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Differentiation of Edible Fats from Selected Sources after Heating Treatments using Fourier Transform Infrared Spectroscopy (FTIR) and Multivariate Analysis

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Abstract. Lard consumption is forbidden for Muslim people and researches have been done to differentiate lard from other fats for *Halal* authentication. Therefore, this study was conducted to differentiate lard from other edible fats after heating treatments using Fourier transformed infrared spectroscopy (FTIR) and multivariate data analysis techniques. Five samples of fats from different sources; lard, chicken, mutton, tallow and palm based shortening were heated at different temperature (120, 180 and 240 °C) and time (30, 60, 120 and 180 min). The spectra in the form of multivariate data were acquired using FTIR spectroscopy. Principal components analysis (PCA), *k*-mean cluster analysis (*k*-mean CA) and linear discriminant analysis (LDA) were used to compare the ability of each technique to differentiate the fats after the heating treatments. It was found that the combination of PCA with *k*-mean CA was able to differentiate heated fats according to its origin. LDA method was successfully used to classify 80.5 % of samples in its group. Thus, PCA, CA and LDA can be used as multivariate data analysis to differentiate the heated edible fats.

INTRODUCTION

Edible fats have been used as energy source for human and animals. The production of bulk animal fats and poultry fats are intended for many purposes such as edible, animal feeds, and industrial applications. Fats obtained from animals or oils from plants undergo rendering process which requires certain temperature and time. Fats derived from animals often categorized as by-products from meat and the rendering process caused changes and destruction from the origin fats. However, the stability of fats against oxidation and other mechanisms such as heating and boiling process lead to the reduced quality of fats in animal species, diet, and feed quality [1].

Physically, fats rendered from different animals are in white solid after undergone rendering process involving crushing of raw fat tissues at low temperature. The main composition of fat is triacylglycerol (TAG) which is esterified fatty acids (FAs) with glycerol. FAs are divided into saturated monounsaturated and polyunsaturated types which composition differs in animals and plants. However, it is impossible to identify different species of animal fats by visualization especially after heated at extreme temperatures. The differentiation of animal fats is important to know the origin of animal species used for religious and regulatory purposes.

Lard is a fat derived from pork and has been used in the domestic and industrial products. However, lard consumption is forbidden among Muslim. Many kinds of research focus on classification of lard with other fats to establish the database for the *Halal* purpose. The discrimination between lard and other fats is possible by modern instrumentations. Such the instrumentations include nucleus magnetic resonance (NMR) [2, 3], gas chromatography mass spectroscopy (GCMS) [4], high pressure liquid chromatography (HPLC) [5, 6] and FTIR spectroscopy [7-10] that generate high multi dimensionalities data which are used to determine the *Halal* status of food products. A lot of work has been conducted using infrared (IR) spectroscopy and multivariate analysis in order to identify the origin of fats and oils [11-13].

Chemometric technique is by far the most commonly used for IR data processing. Principal component analysis (PCA) is the main technique to exploit huge data matrix into low-dimensional space and by axes known as principal components (PCs). In matrix representation, the model with a given number of components has the following equation:

$$X=TP^t + E \quad (1)$$

T is the score matrix, P is the loading matrix and E is the error matrix. Results of PCA are displayed by visualization of score plot and loading plot in order to relate sample and variable distribution in a dataset. From PCA the clustering process can be utilised for *k*-mean cluster analysis (*k*-mean CA) as unsupervised while LDA was used for supervised pattern recognition by introducing numbers of categories or groups before assembling the data. Training set and test set are validated to gain percentages of satisfactory LDA models. *k*-mean CA on the spectra data matrix is reduced to one dimension by matching similar pairs until all points in a single group are matched and display numbers of cluster group at observation or samples. Another classification is PLS method projection on latent structures such as partial least square discriminant analysis (PLS-DA), principal component regression (PCR), multiple linear regression (MLR) technique, and support vector machines (SVMs) which are not discussed in this paper. Classifying lard by spectral analysis has been proven successfully solved with chemometrics techniques [14-17].

