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Which method is appropriate for lard detection in halal foods: Fourier transform infrared, differential scanning calorimetry, or polymerase chain reaction?

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Abstract

Background and objective: Lard is commonly used in food industry due to its availability, low cost, and ability to enhance food quality. However, certain religious groups such as Muslims do not eat it and consider lard as non-halal food. In the present study, for the first time, we conducted chemical and molecular analysis to detect different percentages of lard simultaneously.

Materials and methods: To determine origin of a sample containing lard, various percentages of lard including 100, 50, 40, 30, 20, 10, 5, 3, 1 and 0% w/w were prepared in triplicate and tested for Fourier transform infrared, differential scanning calorimetry, and polymerase chain reaction analysis. Fourier transform infrared spectral analysis was carried out in the mid-infrared range (2-25 µm). Differential scanning calorimetry analysis was done from -60 to 60 ºC at scan rate of 10 ºC/min. The reactions in polymerase chain reaction analysis were amplified by specific primers which designed from cow and pig.

Results and conclusion: Our results demonstrated that all three methods could detect various quantities of lard, but Fourier transform infrared and differential scanning calorimetry had some exceptions in their results. In comparison, polymerase chain reaction could amplify the pig-specific band (212 bp) in all samples containing lard. Therefore, our results showed that polymerase chain reaction approach is more reliable and accurate than two other methods.

Keywords: Differential scanning calorimetry, Fourier transform infrared, Halal, Lard, Polymerase chain reaction

1. Introduction

Lard is a widely used ingredient in food industry due to its availability and affordability. However, consuming pig-derived products is not permitted among Muslim, Judaism, and Hinduism populations. According to the Islamic rules, eating little amounts of haram ingredients is forbidden [1]. In addition, adulteration is a big challenge in food industry, which motivates researchers to conduct numerous investigations in this regard [2-4]. Currently, two billions of the world's population are Muslims, in which Egypt, Afghanistan, Syria, Pakistan, Turkey, and Iran are the countries with over 90% Muslims. In Iran, where the population is predominantly Muslim (99.4%), using halal food is highly important, particularly for the imported foods [5].

The global halal market is increasing very fast (growth rate of 20%), and the current halal trade is estimated

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as 2.3 trillion USD annually [5]. Therefore, distinguishing the non-halal ingredients in food products is important to meet the needs of consumers. Halal confirmation cannot be achieved by physical control, and chemical and molecular analysis are required. Detection of lard has been done by chemical approaches such as Fourier transform infrared spectroscopy (FTIR) [6-20], differential scanning calorimetry (DSC) [21-24], gas chromatography (GC) [25,26], and liquid chromatography (LC) [27,28]. Despite some limitations in DNA-based methods [29-31], polymerase chain reaction (PCR) analysis as a highly accurate and sensitive technique has been used to identify the origin and adulteration of foods including pig fat [32-35]. It is worth mentioning that none of the previous studies relied on both chemical and molecular analytical methods simultaneously. In the current work, different ratios of lard were studied by FTIR, DSC, and PCR for the first time to find out the most accurate approach.

2. Materials and methods

2.1. Sample preparation

Halal Research Center of Iran provided us with pure lard and samples containing various concentration of lard including 100, 50, 40, 30, 20, 10, 5, 3, 1 and 0% w/w. Cow butter was used to prepare the samples with different quantities of lard. In practice, different amounts of lard and cow butter were weighed, mixed, heated at 70 ºC. After liquefaction, the samples were vortexed vigorously to make a homogenous mixture. The samples were prepared in triplicate.

2.2. Fourier transform infrared

FTIR spectral analysis of the samples was carried out in the mid-infrared range (2-25 µm) by using Perkin-Elmer Spectrum ONE FT-IR spectrometer equipped with a deuterated triglycine sulphate detector and a KBr beam splitter. FTIR spectra were collected at 450-4000 cm^{-1} with resolution of 4 cm^{-1}. Each spectrum was obtained by average of 16 scans at 25 ºC. Final spectra were then processed by OMNIC 8.2 software.

