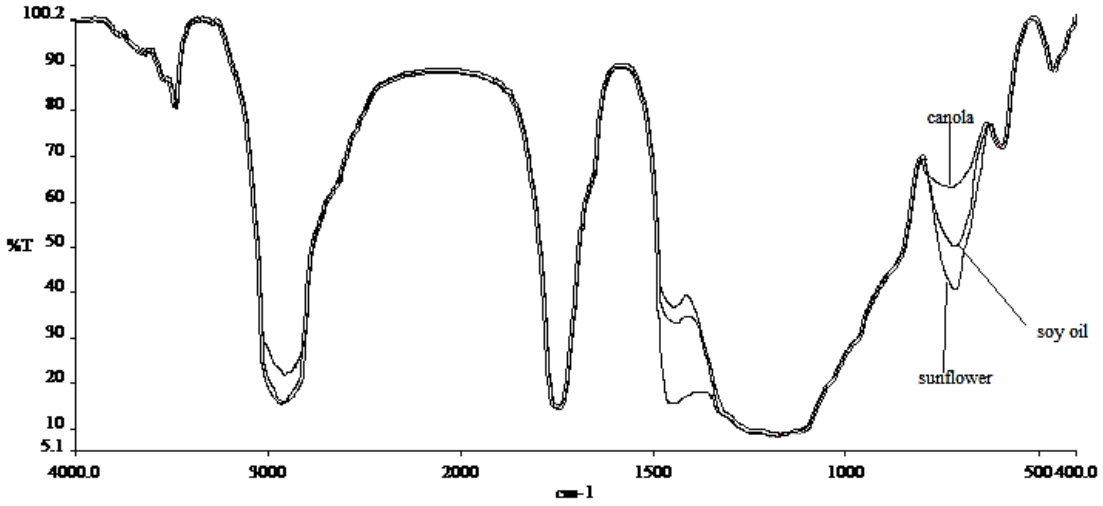

Fig. 2. FTIR spectra of pure vegetable oils.

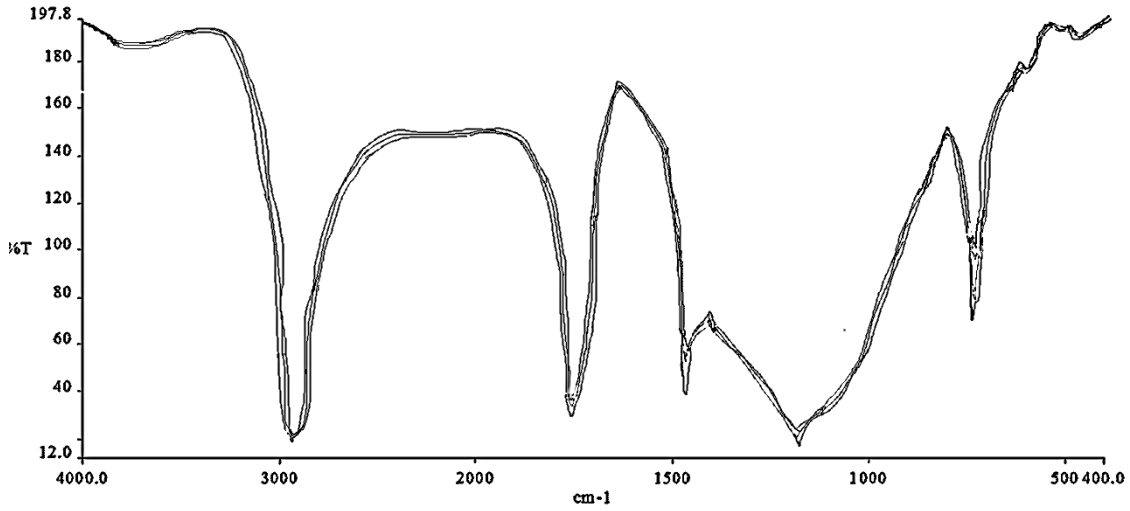

Fig. 3. FTIR spectra of adulterated ghee with 10-30% soya bean oil.