Heating treatments have been carried out to evaluate differentiation of various frying oils at certain temperatures and periods using multivariate technique. The effect of long heating time for classification of canola, corn, and sunflower was successfully calibrated at 94% cross by canonical variate analysis (ECVA) compared to PCA and PLSDA for the heating process times of 24 – 27 and 33 – 36 hours at 170 °C [18]. The heating treatment on Algerian extra virgin olive oil at 60 °C and evaluated during 90 days using PLS models was able to distinguish the different oils' regions quality [19]. To the best of our knowledge, varying parameters of heating treatments on animal's fats for *Halal* determination have scarcely been studied by multivariate technique.

Therefore a substantial study focused on the changes in chemical functional groups of lard compared to the other edible fats in order to develop a model to understand the relative importance of heating parameter effects. The main observation is to differentiate fats from animal's species which are physically solid at room temperature and shortening based palm oils as well. Considering the fact that the multivariate data involved high dimensionality in monitoring the different types of fats, temperatures and heating time to access information, suggested that PCA [20] *k*-mean CA [21], and LDA could be used for evaluating the best selection multivariate application. The main objective of this research is to classify lard and other edible fats after heating treatments using FTIR data by multivariate analysis.

MATERIALS AND METHODS

Samples and Heating Procedures

Lard, chicken, mutton, tallow and shortening based palm oil were purchased from a local supermarket at Nilai, Negeri Sembilan. Fat was separated from meat residues, minced and weighted at 100 g in a 250 ml quartz beaker. Heating of fat was done using a digital hotplate with temperature probe contact to the fat in the beaker. Frying protocols were applied with the heating time 30, 60, 120 and 180 minutes at 120 °C, 180 °C and 240 °C as shown in Table 1. Approximately 20 g w/w of heated fats in triplicate of each treatment were cooled at room temperature before being kept in a universal bottle and refrigerated at 4 °C. Raw samples of each edible fats were included as a blank.

TABLE 1. Marking of 5 type edible fat with temperatures and times

TEMPERATURES (°C)	raw	120				180				240			
TIMES (MINUTES)		30	60	120	180	30	60	120	180	30	60	120	180
Lard (L)	L0	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12
Tallow (B)	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
Mutton fat (M)	M0	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Chicken fat (C)	C0	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
Shortening (V)	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12

Fat Extraction

Fats were extracted using Folch *et al.* method [22]. The fat tissues were homogenized with chloroform:methanol (2:1) to a final volume 20 times of the tissues sample (10 g in 200 ml of solvent mixture). After dispersion, the whole mixture was agitated for 15-20 min in a shaker at room temperature. The homogenate was filtrated by funnel with a filter paper to recover the liquid phase. Liquid phase contained chloroform/methanol was washed with 2 volume of distilled water contained 0.9% w/v of NaCl and left for 30 minutes until the mixtures separated into two layers. The lower chloroform phase containing lipids were collected and dried under vacuum in a rotary evaporator.

Acquisition of Spectra in the Infrared (FTIR)

The infrared spectra were acquired on a Perkin Elmer model Spectrum 400 FTIR Spectrometer using attenuated total reflectance technique (ATR-FTIR). A range from 4,000 to 650 cm^{-1} was scanned, with a resolution of 4 cm^{-1} and 32 scans. Background of air spectrum was taken for after each scan. The spectra of each sample were acquired with replications.