2.3. Differential scanning calorimeter

DSC analysis of the samples was performed by using DSC 214 *Polyma* differential scanning calorimeter (NETZSCH, Germany) to determine thermodynamic transitions and heat capacities of the samples. In brief, 5-8 mg of each sample was accurately weighed and heated from -60 to 60 ºC at scan rate of 10 ºC/min. AutoEvaluation software was used for thermos-analytical measurements.

2.4. Polymerase chain reaction

DNA of the samples were extracted using a modified CTAB (cethyltrimethylammonium Bromide) method. Purity of the extracted DNA was determined by NanoDrop™ 2000c spectrophotometers (Thermo Fisher, USA). PCR assay was carried out on a thermal cycler Corbett CGI-96 with 20 µl reaction mixture including PCR master mix (AMPLIQON, Denmark), 0.5 µM of each primer (Table 1), and 50-100 ng of the extracted DNA. PCR program was included to: first step at 95 ºC for 3 min, second step for 35 cycles at 95 ºC of 25 s, 58 and 66 ºC for 25 s (annealing condition for cow and pig primers, respectively) and 72 ºC for 25 s, and third step at 72 ºC for 5 min. As well, GelRed® nucleic acid gel stain (Biotium, California, United States) was used to visualize 5 µl PCR product on 2% agarose gel. Cow and pig PCR products were expected to have size of 271 and 212 bp, respectively.

Table 1- Characteristics of the primers used in PCR assay of the samples containing different concentrations of lard

Primer	Sequence (5'-3')	PCR product (bp)
Forward (cow)	GCCATATACTCTCCTTGGTGACA	271
Reverse (cow)	GTAGGCTTGGGAATAGTACGA	
Forward (pig)	GCCTAAATCTCCCCTCAATGGTA	212
Reverse (pig)	ATGAAAGAGGCAAATAGATTTTCG	

3. Results and discussion

3.1. Fourier transform infrared

FTIR spectroscopy follows the Beer's law, which states that intensity of peaks in the IR spectrum is directly proportional to the concentration of analytes at mid-infrared wavelengths. This method has been widely used by researchers to identify presence of lard in fats and oils, since position of fatty acids, chain saturation, and specific compounds in fats and oils can reveal the origin of fat [1,14,15]. In our study, 12 distinct peaks were detected in the FTIR spectra (Figure 1). Specifically, the peak at 864.93 cm^{-1} related to C-H bond was only observed in the pure lard samples, and it was not detected in the samples containing 100% cow fat or different concentrations of lard. In addition, the peak at 720 cm^{-1} indicated a bond between carbon and halogen, and was found in the samples with different concentrations of lard (not in the samples with 100% or 0% lard). Moreover, the observed peak at 1417 cm^{-1} was only observed in the samples free of lard, which can be used to confirm the halal status of the samples. Interestingly, the samples containing 100% and 0% lard displayed the peaks at 1743 and 1746 cm^{-1}, respectively, while the other samples with different concentrations of lard showed the peaks at average wave numbers, except for the sample containing 3% lard. Nonetheless, the peak at 3006 cm^{-1} was only observed in the samples containing 100% and 50% lard.