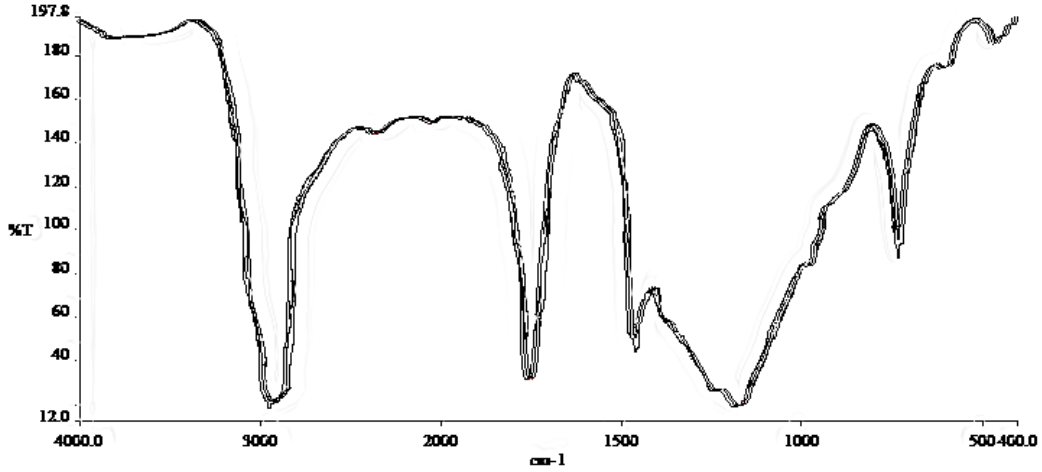

Fig. 4. FTIR spectra of adulterated ghee with 10-30% canola oil.

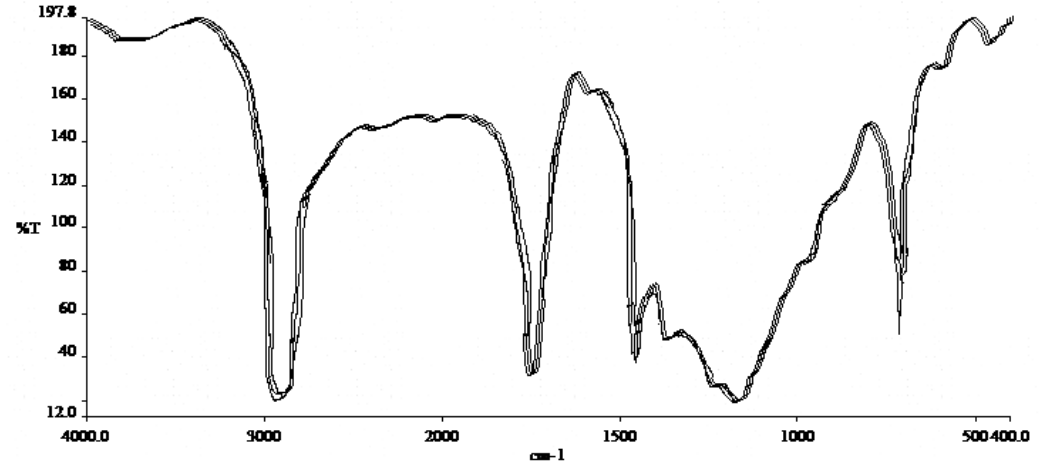

Fig. 5. FTIR spectra of adulterated ghee with 10-30% sunflower oil.

$y= b_0+ x_1b_1+ x_2b_2+ x_3b_3+ \ldots\ldots x_nb_n$

Where y is the dependent variable (percent oil compositions in this study), x_1, $x_2\ldots\ldots x_n$ are the independent variables (FTIR intensity at various wave numbers in this study), b_0 is the y-intercept of the regression

Equation, and b_1, b_2 … … … b_n are the regression coefficients of x variables.

The result of the validation study of the determined and actual percent composition of vegetable oils in pure ghee samples adulterated with vegetable oil is shown in Tables 2-4. The capability of the PLS regressions to accurately predict the percent composition of vegetable oils in the adulterated validation samples were evaluated using a root-mean-square-relative-percent error (RMS%RE) in equation

$$\text{RMS\%RE}= \sqrt{\frac{\sum(\%RE_i)^2}{n}}$$

Where, %REi is the percent relative error calculated from the known and predicted values for the validation sample, and n is the number of validation samples in the set. The PLS regression models correctly determined percent compositions with RMS%RE error of 0.94% and 1.192 and 2.54% for soya bean oil, canola and sunflower oil, respectively. The values of R^2 was 0.986, 0.997 and 0.991 for soya bean oil, canola and sunflower oil, respectively. The results of PLS analysis of adulterated ghee with tested vegetable oils are presented in Table 2-4.

