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Lard classification from other animal fats using Dielectric Spectroscopy technique

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Abstract

Lard adulteration in processed foods is a major public concern as it involves religion and health. Most lard discriminating works require huge lab-based equipment and complex sample preparation. The objective of the present work was to assess the feasibility of dielectric spectroscopy as a method for classification of fats from different animal sources, in particular, lard. The dielectric spectra of each animal fat were measured in the radio frequency of 100 Hz – 100 kHz at 45°C to 55°C. The fatty acid composition of each fat was studied by using data from gas chromatography mass spectrometry (GCMS) to explain the dielectric behaviour of each fat. The principal component analysis (PCA) and artificial neural network (ANN) were used to classify different animal fats based on their dielectric spectra. It was found that lard showed the highest dielectric constant spectra among other animal fats, and was mainly affected by the composition of C16 and C18 fatty acids. PCA classification plot showed clear performance in classifying different animal fats. Finally, ANN classification showed different animal fats were classified into their respective groups effectively at high accuracy of 85%. Dielectric spectroscopy, in combination with quantitative analysis, was concluded to provide rapid method to discriminate lard from other animal fats.

Introduction

In 2015, IHS Inc. (Information Handling Services) reported that animal-based fats and oils such as butter, lard and tallow had been ranked 5th, 6th and 7th most consumed fats and oils globally (Manning, 2016). Shockingly, the food industry blended lard with plant oils in order to achieve lower production costs as well as to produce cost-effective shortenings, margarines and variety food products that contain oil (Aida *et al*., 2005). Lard adulteration is a very sensitive and serious problem because it involves religious faith. The consumption of pig or its product in any form is prohibited for both Muslims and Jews (Siddiqui, 2012). In addition, Hindus, Buddhists and consumers who practice vegetarian will also be affected by this.

Several approaches to distinguish halal food products (i.e. suitable to be consumed by Muslims and Jews) had been introduced in previous studies. These approaches can be split into two categories which are the labelling-based and the spectral-based. Polymerase Chain Reaction (PCR) is an example of the labelling-based method which observes the presence of DNA, and has been utilised to study the pig's meat and fat (Aida *et al*., 2005), and the pig's derivatives in foods including breads, biscuits and sausages (Che Man *et al*., 2007). The spectral-based method is basically a method that obtains physical and chemical information from the interaction of molecules with electromagnetic radiation (EMR) (Sneddon, 2002) which includes electronic nose (E-Nose), liquid chromatography, differential scanning calorimetry

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(DSC), gas chromatography and spectroscopy. E-nose technology has been utilised in the detection of pig's meat in foods and lard adulteration in palm oil by evaluating the odour pattern (Che Man *et al*., 2005a; Nurjuliana *et al*., 2011). DSC has been used in the detection of the presence of lard/randomised lard as adulterants in RBD palm oil (Marikkar *et al*., 2001). There are several types of gas chromatography techniques that have been used in the halal product analysis including gas chromatography-flame ionisation detector (GC-FID) used to differentiate lard from other edible fats (Dahimi *et al*., 2014), Gas chromatography coupled with time-of-flight mass spectrometry (GC-ToF) has been used to detect lard based on fatty acid profile (Indrasti *et al*., 2010), and gas chromatography mass spectrometry (GCMS) and element analyser-isotope ratio mass spectrometry (EA-IRMS) h used to distinguish chicken, pig, mutton and beef fats (Ahmad Nizar *et al*., 2013). Liquid chromatography technique has been used to differentiate lard from different animal fats in the presence of plant oils (Marikkar *et al*., 2005). Fourier Transform Infrared (FTIR) spectroscopy is an example of a spectroscopy technique which has been vastly used in halal verification and detection such as in chocolates (Che Man *et al*., 2005b), meatballs (Rohman *et al*., 2011), cod-liver oils (Rohman and Che Man, 2009), cake formulation (Syahariza *et al*., 2005), fat mixtures (Rohman and Che Man, 2011), inks for food packaging (Ramli *et al*., 2015) and many more. The above mentioned approaches to distinguish halal food products require huge lab equipment and complex sample preparation using expensive chemicals and reagents.