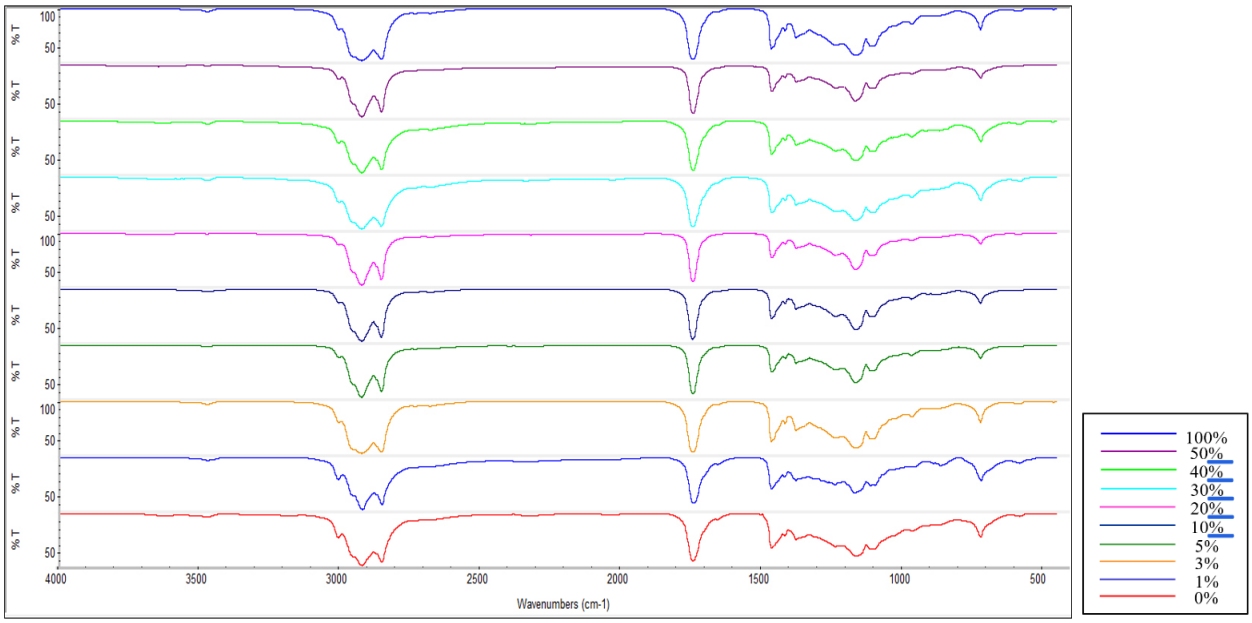

Figure 1- FTIR spectra of the samples containing different concentrations of lard

3.2. Differential scanning calorimeter

DSC is a method used to measure the changes in heat capacity under constant pressure and temperature conditions. The method involves exposing a sample with a certain mass to heating or cooling and measuring the changes in heat flow in response to the temperature changes. Thus, it investigates melting, heat transfer temperature, and phase changes. Table 2 shows the information about DSC analysis of the samples. Analysis of the sample containing 100% lard showed three distinct peaks with a unique peak at 36.9 °C, which corresponded to the glass transition temperature of the sample. In contrast, the other samples showed two peaks in the DSC graph. TG of the samples containing 100% and 0% lard were similar (around 23 °C), whereas it was higher than 23 °C in the other samples except for the sample containing 1% lard (19.1 °C). It

showed that the third peak which appeared at temperatures over 45 ºC would be of interest in authentication of halal foods. Although, it does not determine inclusion of lard as a component of fat mixture in a food product.

Table 2- Results of DSC analysis of the samples containing different concentrations of lard

	First peak			Second peak			Third peak		
Lard concentration	TG (ºC)	ΔCP (J/g*K)	ΔH (J/g)	TG (ºC)	ΔCP (J/g*K)	ΔH (J/g)	TG (ºC)	ΔCP (J/g*K)	ΔH (J/g)
100%	-3.6	1.44	-34.9	23.3	1.458	-34.43	36.9	0.174	-11.5
0%	-4.4	0.211	-38.8	23.9	0.144	-42.03	-	-	-
50%	-9.8	0.152	-32.4	29	0.204	-38.63	-	-	-
40%	-8.4	0.026	-39.45	25.7	2.1	-27.42	-	-	-
30%	2.7	0.127	-12.49	27.1	1.18	-15.82	-	-	-
20%	4.2	0.374	-17.99	26.9	0.599	-14.01	-	-	-
10%	-23.9	0.086	-47.26	24.2	0.448	-20.66	-	-	-
5%	-2.2	0.019	-40.25	26.2	0.418	-23.39	-	-	-
3%	-2.2	0.313	-44.92	26.1	0.507	-14.22	-	-	-
1%	-4.7	0.024	-15.15	19.1	0.027	-19.91	-	-	-

3.3. Polymerase chain reaction

PCR is one of molecular techniques for detection of pig DNA, which can produce millions of copies from a single copy or a small piece of DNA [2]. This technique can be used to determine origin of foods because DNA is relatively stable and traceable in many processed products. According to our results, the pig-specific primers amplified the pig-specific band (212 bp) in the all samples containing lard, while no band was observed in the sample free of lard (Figure 2). In addition, except for the sample containing 100% lard, the others produced 271 bp band that is indicator of cow genome (Figures 3).