Table 2. The actual and predicted (% compositions) of adulterated ghee with soya bean oil

Determined % soya bean oil	Actual % soya bean oil	% RE
1.71	0%	-1.71
8.88	10%	1.11
19.92	20%	0.074
29.032	30%	0.967

Table 3. The actual and predicted (% compositions) of adulterated ghee with canola oil

Determined % canola oil	Actual % canola oil	% RE
0.206	0%	-0.206
9.8	10%	0.198
18.87	20%	1.12
29.70	30%	0.295

Table 4. The actual and predicted (% compositions) of adulterated ghee with sunflower oil

Determined % sunflower oil	Actual % sunflower oil	% RE
1.47	0%	-1.47
8.12	10%	1.87
20.13	20%	-0.13
29.10	30%	0.89

The scatter plot for the relationship between actual and predicted concentration of soya bean, canola and sunflower oil in ghee in validation model are shown in Figure 6-8. Associated regression equation, also, is shown and x_1, x_2 and x_3 are FTIR intensity at 3006, 1459 and 722 cm^{-1}, respectively.

According to low values of RMS%RE for each experiment (soya bean, canola and sunflower oils are 0.94% and 1.192 and 2.54%, respectively) along with R^2values (0.986, 0.997 and 0.991 for soya bean, canola and sunflower oils, respectively) indicate that changes in the predictions are related to changes in the response variable and that these models could explain a lot of the response variability.

Conclusion

Combination of FTIR spectroscopy and multivariate PLS regression analysis could be a rapid, low cost and accurate method for identification of adulterated ghee with partially hydrogenated vegetable oils. This method offers advantages over other proposed methods like gas chromatography. In addition, this approach is capable to show percent composition of adulterated ghee with vegetable oils. A PLS multivariate regression was performed for the adulterated samples. The PLS regression models precisely determined the percent compositions of adulterated vegetable oils with low error of determination. Accordingly, it can be stated that PLS appears to be a reasonable approach for identification of adulteration in ghee.

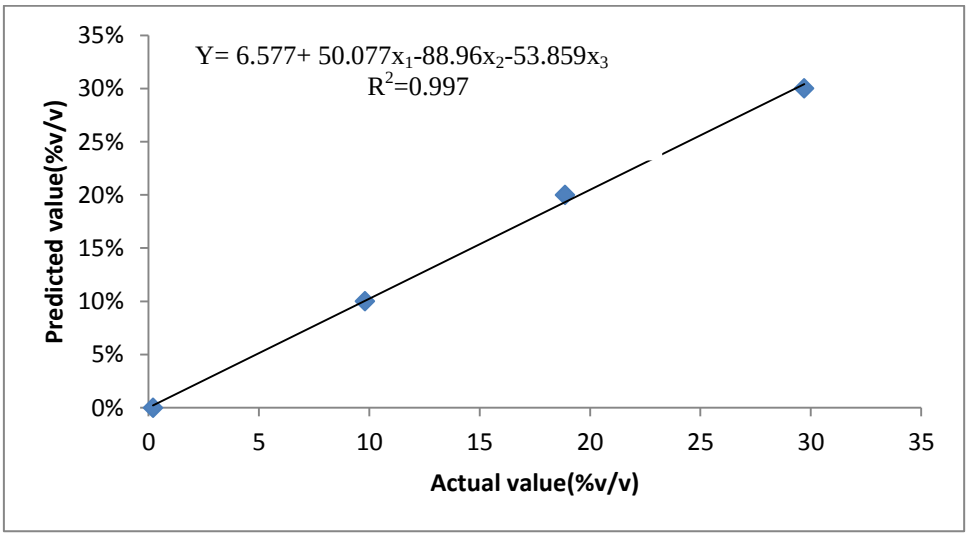

Fig. 6. PLS calibration model for the relationship between actual and predicted concentrations of canola oil in ghee.