Research works in dielectric study can be categorised into two; non-spectroscopic and spectroscopic. The non-spectroscopic dielectric study analyses material's dielectric properties at a specific frequency (Bengtsson and Risman, 1971; Ohlsson *et al*., 1974; Rzepecka and Pereira, 1974; O'Connor and Synnott, 1982; Ndife *et al*., 1998; Liao *et al*., 2003a; 2003b), while the spectroscopic dielectric study utilises and analyses dielectric data of the materials in a range of frequency. In analysing dielectric data, statistical analysis on dielectric spectra can be utilised for sample characterization and classification. Studies have been conducted on several materials including foods (Nelson, 1991; Bodakian and Hart, 1994; Feng *et al*., 2002; Wang *et al*., 2003; 2005; 2008; Nelson, 2005; Nunes *et al*., 2006; Ahmed *et al*., 2007; Zhuang *et al*., 2007; Agranovich *et al*., 2016) and non-foods (Arai *et al*., 1992; Mijovic and Fitz, 1998; Hollertz, 2014). Compared to conventional techniques in halal authentication, which are lab-based, time-consuming and require multiple chemicals and processes for sample preparation, dielectric spectroscopy offers a simpler method to capture data from sample. With simple sample preparation, dielectric spectroscopy technique promotes a rapid and low-cost lard classification method.

Most of dielectric spectroscopy works studied the dielectric properties of vegetable oils (Bansal *et al*., 2001; Lizhi *et al*., 2008; 2010; Cataldo *et al*., 2009; Shah and Tahir, 2011). Only one work was found using dielectric spectroscopy to study animal fats, which was at a high radio frequency (Sucipto *et al*., 2013). Nevertheless, the research in classifying lard from other animal fats at a low radio frequency is yet to be explored.

In the present work, dielectric spectroscopy technique at radio frequency was used as an approach to classify lard from other edible animal fats. The present work was aimed to access the feasibility of dielectric spectroscopy as a straightforward and rapid tool for classification of oil from different animal sources. The spectroscopy measurements were run at a low frequency range (100 Hz – 100 kHz), hence minimum cost was required for the instrumentation for industrial application. With that, the present work provided significant help in the development of rapid and portable halal detection system for halal regulators in ensuring efficient halal food manufacturing.

Materials and methods

Sample preparation

A total of 20 adipose tissues of animals with five of each of lard, chicken fat, mutton tallow and beef tallow were rendered to extract the fats. The adipose tissues were collected from local markets. The rendering was conducted following the method proposed by Marikkar *et al*. (2001). To remove impurities, the melted fats were filtered through double-folded muslin cloth. The moisture in the samples was removed by filtering through Whatman No. 4 filter paper with anhydrous sodium sulphate. Filtered samples were kept in tightly closed container at -20°C until further analysis. All animal fats were thawed in a temperature-controlled chamber at 45°C before being used for analysis. Analytical grade chemicals were used in the present work.

Fatty Acid Methyl Ester (FAME) analysis

The preparation of FAME was conducted following the JOCS Standard Method 2.4.1.3-2013 (Japan Oil Chemists' Society, 1996) by dissolving 50 mg fat in 0.8 mL hexane, and converted to FAME by adding 0.2 mL 1 M sodium methoxide. Hydrolysis and derivatization reactions took place through a 1-min vortex. The clear upper layer supernatant of the solution was transferred into a 2 mL vial before proceeded to GCMS analysis. Standard FAME of 37 compounds (C4 to C24) was purchased from Supelco, Sigma-Aldrich, Bellefonte, PA. Chromatography grade of n-hexane and sodium methylate were purchased from Merck Chemicals (Darmstadt, Germany).

The determination of fatty acid composition was done using Agilent 7890A gas chromatography (Agilent Technologies, Palo Alto, CA, USA) equipped with Agilent 5975 mass spectrometry detector (Agilent Technologies, Palo Alto, CA, USA) and a polar capillary column HP88 with 0.25 mm internal diameter, 100 m length and 0.25 µm film thickness (Agilent Technologies, USA). Samples were injected with a split ratio of 30:1 using helium as a carrier gas at a flowrate of 0.8 mL/min. The initial temperature was set at 150°C for 5 min, and programmed to increase to 240°C at 4°C/min. The final temperature was maintained at 240°C for 15 min. The analysis was conducted at three injection replications. The run time for one injection was 42.5 min. The FAME peaks were identified by comparing their retention time with certified reference standards of FAME. The percentage of fatty acid was calculated based on the peak area of a fatty acid species to the total peak area of all the fatty acids in the fat sample.

Dielectric spectroscopy measurement

Dielectric constant (ε') of 3.4 mL samples were measured using a liquid test fixture (Agilent, 16452A, Agilent Technologies, Hyogo, Japan) and an LCR meter (Agilent 4263B, Agilent Technologies, Hyogo, Japan) that was connected to a personal computer for control and data logging. The ε' were measured at six discrete frequencies; 100 Hz, 120 Hz, 1 kHz, 10 kHz, 20 kHz and 100 kHz (Sairin *et al*., 2015). The dielectric constant of the samples was measured at 45, 50 and 55 ± 0.1°C in a temperature-controlled chamber (Espec SU221, Michigan, USA) to make sure all of the samples were in liquid form when the measurement was taken.

