PLATFORM B (FOOD BIOTECHNOLOGY)

COMPARISON OF HALAL FATS AND OILS WITH LARD USING FTIR AND CHEMOMETRICS

Fauzi. A.H.,[1] Yahaya, N.[1,2], Hassan, S.[1,2], Yahya, H.[1] and Yahya, H.N.[1,2]*

[1]*Faculty of Science and Technology,*
Universiti Sains Islam Malaysia,
Bandar Baru Nilai, Nilai, 71800, Malaysia
[2] *Institute of Fatwa and Halal (IFFAH)*
Universiti Sains Islam Malaysia,
Bandar Baru Nilai, 71800, Nilai, Negeri Sembilan
**Corresponding author: hanisnadia@usim.edu.my*

Keywords: *Halal, lard, FTIR, chemometrics.*

ABSTRACT

Fats and oil adulteration in the food industry is a recurring problem that negatively impacts the quality and safety of the food product. Efforts to ensure fats and oil authentication has involved investigating different analytical methods of adulteration detection. Fourier transform infrared spectroscopy (FTIR) is reported to be the favorable method when coupled with chemometrics. Focusing on halal food as a compulsory dietary requirement for Muslims, concerns regarding halal authenticity of fats and oil have been highlighted. This study aimed to compare halal fats with lard. The objective of this study was to identify the lipid profile of lard using FTIR analysis, compare the lipid profile of lard with halal fats and oil and to determine the differences of lipid profile between halal fats and oil and lard through chemometric analysis. The FTIR analysis was successful in obtaining a lard profile. FTIR data was then analyze through principal component analysis (PCA). Clear separations on the score plot between the different fats and oil samples. This study showed that lipid profile is highly similar to halal fats and oils but can be differentiated through chemometrics. PCA is a suitable tool to support FTIR analysis in differentiating fats and oils.

INTRODUCTION

In the food industry, fats and oil are highly vulnerable to adulteration and for decades this have been a recurring problem [1]. It is intolerable in the food industry because it compromises the quality of the product and poses as a food safety issue where it can be detrimental to health [2] or cause allergic reactions [3]. Adulteration of food product with non-halal ingredients like the addition of lard to butter could also lead to mistrust from the Muslim community [4]. To detect the adulteration of fats and oils, several analytical methods have been identified [5]-[7]. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been used to authenticate cocoa butter [8], olive oil [9], argan oil, soya bean oil, and sunflower oil [10]. Chromatographic methods require highly skilled personnel, tedious sample pre-treatments and preparation, and demands long processing time [11]. Fourier transform infrared spectroscopy (FTIR) is the most common method used to investigate fats and oil authentication. Studies employing FTIR have determined adulteration of cod-liver oil with lard [12], adulteration of olive oil [13], and adulteration of cold press sesame oil [14]. This method is rapid

and relatively easy to conduct with minimal sample preparation. Data obtained from FTIR are usually insufficient without further analysis [15]. With regards to halal assurance, halal products must be free of non-halal ingredients. Among others, butter, sunflower oil, canola oil, olive oil, coconut oil, and mustard oil are vulnerable to non-halal adulterations due to the high compositional similarities [16]. Deliberate addition of lard in butter and other plant oils that are subsequently used for bread products have been reported [17]-[18]. Although literature is available on detection of lard adulteration in fats and oil, information on differences in breed is still lacking for reference. The aim of this study is to compare halal fats and with lard. The objective of this study is to identify the lipid profile of lard using FTIR analysis, compare the lipid profile of lard with halal fats and oil, and to determine the differences of lipid profile between halal fats and oil and lard through chemometric analysis.

METHODOLOGY

Samples and preparation

Pigs were identified and purchased from MyBarn, Malaysia (https://www.mybarn.com.my/). The breeds purchased were Berkshire (black pork), Iberico (black pork), Olive fed (white pork), and White local (white pork). Both the Berkshire (B) and White local (W) are pork raised in Malaysia while Iberico (I) and Olive (O) are pork imported from Spain. Pure butter (PB), olive oil (OO), palm oil (PO), and sunflower oil (SO) were purchased from the local supermarket as halal fats and oil samples. All lipid samples from the fats and oils were extracted using the Folch method [19]. Briefly, 20 mL of chloroform-methanol mixture (2:1 v/v) is used to dissolve 1 g of fat sample. The mixture is periodically stirred at room temperature for 15 min before the mixture is filtered through filter paper. The mixture is then washed with 4 mL of 0.9% sodium chloride (NaCl), vortexed for 10 sec and left to stand at room temperature for 20 min. The bottom layer containing the extracted lipids were removed and stored in glass vials with Teflon lined caps. The extracted lipids were stored at -18 °C prior to analysis.