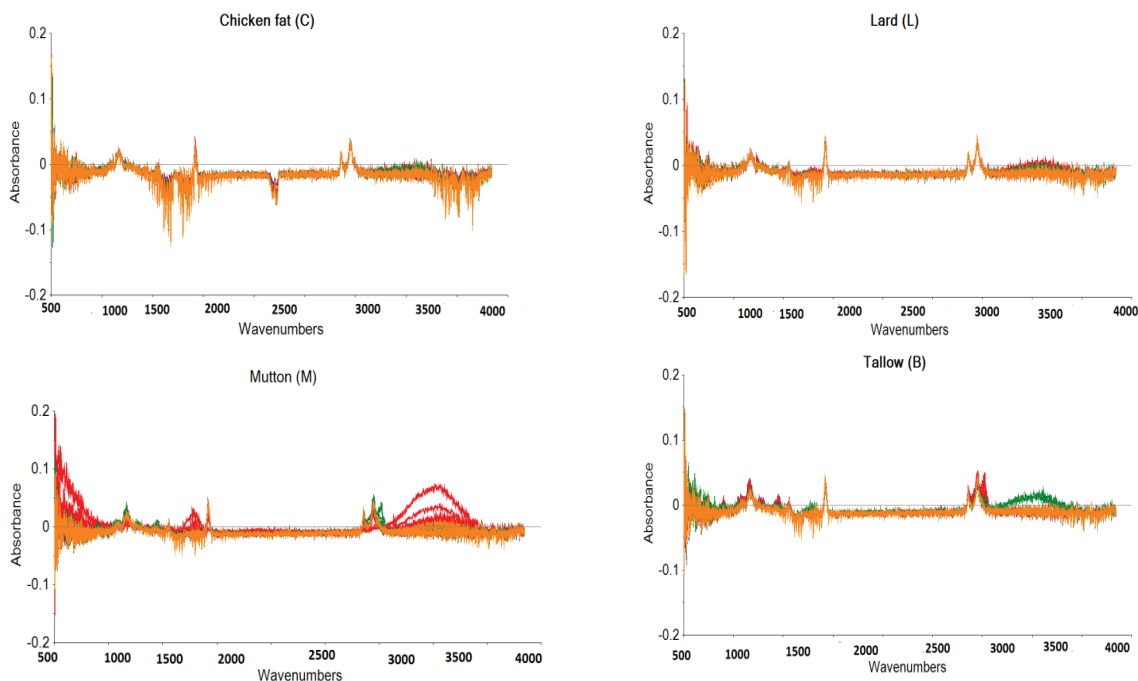
Multivariate Data Analysis

The obtained spectra were subjected to PCA, *k*-mean CA and LDA using chemometrics software Unscrambler® 10.3 X by CAMO USA.

RESULTS AND DISCUSSION

Raw Data

The entire spectra range 4000-650 cm^{-1} was used as starting point for spectral acquisition (Fig. 1). Mutton fat (M) and tallow (B) samples show high intensity between 3100 and 3340 cm^{-1} . Chicken fat (C) shows high noisy at 3359 – 3390, 2240 – 2250 and 1230 – 1900 cm^{-1} . Observation at 2800 – 3200 cm^{-1} shows a similarity between chicken and lard by two identical peaks. Both mutton and tallow fats display split spectral peaks at these frequencies which are required pre-processing data before applying chemometrics.



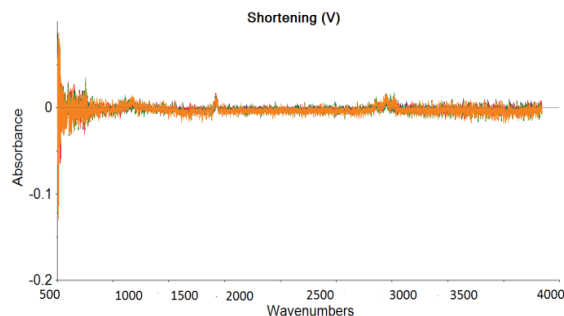


FIGURE 1. Raw data of FTIR from edible fats at spectra range 4000-650 cm^{-1} after heating treatments