The measurement was performed in three replications. The average of the three replications was calculated and used for statistical analysis. The experiment was randomly conducted at three different temperatures. After each sample was measured, the liquid test fixture was cleaned. For measurement of lard sample, the equipment was appropriately and thoroughly cleaned based upon the Islamic requirements standard (Department of Standards Malaysia, 2009; 2010).

Statistical analysis

Relative standard deviation (RSD) was utilised to characterise the ability of measurement and validate precision of the technique, and was measured in percentage. One-way ANOVA analysis with Tukey's range test was conducted as the classification method where the former gave the significance difference among different sample groups while the latter classified them into groups. In the present work; lard, chicken fat, beef tallow and mutton tallow were the sample groups. The effects of frequency and temperature on dielectric properties of animal fats were also determined and $p < 0.05$ was defined as significant difference.

Multivariate data analysis, Principal Component Analysis (PCA), was used to study the variation and classify the dielectric data. In the present work, multiple frequencies coupled with three different temperatures created 18 variables, which were known as multivariate. PCA classification of the dielectric spectral data was done by using MATLAB toolbox. Other than that, Artificial Neural Network (ANN) was also used to get a comparison of different classification performance. ANN classification of the dielectric spectral data was done by using Weka 3.8.1 software.

Results and discussion

Precision of method

The precision of the method was computed using RSD on three replicates measurement. From the calculation, the RSD achieved was lower than 0.07%, which met the limit established by the Food and Drug Administration of 15% (United States Food and Drug Administration, 2001). Therefore, the measured dielectric data for each sample can be concluded to have small variation and high precision. It also showed that the method had good repeatability and reproducibility.

Fatty acid methyl ester (FAME) composition

The saturation level of the fatty acid chains can be used to differentiate lard from other fats and oils (Che Man *et al*., 2011). Table 1 lists the fatty acid compositions of animal fats. Due to the similarity in fatty acid composition, it was not possible to differentiate lard from other fats by using fatty acid profile alone. Dielectric spectroscopy in combination with statistical analysis technique of PCA was used as a tool for such differentiation.

Table 1 shows the fatty acid composition of the animal fats classified according to their fatty acid length and saturation. Based on the table, methyl trans-9-octadecenoate (C18:1 n9t) and methyl cis-9-otadecenoate (C18:1 n9c) were observed as the highest peaks in all samples, i.e.: 39.5% - 42.5% and 16.5% - 19.0%, respectively, which is in accordance to Marikkar *et al*., 2001; Nurjuliana *et al*., 2011; Ahmad Nizar *et al*., 2013. Certain fatty acids were shown to be high in particular animal fats such as C18:0 in mutton (15.6%) and beef tallow (13.2%); and C18:2 n6c in chicken fat (16.5%) and lard (17.6%).

From the fatty acid composition, each fatty acid was grouped into different length. Each fatty acid length included saturated, unsaturated and cis-trans isomers. In all samples, C18 fatty acid contributed to the highest percentage (77% - 89%) followed by C16 fatty acid (7% - 13%). Since C18 yielded the highest composition in fatty acid mixture, the physico-chemical properties such as melting points, solubility and many more should be mainly affected by the C18 fatty acids. This agrees with Lizhi *et al*. (2008).

Table 1. Fatty acid methyl ester (FAME) composition of animal fats.