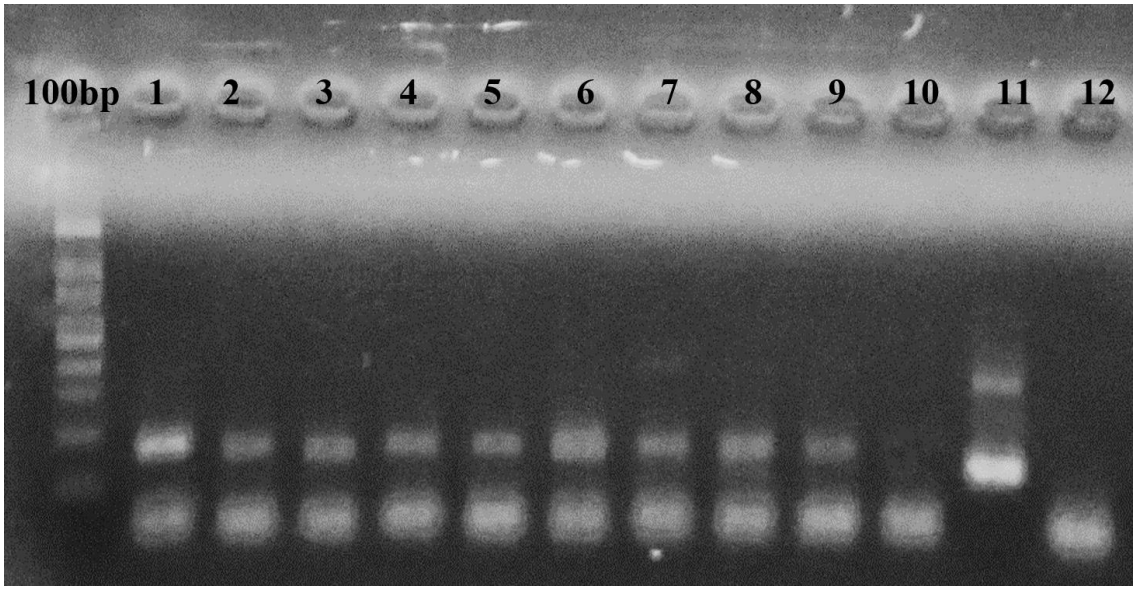

Figure 2- Agarose gel electrophoresis of PCR products with pig specific primer. Left to right: 100 bp ladder, 1 to 10 lines are PCR product of the extracted DNA from the samples containing 100, 50, 40, 30, 20, 10, 5, 3, 1, and 0% lard, respectively. Line 11 is a positive control, and line 12 is a negative control.