Ftir spectroscopy and chemometric analysis

FTIR analysis was carried out using an attenuated total reflectance (ATR-FTIR) (Perkin Elmer, MA, USA). Samples were scanned in the range of 4,000 to 650 cm^{-1} with a resolution of 4 cm^{-1} and a total accumulation of 32 scans. Prior to sample analysis, background scans of surrounding air were acquired. The platform was also cleaned using methanol to avoid contamination. FTIR spectra are collected and recorded as transmittance percentage (T%). The multivariate data from the FTIR analysis were investigated using chemometric, specifically PCA analysis. Data were managed in Microsoft Excel before being imported into the Unscrambler 10.3 X software (CAMO, USA) for chemometric analysis. Suitable data pre-processing was applied prior to PCA analysis.

RESULTS AND DISCUSSIONS

Lipid profile of lard

From the FTIR spectrum, peaks at specific wavenumbers correlate to specific functional groups. Based on the data obtained, FTIR spectrum of 100% lard appears in the range of 4,000 to 650 cm^{-1}. All the peaks identified in the study collectively shows that the sample is animal fat metabolite. Wavenumbers where the peaks forms that correlates to the functional groups found in the lard samples were identified and were compared to other studies where similar peaks were found to show that this is a lipid profile for lard [20]-[21].

Lipid profile comparison of halal fats and oils with lard from different pig breeds

Lard samples from the different breed of pigs Berkshire (B), Iberico (I), Olive (O), and White (W) and fats and oils (pure butter (PB), olive oil (OO), palm oil (PO), and sunflower oil (SO)) were analyzed with FTIR. The spectrum obtained for lard samples B, I, O, and W combined with the spectrum obtained for OO, PB, PO, and SO. Triglycerides are the major constituents of fats and oils, and it is observed in all samples making it difficult to distinguish individual spectrum. The shared peaks from the analysis are a result of the same functional groups present in the samples. Other studies investigating fats and oil adulteration have also reported the difficulty in differentiating samples through FTIR spectrum by visual analysis alone [22]-[24].

Chemometric analysis of halal and non-halal fats and oils

A principal component analysis (PCA) was carried out with the objective of determining whether lipids from different pig breeds are distinguishable between each other and between other fats and oil. Based on the analysis, there is clear grouping of lard with other fat and oils. Similarly, other studies have reported that it is possible to differentiate between animal fats and vegetable oils through spectroscopy followed by PCA analysis [25]-[27]. The breed of the pig has significant influence on the meat's quality [28] and the genotype that affected the quality of the meat was successfully differentiated through a PCA analysis in a separate study [29]. The differences in types of lard and other types of fats could suggest differences in the lipid metabolite in each samples.

CONCLUSION

The FTIR spectroscopy analysis were able to qualitatively identify lard based on the spectrum obtained. The peaks identified in the spectrum corresponds to the functional group present in the lipid samples. By comparison to past reports, it was confirmed that the spectrum belongs to animal fat lard. The FTIR spectrum of the lard in comparison to other halal fats and oil specifically olive oil, pure butter, palm oil, and sunflower oil was difficult to differentiate. The samples were almost non-distinguishable due to the high similarity of triglycerides present in fats and oil. Further analysis through PCA proved that there is visible cluster pattern that could group the different samples. Different types of fats and oil were seem clustered together. The sample pure butter is observed to stand alone while the oils are all separated but generally in the same region of the score plot. The PCA score plot emphasizes that chemometric analysis is important in supporting the FTIR analysis to differentiate between the halal fat and oil samples with the lard samples.