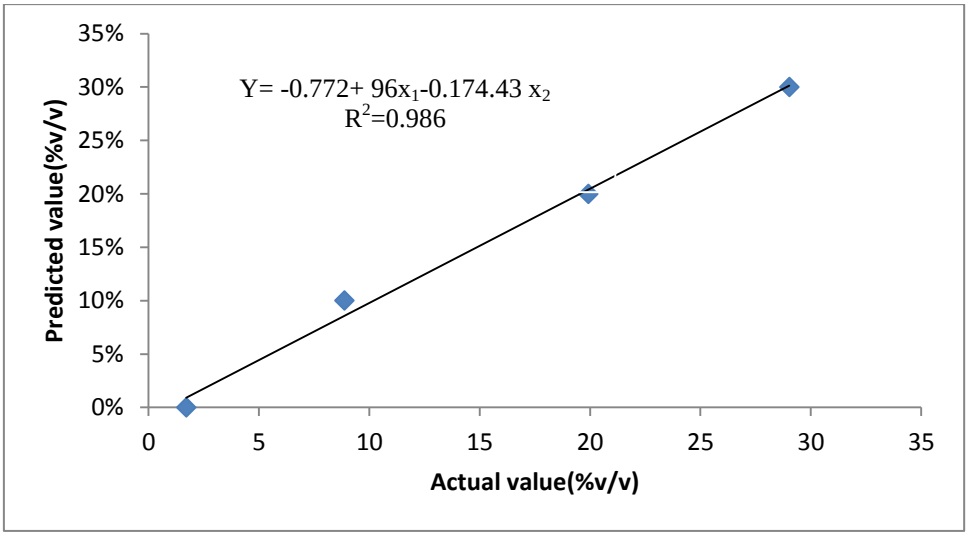

Fig. 7. PLS calibration model for the relationship between actual and predicted concentrations of soya bean oil in ghee.

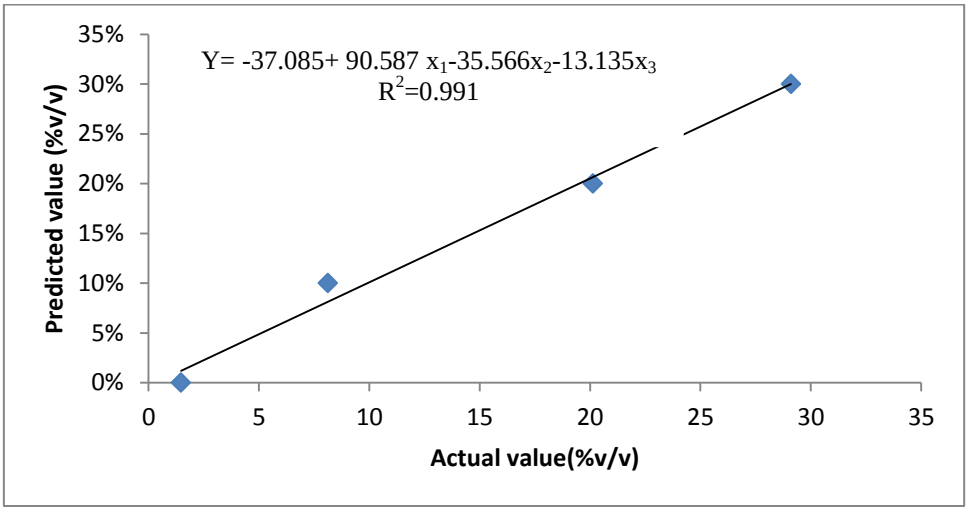

Fig. 8. PLS calibration model for the relationship between actual and predicted concentrations of sunflower oil in ghee.

References

Anon. (2009). FDA, US Food and Drug Administration. Addressing challenges of economically-motivated adulteration. In Paper presented at the public meeting on economically motivated adulteration, College Park, MD, request for comment. Federal register (Vol. 15497-15499).

Anon. (2014). Report e On the food crisis, fraud in the food chain and the control there of Rapporteur (chair). Esther de Lange, Committee on the Environment, Public Health and Food Safety.

Apetrei, I. M. & Apetrei, C. (2014). Detection of virgin olive oil adulteration using a voltammetric e-tongue. Computers and Electronics in Agriculture, 108, 148-154.

Belitz, H. D., Grosch, W. & Schieberle, P. (2009). Food chemistry. Springer. Pages: 650-654.

Bendini, A., Cerretani, L., Di Virgilio, F., Belloni, P., Bonoli-Carbognin, M. G. & Lercker, G. (2007). Preliminary evaluation of theapplication of the FTIR spectroscopy to control the geographicorigin and quality of virgin olive oils, Journal of Food Quality, 30(4), 424–437.

Che Man, Y. & Rohman, A. (2013). Analysis of Canola Oil in Virgin Coconut Oil Using FTIR Spectroscopy and Chemometrics. Journal of Food and Pharmaceutical Sciences. 1, 5-9.

Ding, X., Ni, Y. & Kokot, S. (2015). NIR

spectroscopy and chemometrics for the discrimination of pure, powdered, purple sweet potatoes and their samples adulterated with the white sweet potato flour. Chemometrics and Intelligent Laboratory Systems, 144, 17-23.