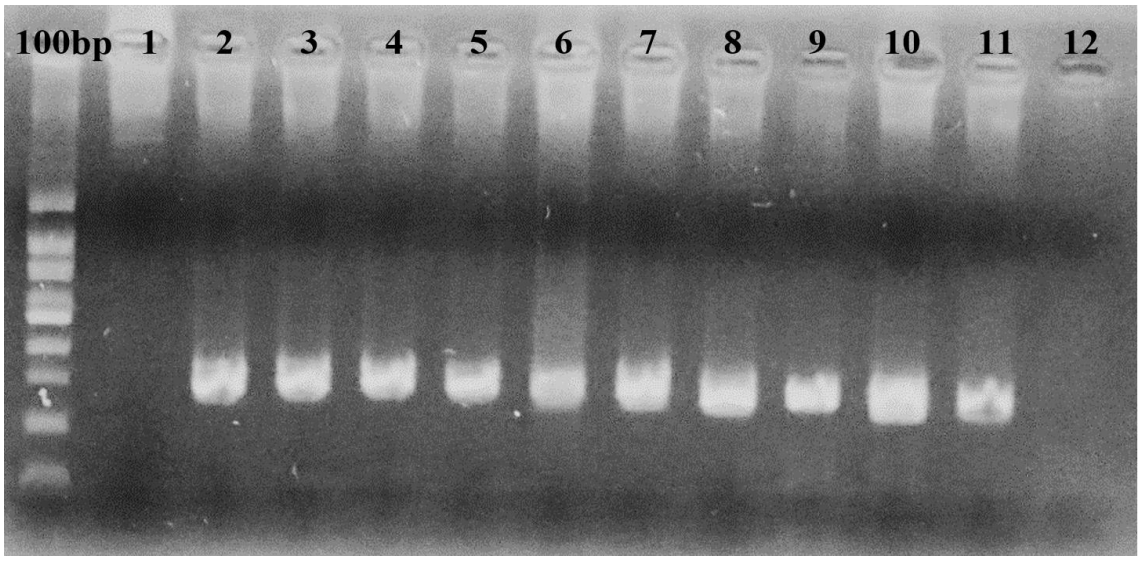

Figure 3- Agarose gel electrophoresis of PCR products with cow specific primer. Left to right: 100 bp ladder, 1 to 10 lines are PCR product of the extracted DNA from the samples containing 100, 50, 40, 30, 20, 10, 5, 3, 1, and 0% lard, respectively. Line 11 is a positive control, and line 12 is a negative control.

4. Conclusion

Nowadays, identification of halal foods is of great importance particularly for Muslims around the world. In this study, different percentages of lard were detected simultaneously by using FTIR, DSC, and PCR approaches for the first time. FTIR results showed that origin of pig can be traced by different wave numbers, but there were some exceptions and it was not specific enough. Similarly, DSC could not distinguish significantly the samples containing different concentrations of lard, because just a unique peak was observed exclusively in the sample containing 100% lard. However, PCR analysis could detect lard accurately in the samples. Hence, PCR approach with its high accuracy, precision, and speed is better than FTIR and DSC to confirm the presence of lard in food products.

5. Acknowledgment

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6. Conflicts of interest

The authors declare that they have no conflict of interest.

References

1. Harun FW. Fourier transform infrared spectroscopy as a technique for multivariate analysis of lard adulteration in food products: a review. Journal of Fatwa Management and Research. 2019; 17(1): 1-13. http://dx.doi.org/10.33102/jfatwa.vol17no1.1

2. Karim NA, Muhamad II, editors. Detection methods and advancement in analysis of food and beverages: A short review on adulteration and Halal authentication. Proceedings of the 3rd International Halal Conference (INHAC 2016). 2018. Springer.

3. Ha J, Kim S, Lee J, Lee S, Lee H, Choi Y, et al. Identification of Pork Adulteration in Processed Meat Products Using the Developed Mitochondrial DNA-Based Primers. Korean Journal Food Science of Animal Resource. 2017; 37(3): 464-468. https://doi.org/10.5851/kosfa.2017.37.3.464

4. Mortas M, Awad N, Ayvaz H. Adulteration detection technologies used for halal/kosher food products: an overview. Discover Food. 2022; 2: 15. https://doi.org/10.1007/s44187-022-00015-7

5. Azam MSE, Abdullah MA. Global halal industry: relalities and opportunities. International Journal of Islamic Business Ethics. 2020; 5: 47-59. http://dx.doi.org/10.30659/ijibe.5.1.47-59

6. Guntarti A, Ahda M, Kusbandari A, Sauri1 AS. Fourier-transform infrared spectroscopy combined with chemometrics for detection of pork in beef meatball formulation. International Journal of Green Pharmacy. 2018;12 (3): 153.

7. Erwanto Y, Muttaqien AT, Sugiyono, Sismindari, Rohman A. Use of fourier transform infrared (ftir) spectroscopy and chemometrics for analysis of lard adulteration in "rambak" crackers. International Journal of Food Properties. 2016; 19(12): 2718-2725. https://doi.org/10.1080/10942912.2016.1143839

8. Che Man Y, Mirghani ME. Detection of lard mixed with body fats of chicken, lamb, and cow by Fourier transform infrared spectroscopy. Journal of the American Oil Chemists' Society. 2001; 78(7): 753-761.