REFERENCES

[1] Rohman A., Ghazali MA., Windarsih A., Irnawati, Riyanto S., Yusof FM., Mustafa S. "Comprehensive review on application of FTIR spectroscopy coupled with chemometrics for authentication analysis of fats and oils in the food products". *Molecules*, vol. 25(22). 5485. 2020. https://doi.org/10.3390/molecules25225485

[2] Gelpi E., de la Paz MP., Terracini B., Abaitua I., de la Camara AG., Kilbourne EM., Lahoz C., Nemery B., Philen RM., Soldevilla L., Tarkowski S. "The Spanish toxic oil syndrome 20 years after its onset: A multidisciplinary review of scientific knowledge". *Environmental Health Perspectives*, vol. 110(5), pp. 547-464. 2002.

[3] Chogale R. "Food adulteration: effects and detection. *Advances in Chemical Science,* vol. 1, pp. 66-73. 2022.

[4] Taylan, O., Cebi, N., Yilmaz, M.T., Sagdic, O., & Bakhsh, A.F. "Detection of lard in butter using Raman spectroscopy combines with chemometrics". *Food Chemistry,* 332, 127344. 2020.

[5] Berti, L, Yetti, R. D., & Asra, R. "Chemical analysis methods to detect pork DNA and derivative contents in food: A Review". *Asian Journal of Pharmaceutical Research and Development*, vol. 8(9), 10-19. 2020."http://dx.doi.org/10.22270/ajprd.v8i6.850

[6] Rohman, A. & Windarsih, A. (2020). The application of molecular spectroscopy in combination with chemometrics for halal authentication analysis: A review". *International Journal of Molecular Science*, vol. 21, 5155. 2020. doi:10.3390/ijms21145155

[7] Zia, Q., Alawami, M., Mokhtar, N. F. K., Nhari, R. M. H. R. & Hanish, I. "Current analytical methods for porcine identification in meat and meat products". *Food Chemistry*, vol. 324, 126664. 2020. https://doi.org/10.1016/j.foodchem.2020.126664

[8] Azir, M., Abbasiliasi, S., Ibrahim, T.A.T., Manaf, Y.N.A., Sazili, A.Q.,and Mustafa, S. "Detection of lard in cocoa butter—its fatty acid composition, triacylglycerol profiles, and thermal characteristics." *Foods*, vol. 6, 98. 2017.

[9] Yang, Y., Ferro, M.D., Cavado, I., Liang, Y. "Detection and identification of extra virgin olive oil adulteration by GC-MS combined with chemometrics". *J. Agric. Food Chem*, vol. 61, 3693-3702. 2013.

[10] Hilali, M., Charrouf, Z., Soulhi, A.E.A., Hachimi, L., Guillaume, D. "Detection of argan oil adulteration using quantitative campesterol GC-analysis*". J. Am. Oil Chem. Soc*, vol. 84, 761-764. 2007.

[11] Tian, L., Zeng, Y., Zheng, X., Chiu, Y., Liu, T. "Detection of peanut oil adulteration mixed with rapeseed oil using gas chromatography and gas chromatography–ion mobility spectrometry". *Food Anal*ytical *Methods*, vol. 12, pp. 2282- 2292. 2019.

[12] Rohman, A., Che Man, Y.B. "Analysis of cod-liver oil adulteration using Fourier transform infrared (FTIR) spectroscopy. *J. Am. Oil Chem. Soc,* vol. 86, pp. 1149-1153. 2009.

[13] Zhang, X.F., Zou, M.Q., Qi, X.H., Liu, F., Zhang, C., Yin, F. "Quantitative detection of adulterated olive oil by Raman spectroscopy and chemometrics". *J. Raman Spectrosc*, vol. 42, pp. 1784-1788. 2011.

[14] Ozulku, G., Yildirim, R.M., Toker, O.S., Karasu, S., Durak, M.Z. "Rapid detection of adulteration of cold pressed sesame oil adulterated with hazelnut, canola, and sunflower oils using ATR-FTIR spectroscopy combined with chemometric". *Food Control*, vol. 82, pp. 212-216. 2017.

[15] Silva, L.C.R., Folli, G.S., Santos, L.P., Barros, I.H.A.S., Oliveira, B.G., Borghi, F.T., dos Santos, F.D., Filgueiras, P.R., Romão, W. "Quantification of beef, pork, and chicken in ground meat using a portable NIR spectrometer. *Vibrational Spectroscopy*, 111, 103158. 2020.

[16] Munir, F., Musharraf, S.G., Sherazi, S.T.H., Malik, M.I., & Bhanger, M.I. "Detection of lard contamination in five different edible oils by FT-IR spectroscopy using a partial least squares calibration model". *Turkish Journal of Chemistry*, vol. 23, 1098-1108. 2018.