The typical spectra of edible fats by absorption band for common TAG shown in Fig. 1 illustrates that bands at 2922 and 2852 cm^{-1} can be associated with the stretching vibrations of methylene ($-\text{CH}_2$) and methyl ($-\text{CH}_3$) groups, respectively. Methylene group shows bands at 1465 and 1375 cm^{-1} due to bending vibrations. The sharp carbonyl $\text{C}=\text{O}$ absorption peak of ester linkage at 1740 cm^{-1} clearly observed for all animals fat. Five important wavenumbers were observed at 1235, 1160, 1117 1098 and 721 cm^{-1} are overlapping ($-\text{CH}_2$) by rocking vibrations shows the bending of *cis* disubstituted olefin [23]. Shortening (V) has almost lowest intensity at these frequencies than others edible fats. This suggested that fat is undergone hydrogenation process and lost its origin characteristics. The wavenumbers of 1500-1000 cm^{-1} have been used as the fingerprint for edibles oil classification by previous study [24-26].

Pre-processing Data

The pre-processing raw data stage is aimed to remove variation within the data that does not pertain to the analytical information. Dataset of 195 spectra measured at 1743 different wavenumbers containing 339 885 data points. Spectra were normalized to produce an intensity range from 0 to 1 and hence easy to read. Then spectra were treated using the Savitzky–Golay second derivative procedure with a second-order polynomial and a 15-point window as pre-processing tools for multivariate analysis [27-29] (Fig. 2) to remove noise. Chicken fat shows dominant at wavenumbers 3488 – 3980, 2160 – 2300 and 1200 – 1900 cm^{-1} .

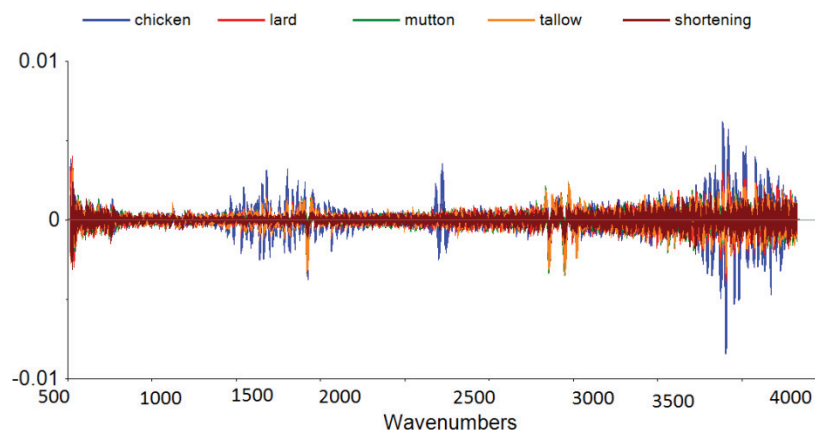


FIGURE 2. FTIR spectra of all edibles fats after pre-processing using the Savitzky–Golay second derivatives

Principal Components Analysis (PCA)

The spectral ranges at 715 cm^{-1} and above were selected because the other regions (650 – 714 cm^{-1}) have no spectral information because of the unvarying spectrum. The pre-processed FTIR spectral were projected by the PCs axes to reduce dimensions. Separation different types of edible fats were observed only through scores plots. The combination of results of Scores plots between the PCs: PC1 versus PC2, and PC2 versus PC3, are evaluated (Fig. 3).