9. Manaf MA, Man YBC, Hamid NSA, Ismail A, Abidin SZ. Analysis of adulteration of virgin coconut oil by palm kernel olein using Fourier transform infrared spectroscopy. Journal of Food Lipids. 2007; 14(2): 111-121. https://doi.org/10.1111/j.1745-4522.2007.00066.x

10. Naquiah ANN, Marikkar JMN, Mirghani MES, Nurrulhidayah AF, Yanty NAM. Differentiation of fractionated components of lard from other animal fats using different analytical techniques. Sains Malaysiana. 2017; 46(2): 209-216. http://dx.doi.org/10.17576/jsm-2017-4602-04

11. Muttaqien A, Erwanto Y, Rohman A. Determination of buffalo and pig "rambak" crackers using FTIR spectroscopy and chemometrics. Asian Journal of Animal Science. 2016; 10: 49-58. http://dx.doi.org/10.3923/ajas.2016.49.58

12. Nurrulhidayah A, Rohman A, Amin I, Shuhaimi M, Khatib A. Analysis of chicken fat as adulterant in butter using Fourier transform infrared spectroscopy and chemometrics. Grasas y Aceites. 2013; 64(4): 349-355. http://dx.doi.org/10.3989/gya.072812

13. Rahayu WS, Rohman A, Martono S, Sudjadi S. Application of FTIR spectroscopy and chemometrics for halal authentication of beef meatball adulterated with dog meat. Indonesian Journal of Chemistry. 2018; 18(2): 376-381. https://doi.org/10.22146/ijc.27159

14. Rohman A, Che Man Y. Monitoring of virgin coconut oil (VCO) adulteration with palm oil using Fourier transform infrared spectroscopy. Journal of Food Lipids. 2009; 16(4): 618-628. https://doi.org/10.1111/j.1745-4522.2009.01170.x

15. Rohman A, Che Man Y. Analysis of chicken fat as adulterant in cod liver oil using Fourier transform infrared (FTIR) spectroscopy and chemometrics. CyTA-Journal of Food. 2011; 9(3): 187-191. https://doi.org/10.1080/19476337.2010.510211

16. Rohman A, Himawati A, Triyana K, Sismindari, Fatimah S. Identification of pork in beef meatballs using Fourier transform infrared spectrophotometry and real-time polymerase chain reaction. International Journal of Food Properties. 2017; 20(3): 654-661. https://doi.org/10.1080/10942912.2016.1174940

17. Suparman S. The use of Fourier transform infrared spectroscopy (FTIR) and gas chromatography mass spectroscopy (GCMS) for halal authentication in imported chocolate with various variants. Journal of Food and Pharmaceutical Sciences. 2015; 3(1): 6-11. https://doi.org/10.14499/jfps

18. Zhang Z, Lunter DJ. Confocal Raman microspectroscopy as an alternative to differential scanning calorimetry to detect the impact of emulsifiers and formulations on stratum corneum lipid conformation. European Journal of Pharmaceutical Sciences. 2018; 121: 1-8. https://doi.org/10.1016/j.ejps.2018.05.013

19. Guntarti A, Prativi SR. Application method of fourier transform infrared (FTIR) combined with chemometrics for analysis of rat meat (Rattus diardi) in meatballs beef. Pharmaciana. 2017;7(2):133-140. http://dx.doi.org/10.12928/pharmaciana.v7i2.4247

20. Lukitaningsih E, Sa'adah M, Rohman A. Quantitative analysis of lard in cosmetic lotion formulation using FTIR spectroscopy and partial least square calibration. Journal of the American Oil Chemists' Society. 2012; 89(8): 1537-1543. https://doi.org/10.1007/s11746-012-2052-8

21. Marikkar J, Lai O, Ghazali H, Che Man Y. Detection of lard and randomized lard as adulterants in refined-bleached-deodorized palm oil by differential scanning calorimetry. Journal of the American Oil Chemists' Society. 2001;78(11): 1113-1119. https://doi.org/10.1007/s11746-001-0398-5

22. Nurrulhidayah A, Arieff S, Rohman A, Amin I, Shuhaimi M, Khatib A. Detection of butter adulteration with lard using differential scanning calorimetry. International Food Research Journal. 2015; 22(2): 832-839.