[17] Fadzlillah, N. A., Man, Y. B. C., Rohman, A., Rosman, A. S., Ismail, A., Mustafa, S., & Khatib, A. "Detection of Butter Adulteration with Lard by Employing (1)H-NMR Spectroscopy and

Multivariate Data Analysis". *Journal of Oleo Science*, 64(7), 697–703. 2015. https://doi.org/10.5650/JOS.ESS14255

[18] Rohman, A., & Man, Y. B. C. "Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin olive oil adulterated with palm oil". *Food Research International*, 43(3), 886–892. 2010. https://doi.org/10.1016/j.foodres.2009.12.006

[19] Folch, J., Lees, M., & Stanley, G.H.S. "A simple method for the isolation and purification of total lipides from animal tissues". *Journal of Biological Chemistry*, vol. 226, pp. 497-509. 1957.

[20] Salleh, N.A.M., Hassan, M.S., Jumal, J., Harun, F.W., Jaafar, M.Z. "Heating treatments using Fourier Transform Infrared Spectroscopy (FTIR) and multivariate analysis". *Recent Advancement on Applied Physics, Industrial Chemistry, and Chemical Technology*. AIP Conference Proceedings. 1972. 2018. 030015. 10.1063/1.5041236.

[21] Upadhyay, N., Jaiswal, P., Jha, S.N. "Application of attenuated total reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR) in MIR range coupled with chemometrics for detection of pig body fat in pure ghee (heat clarified milk fat)". *Journal of molecular Structure*, vol. 1153, pp. 275-281. 2018. doi:10.1016/j.molstruc.2017.09.116

[22] Yang, D., & Ying, Y. "Applications of Raman spectroscopy in agricultural products and food analysis: A review". *Applied spectroscopy reviews*, vol. 46(7), pp. 539-560. 2011. https://doi.org/10.1080/05704928.2011.593216

[23] Ye Q., Meng, X. "Highly efficient authentication of edible oils by FTIR spectroscopy coupled with chemometrics". *Food Chemistry*, vol. 385, 132661. 2022.

[24] Smithson SC., Fakayode BD., Henderson S., Nguyen J., Fakayode SO. "Detection, purity analysis, and quality assurance of adulterated peanut (Arachis hypogaea) oils". *Foods*, vol 7(8), 122. 2018. https://doi.org/10.3390/foods7080122

[25] Salleh, N.A.M., & Hassan. M.S. "Discrimination of lard and other edible fats after heating treatments using partial least square regression (PLSR), Principal component regression (PCR), and linear support vector machine regression (SVMR)". *Journal of Physics: Conference Series*, vol. 1366. 2019. doi:10.1088/1742-6596/1366/1/012114

[26] Meira, M., Quintella, C.M., Ribeiro, E.M.O., Guimarães, A.K., Santos, M.A., Cerqueira, G.S., Cid, A.L.D., Luna, S., Silva, W.L. "Prediction of concentration of animal fat in mixture with vegetable oil by multivariate calibration associated with spectroflourimetry". *American Journal of Analytical Chemistry*, vol. 4, pp. 99-103. 2013.

[27] Nasyrah AR., MArikkar JMN., Dzulkifly MH. "Discrimination of plant and animal derived MAG and DAG by principal component analysis of fatty acid composition and thermal profile data". *International Food Research Journal*, vol. 19(4), pp. 1497-1501. 2012.

[28] Kim, J.M, Lee, S.H., Ryu, Y.C. "Comparison of meat quality and muscle fibre characteristics on multiple pig breeds and sexes using principal component analysis". *Animal Production Science*, vol. 58(11). 2017. http://dx.doi.org/10.1071/AN16223

[29] Jonsäll, A, Johansson, L, Lundstrom, K., Anderson, K., Nilsen, A.N, Risvik, E. "Effects of genotype and rearing system on sensory characteristics and preference for pork (*M. Longissimus dorsi*)". *Food Quality and Preference*, vol. 13, pp. 73-80. 2002. http://dx.doi.org/10.1016/S0950-3293(01)00060

ACKNOWLEDGEMENT

This research was funded by the Ministry of Higher Education (MOHE) under the Fundamental Research Grant Scheme (FRGS) grant FRGS/1/2020/WAB12/USIM/02/1.