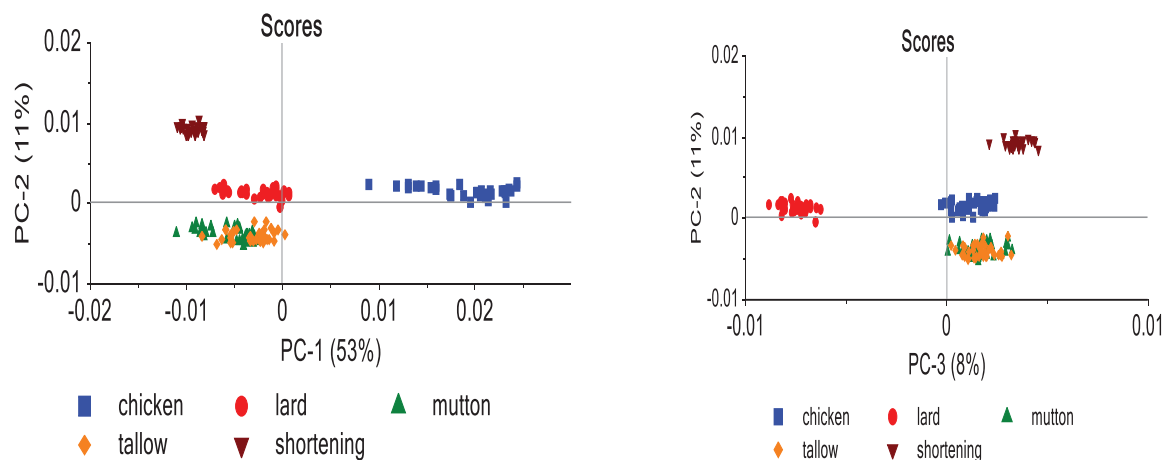


FIGURE 3. Score plots of PC1 versus PC2 and PC2 versus PC3 of edibles fats after heating treatments

Through the scores plots from PCA, it was observed that 71% of data variances were explained by the analysis of the first three PCs. Score plot (PC1 versus PC2) separates the chicken samples in the positive values while others samples in negative values at PC1. On the other hand, score plot (PC3 versus PC2) separates lard in negative values while other fats in positive values at PC3. Loadings plots (defined frequencies regions) were displayed poorly and unable to identify specific frequencies regions. Differentiation of lard with other animal fats and vegetable oils using PCA are possible by selecting specific frequencies regions [30] contrast with these results. However, the other previous study [31] was done using full spectra to discriminate various fats with lard without specifying frequencies regions. PLS technique has been suggested in spectral analysis to identify specific frequencies regions that contribute classification of edible fats [8, 34]. Meanwhile, the samples of mutton and tallow were found to be scattered in one cluster for both Score plots. Four groups of edible fats were utilized further in the *k*-mean CA and LDA classification method.

***k*- mean CA**

The Euclidean distance method was used to obtain the CA. The separation among the edible fats by CA is more objective because it provides numerical results. For convenience visualisation, *k*-mean CA was plotted using dendrogram and produced relative distance at scale 7.5 into four clusters (Fig. 4). All fats are classed accordingly except mutton (M) and tallow (T) where they are classed into one same cluster. Pure lard, beef fats, and chicken fats were successfully discriminated by *k*-mean CA [21]. Tallow and mutton were systematically clustered in one group by CA [30].