Domingues, D. S., Pauli, E. D., de Abreu, J. E. M., Massura, F. W., Cristiano, V. & Santos, M. J. (2014). Detection of roasted and ground coffee adulteration by HPLC by amperometric and by post-column derivatization UV-Vis detection. Food Chemistry, 146, 353-362.

Elzey, B., Pollard, D. & Fakayode, S. (2016). Determination of adulterated neem and flaxseed oil compositions by FTIR spectroscopy and multivariate regression analysis. Food Control, 68, 303-309.

Fadzillah, N. A., Man, Y. B., Rohman, A., Rosman, A. S., Ismail, A., Mustafa, S. & Khatib, A. (2015). Detection of Butter Adulteration with Lard by Employing (1)H-NMR Spectroscopy and Multivariate Data Analysis. Journal of Oleo Science, 64(7), 697-703. doi: 10.5650/jos.ess14255. Epub 2015 May 21.

Fakayode, S. O., Hordge, L. N., McDaniel, K. L. & Jones, D. D. (2016). Multicomponent and simultaneous determination of estrogens (ethinylestradiol and Norgestimate) concentrations in human and bovine serum albumin by use of fluorescence spectroscopy and partial-least square regression analysis. Talanta, 152, 401-409.

Kim, J. M, Kim, H. J. & Park, J. M. (2015). Determination of Milk Fat Adulteration with Vegetable Oils and Animal Fats by Gas Chromatographic Analysis. Journal of Food Science, 80(9), C1945-51. doi: 10.1111/1750-3841.12979.

Kurniawati, E., Rohman, A. &Triyana, K. (2014). Analysis of lard in meatball broth using Fourier transform infrared spectroscopy and chemometrics. Meat Science, 96(1), 94-98.

Liang, P., Wang,H., Chen, C., Ge, F., Liu, D., Li, S., Benyong Han, Xiong, X. & Zhao, S. (2013). The Use of Fourier Transform Infrared Spectroscopy for quantification of adulteration in virgin Walnut oil. Available at: http://dx.doi.org/10.1155/2013/305604.

Mehta, M. (2013). Consumption pattern and fatty acid composition of ghee. Food Science Research Journal, 4(2), 116-120.

Mirrezaie Roodaki, M. S., Sahari, M. A., Ghiassi Tarzi, B., Barzegar, M. & Gharachorloo, M. (2016). Effect of refining and thermal processes on olive oil properties. Journal of Agricultural Science and Technology, 18, 629-641.

Orsavova, J., Misurcova, L., Ambrozova, V. A., Robert Vicha, R. & Mlcek, J. (2015). Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. International Journal of Molecular Sciences, 16, 12871-12890; doi:10.3390/ijms160612871.

Rohman, A. & Che Man, Y. B. (2010). Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin oliveoil adulterated with palm oil. Food Research International, 43, 886–892.

Rohman, A. & Che Man, Y. B. (2012). The chemometrics approach applied to FTIR spectral data for the analysis of rice branoil in extra virgin olive oil. Chemometrics and Intelligent Laboratory Systems, 110, 129–134.

Rohman, A. Larasati Setyaningrum, D. & Riyanto, S. (2014). FTIR Spectroscopy Combined with Partial Least Square forAnalysis of Red Fruit Oil in Ternary Mixture System. International Journal of Spectroscopy. Available at: http://dx.doi.org/10.1155/2014/785914.

de Sa Oliveira, K., de Souza Callegaro, L., Stephani, R., Almeida, M. R. & deOliveira, L. F. C. (2016). Analysis of spreadable cheese by Raman spectroscopy and chemometric tools. Food Chemistry, 194, 441-446.

Sulieman, A. M. E., Mohammed, M. B. & Ali, A. O. (2013). Physicochemical and Sensory Properties of Traditionally and Laboratory Made Ghee (Samin) of the Sudan. International Journal of Food Science and Nutrition Engineering, 3(1), 7-11.

Suwarat, N. & Tungjaroenchai, W. (2013). Characteristic of Ghees Obtained from Different Post-Clarification Temperatures. International Journal of Bioscience, Biochemistry and Bioinformatics. 3(4), 332-334.

Tan, J., Li, R., Jiang, Z. T., Tang, S. H., Wang, Y., Shi, M., Xiao, Y. Q., Jia, B., Lu, T. X. & Wang, H. (2016). Synchronous front-face fluorescence spectroscopy for authentication of the adulteration of edible vegetable oil with refined used frying oil. Food Chemistry, 15, 274-280. doi: 10.1016/j.foodchem.2016.08.053.