23. Mansor T, Che Man Y, Shuhaimi M. Employment of differential scanning calorimetry in detecting lard

adulteration in virgin coconut oil. Journal of the American Oil Chemists' Society. 2012; 89(3): 485-496. https://doi.org/10.1007/s11746-011-1936-3

24. Azir M, Abbasiliasi S, Tengku Ibrahim TA, Manaf YNA, Sazili AQ, Mustafa S. Detection of lard in cocoa butter-its fatty acid composition, triacylglycerol profiles, and thermal characteristics. Foods. 2017; 6(11): 98. https://doi.org/10.3390/foods6110098

25. Indrasti D, Man YBC, Mustafa S, Hashim DM. Lard detection based on fatty acids profile using comprehensive gas chromatography hyphenated with time-of-flight mass spectrometry. Food Chemistry. 2010;122(4):1273-1277. https://doi.org/10.1016/j.foodchem.2010.03.082

26. Dahimi O, Hassan MS, Rahim AA, Abdulkarim SM, and Mashitoh S. Differentiation of lard from other edible fats by gas chromatography-flame ionisation detector (GC-FID) and chemometrics. Journal of Food and Pharmaceutical Sciences. 2014: 27-31. https://doi.org/10.14499/jfps

27. Marikkar JMN, Ghazali HM, Che Man YB, Peiris TSG, Lai OM. Distinguishing lard from other animal fats in admixtures of some vegetable oils using liquid chromatographic data coupled with multivariate data analysis. Food Chemistry. 2005a; 91: 5-14. https://doi.org/10.1016/j.foodchem.2004.01.080

28. Marikkar JMN, Ghazali HM, Che Man YB, Peiris TSG, Lai OM. Use of gas liquid chromatography in combination with pancreatic lipolysis and multivariate data analysis techniques for identification of lard contamination in some vegetable oils. Food Chemistry. 2005; 90(1-2): 23-30. https://doi.org/10.1016/j.foodchem.2004.03.021

29. Norrakiah A, Azim MS, Sahilah A, Salam BA. Halal analysis of raw materials, ingredients and finished bakery products using PCR and gene chip southern-hybridization for detection of porcine DNA. International Food Research Journal. 2015; 22(5): 1883-1887.

30. Aida AA, Che Man YB, Raha AR, Son R. Detection of pig derivatives in food products for halal authentication by polymerase chain reaction-restriction fragment length polymorphism. Journal of the Science of Food and Agriculture. 2007; 87: 569-572. https://doi.org/10.1002/jsfa.2699

31. Aida AA, Che Man YB, Wong CMVL, Raha AR, Son R. Analysis of raw meats and fats of pigs using polymerase chain reaction for Halal authentication. Meat Science. 2005; 69: 47-52. https://doi.org/10.1016/j.meatsci.2004.06.020

32. Farag MDEH. Detecting Adulteration in Halal Foods. In: Al-Teinaz YR, Spear S, Abd El-Rahim IHA (eds.). Halal Food. 2020. John Wiley & Sons. https://doi.org/10.1002/9781118823026.ch18

33. Abd-Gani S, Mustafa S, Desa MM, Mokhtar NK, Hanapi U, Zakaria Z, et al. Detection of porcine adulteration in cosmetic cream formulation via taqman probe real-time polymerase chain reaction. International Journal of Engineering and Technology. 2018; 7: 112-115. https://doi.org/10.14419/ijet.v7i4.14.27509

34. Salleh NAM, Hassan MS. 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. 2019; 1366: 1-12. https://doi.org/10.1088/1742-6596/1366/1/012114

35. Azim AA. Detection of pork and lard adulteration in food products using molecular biology techniques for halal authentication. Masters thesis. Universiti Putra Malaysia. 2007.