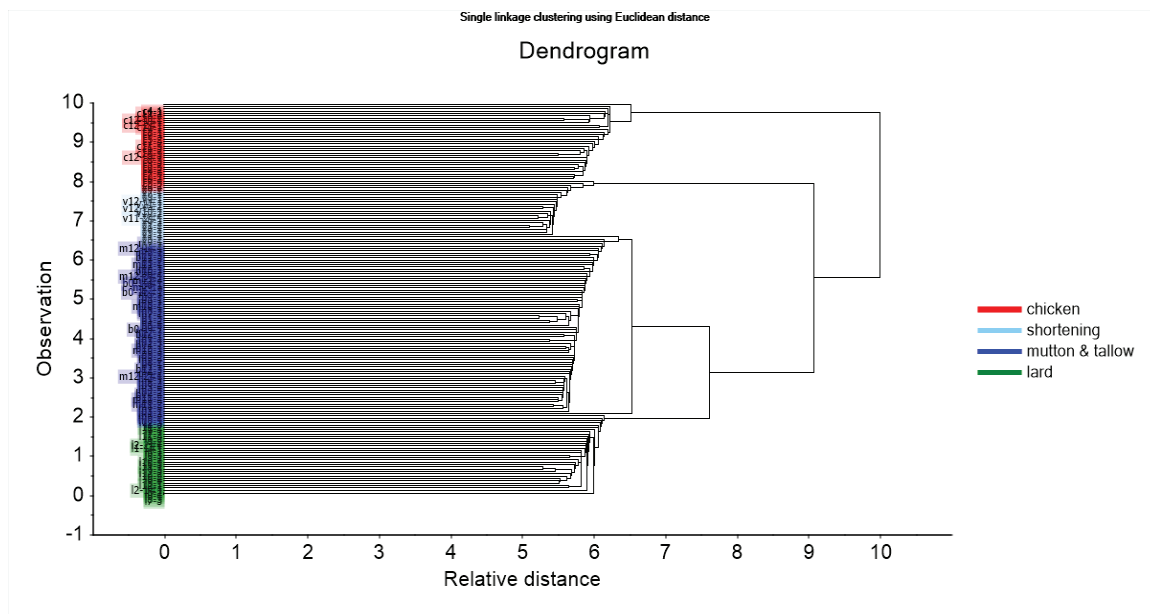


FIGURE 4. Dendrogram illustrating the *k*-mean clustering of edible fats after heating treatments according to classes

Linear Discriminant Analysis (LDA)

The discrimination plot is a visualization of the LDA results for the training samples 5 types of edible fats (Fig. 5). Edible fats are classified by colour-coded. The axes are for two of the classes lard and chicken in the model. Lard and chicken lying close to zero for a class are associated with the class.

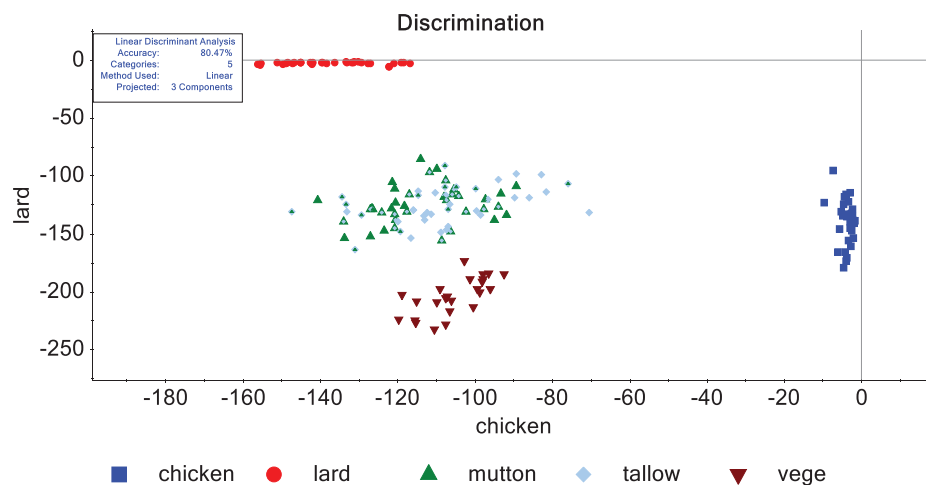


FIGURE 5. LDA Model—Distance between the classes for the training set of edible fats samples after heating treatments. (Class I versus Class II: chicken and lard)

Table 2 presents the results achieved for the testing samples. The results proved satisfactory when 80.47% is correctly classified for the spectra of the samples of the batch of the test set.

TABLE 2. LDA Model—Distance between the classes for testing fats samples

Classified Test set	Chicken	Lard	Mutton	Tallow	Shortening	Class
11-1#2	-130.19	-1.83	-125.97	-125.81	-200.25	Lard
12-1#2	-141.83	-1.97	-123.86	-123.70	-219.14	Lard
12-2#2	-149.69	-3.37	-129.72	-129.00	-239.70	Lard
c12-1#2	-2.56	-132.52	-108.40	-104.20	-86.43	Chicken

c12-2#2	-2.79	-132.99	-104.32	-100.27	-85.81	Chicken
c12-3#2	-5.94	-166.25	-159.77	-153.25	-111.44	Chicken
m12-1#2	-109.89	-93.93	-4.25	-5.35	-198.08	Mutton
m12-2#2	-117.65	-131.12	-2.95	-2.24	-245.37	Tallow
m12-3#2	-106.28	-148.30	-3.79	-2.99	-228.25	Tallow
b0-1a#2	-147.29	-131.30	-5.01	-5.38	-268.30	Mutton
b0-1#2	-107.25	-146.64	-3.52	-2.77	-228.96	Tallow
b0-1b#2	-134.40	-117.90	-3.30	-4.37	-235.75	Mutton
v11-2#2	-99.31	-198.30	-212.27	-216.97	-1.69	Shortening
v12-1#2	-107.08	-204.67	-225.98	-230.92	-1.80	Shortening
v12-2#2	-92.45	-185.41	-198.56	-203.07	-2.14	Shortening

Label – l: lard, c: chicken fat, m: mutton, b: tallow & v: shortening.

Mutton and tallow were not correctly classified. This might be because they are in the same cluster in the score plots. Both fats have correlated at score plots by the composition of saturated and unsaturated FAs [3] and TAG [32] indicated mutton and tallow fats have similar chemical composition. Previous studies [33-35] have proven 100% correctly classified of lard and others edible fats using discriminant analysis based on PLS techniques.

CONCLUSIONS

The present paper suggests it is possible to develop a methodology to identify edible fats selected sources after heating treatments using FTIR and multivariate data analysis techniques. Upon comparing FTIR spectra of lard with other edible fats using the PCA, *k*-mean CA and LDA it becomes evident that lard, chicken and shortening based palm oil can be identified although after being treated at different temperatures for several periods of time. However, mutton and tallow showed the same clustering for all the multivariate techniques which make both fats cannot be identified. Both fats also decreased the percentage of LDA with only 80.5% correctly classification of all the heated edibles fats samples.

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REFERENCES

1. P. Joseph, in *Oxidative Stab. Shelf Life Foods Contain. Oils Fats* (Elsevier, 2016), pp. 233–249.
2. G. Fang, J. Y. Goh, M. Tay, H. F. Lau and S. F. Y. Li, *Food Chem.* **138**, 1461- 1469 (2013).
3. N. A. Fadzillah, A. Rohman, R. A. Salleh, I. Amin, M. Shuhaimi, M. Y. Farahwahida, O. Rashidi, J. M. Aizat and A. Khatib, *Int. J. Food Prop.* **20**, 2147-2156 (2017).
4. A. N. Nina Naquiah, J. M. N. Marikkar and D. M. Hashim, *J. Oleo Sci.* **62**, 459-464 (2013).
5. N. A. M. Yanty, J. M. N. Marikkar and M. S. Miskandar, *Grasas Y Aceites* **63**, 328-334 (2012).
6. K. A. AL-Rashood and R. R. Abou-Shaaban, *J. Am. Oil Chem. Soc.* **73**, 303-309 (1996).
7. Z. A. Syahariza, Y. B. Che Man, J. Selamat and J. Bakar, *Food Chem.* **92**, 365-371 (2005).
8. E. Kurniawati, A. Rohman and K. Triyana, *Meat Sci.* **96**, 94-98 (2014).
9. Y. Erwanto, A. T. Muttaqien, Sugiyono, Sisindari and A. Rohman, *Int. J. Food Prop.* **19**, 2718-2725 (2016).
10. Y. B. Che Man, Z. A. Syahariza, M. E. S. Mirghani, S. Jinap and J. Bakar, *Food Chem.* **90**, 815-819 (2005).
11. K. Javidnia, M. Parish, S. Karimi and B. Hemmateenejad, *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **104**, 175-181 (2013).
12. P. de la Mata, A. Dominguez-Vidal, J. M. Bosque-Sendra, A. Ruiz-Medina, L. Cuadros-Rodríguez and M. J. Ayora-Cañada, *Food Control* **23**, 449-455 (2012).
13. A. Rohman, S. Riyanto, A.M. Sasi and F.M. Yusof, *Food Biosci.* **7**, 64-70 (2014).
14. A. Rohman and Y. B. Che Man, *Chemom. Intell. Lab. Syst.* **110**, 129-134 (2012).
15. A. Rohman and Y. B. Che Man, *Scientific World Journal.* **2012**, 250795 (2012).

16. G. Ozulku, R. M. Yildirim, O. S. Toker, S. Karasu and M. Z. Durak, [Food Control](#) **82**, 212-216 (2017).
17. A. Rohman and Y. B. Che Man, [Eur. J. Lipid Sci. Technol.](#) **113**, 522-527 (2011).
18. K. Javidnia, M. Parish, S. Karimi and B. Hemmateenejad, [Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.](#) **104**, 175-181 (2013).
19. R. Selaimia, R. Oumeddour and S. Nigri, [Int. Food Res. J.](#) **3**, 1301-1307 (2017).
20. O. Dahimi, A. A. Rahim, S. M. Abdulkarim, M. S. Hassan, S. B. T. Z. Hashari, A. S. Mashitoh and S. Saadi, [Food Chem.](#) **158**, 132-138 (2014).
21. O. Dahimi, M. S. Hassan, A. A. Rahimm, S. M. Abdulkarim and A. S. Mashitoh, [J. Food Pharm. Sci.](#) **2**, 27-31 (2014).
22. J. Folch, M. Lees and G.H.S. Stanley, [J. Biol. Chem.](#) **226**, 497-509 (1957).
23. M. D. Guillén and N. Cabo, [J. Am. Oil Chem. Soc.](#) **74**, 1281-1286 (1997).
24. M. A. Allam and S. F. Hamed, [J. Appl. Sci. Res.](#) **73**, 102-108 (2007).
25. A. Rohman and Y. B. Che Man, [Food Res. Int.](#) **43**, 886-892 (2010).
26. A. Rohman, Y. B. Che Man, A. Ismail and P. Hashim, [J. Am. Oil Chem. Soc.](#) **87**, 601-606 (2010).
27. N. Dupuy, L. Duponchel, J. P. Huvenne, B. Sombret and P. Legrand, [Food Chem.](#) **57**, 245-251 (1996).
28. D. Cozzolino, I. Murray, A. Chree and J. R. Scaife, [LWT - Food Sci. Technol.](#) **38**, 821-828 (2005).
29. C. Shiroma and L. Rodriguez-Saona, [J. Food Compos. Anal.](#) **22**, 596-605 (2009).
30. Y. B. Che Man, A. Rohman, and T. S. T. Mansor, [J. Am. Oil Chem. Soc.](#) **88**, 187-192 (2011).
31. S. Bellorini, S. Strathmann, V. Baeten, O. Fumière, G. Berben, S. Tirendi and C. Von Holst, [Anal. Bioanal. Chem.](#) **382**, 1073-1083 (2005).
32. A. Rohman, K. Triyana and Y. Erwanto, [Int. Food Res.](#) **19**, 475-479 (2012).
33. Y. B. Che Man, A. M. Marina, A. Rohman, H. A. Al-Kahtani and O. Norazura, [Int. J. Food Prop.](#) **17**, 354-362 (2014).
34. A. Rohman and Y. B. Che Man, [Int. Food Res. J.](#) **17**, 519-526 (2010).
35. A. Rohman and Y. B. Che Man, [Agritech.](#) **28**, 192-201 (2008).