	Lard	Chicken fat	Mutton tallow	Beef tallow
c4:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
c6:0	0.02 ± 0.02	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
c8:0	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.01	0.01 ± 0.00
c10:0	0.06 ± 0.01	0.06 ± 0.06	0.14 ± 0.07	0.08 ± 0.00
c11:0	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
c12:0	0.09 ± 0.02	0.88 ± 1.45	0.08 ± 0.01	0.09 ± 0.01
c13:0	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.00
c14:0	0.77 ± 0.08	1.20 ± 0.90	1.85 ± 0.33	2.96 ± 0.13
c14:1	0.00 ± 0.00	0.78 ± 0.92	0.04 ± 0.03	1.42 ± 0.47
c15:0	0.08 ± 0.02	0.18 ± 0.24	0.92 ± 0.27	0.86 ± 0.12
c15:1	0.02 ± 0.00	0.01 ± 0.00	0.05 ± 0.08	0.00 ± 0.00
c16:0	7.22 ± 0.37	8.90 ± 1.09	9.99 ± 1.85	10.88 ± 0.27
c16:1	0.76 ± 0.13	3.34 ± 0.18	0.80 ± 0.02	3.00 ± 0.71
c17:0	0.17 ± 0.16	0.16 ± 0.27	3.00 ± 1.12	2.10 ± 0.22
c17:1	0.08 ± 0.07	0.02 ± 0.04	0.58 ± 0.61	0.04 ± 0.03
c18:0	4.01 ± 0.74	2.62 ± 0.79	15.57 ± 3.19	13.16 ± 1.95
c18:1n9t	39.71 ± 5.19	40.24 ± 5.54	42.49 ± 5.18	39.51 ± 2.38
c18:1n9c	16.52 ± 2.32	19.04 ± 1.30	18.95 ± 2.65	18.77 ± 2.42
c18:2n6t	4.98 ± 4.73	0.06 ± 0.09	0.27 ± 0.24	0.06 ± 0.05
c18:2n6c	17.56 ± 3.52	16.50 ± 9.13	1.33 ± 0.53	0.81 ± 0.07
c20:0	0.30 ± 0.08	0.16 ± 0.06	0.17 ± 0.07	0.19 ± 0.07
c18:3n6	2.55 ± 1.95	3.00 ± 0.08	1.84 ± 0.49	2.78 ± 0.36
c18:3n3	4.28 ± 0.98	2.36 ± 1.00	1.12 ± 0.59	2.59 ± 0.24
c20:1	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.02	0.00 ± 0.00
c21:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
c20:2	0.02 ± 0.00	0.01 ± 0.01	0.06 ± 0.01	0.04 ± 0.04
c22:0	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
c20:3n6	0.21 ± 0.02	0.09 ± 0.01	0.09 ± 0.05	0.29 ± 0.22
c20:3n3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.00
c22:1n9	0.12 ± 0.02	0.09 ± 0.01	0.13 ± 0.08	0.09 ± 0.02
c20:4n6	0.31 ± 0.09	0.18 ± 0.06	0.19 ± 0.06	0.17 ± 0.06
c23:0	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
c22:2n6	0.01 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.00
c20:5n3	0.03 ± 0.01	0.02 ± 0.01	0.15 ± 0.30	0.02 ± 0.02
c24:0	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
c24:1n9	0.01 ± 0.00	0.00 ± 0.00	0.04 ± 0.08	0.00 ± 0.00
c22:6n3	0.06 ± 0.03	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.02
∑SFA	12.76	14.2	31.81	30.35
∑MUFA	57.22	63.52	63.09	62.83
∑PUFA	30.01	22.27	5.11	6.82

Figure 1(a) illustrates the distribution of C18 fatty acid concentration in different animal fats. C18 here included all C18 saturations i.e., C18:0, C18:1, C18:2, C18:3 and their isomers. Beef tallow had the highest concentration of C18, followed by mutton tallow, chicken fat and lard ($p < 0.05$). Figure 1(b) illustrates the distribution of C16 fatty acid concentration in animal fats. C16 here included all C16 saturations i.e., C16:0, C16:1 and their isomers. Beef had the highest concentration of C16, while chicken fat and mutton had almost equal concentration of C16 fatty acid, and lard had the lowest concentration of C16 ($p < 0.05$).

Based on the composition of saturated and unsaturated fatty acids for all animal fats, it was observed that both beef tallow and mutton tallow had higher saturated fatty acids composition than chicken fat and lard, while both lard and chicken fat had higher unsaturated fatty acids than beef and mutton tallow. From the composition of different saturation of C18 fatty acids in different animal fats, it was observed that C18:1 was the highest composition that contributed to at least 50% of the population of C18 while C18:2 and C18:3 contributed to lower percentages. C18:1 was observed to have equal composition among different animal fats ($p < 0.05$). C18:2 on the other hand, showed significant difference in composition between different animal fats ($p < 0.05$). Lard had the highest C18:2 followed by chicken fat, mutton tallow and beef tallow. Lard was also observed to have higher C18:3 as ecompared to other animal fats.

Dielectric spectral

The dielectric spectra of lard, chicken fat, beef tallow and mutton tallow studied across the frequency appeared similar. Lard was observed to have higher dielectric constant as compared to other animal fats. Nonetheless, it was still not convenient to distinguish lard using dielectric constant spectra; thus additional data analysis was needed. Table 2 shows the average value of dielectric constant for different animal fats. The range of dielectric constant was observed to be overlapping between different animal fats. For this reason, and to remove redundant and less significant data, the statistical analysis techniques of PCA were utilised for such differentiation.

From the dielectric constant spectra, it was found that the dielectric constant values were almost constant across the frequency. The same pattern was observed by Lizhi *et al*. (2008) where the dielectric constant of various vegetable oil and fatty acids virtually showed no frequency dependence in lower frequency region. A report by Ahmed *et al*. (2007) showed similar behaviour in unsalted butter.

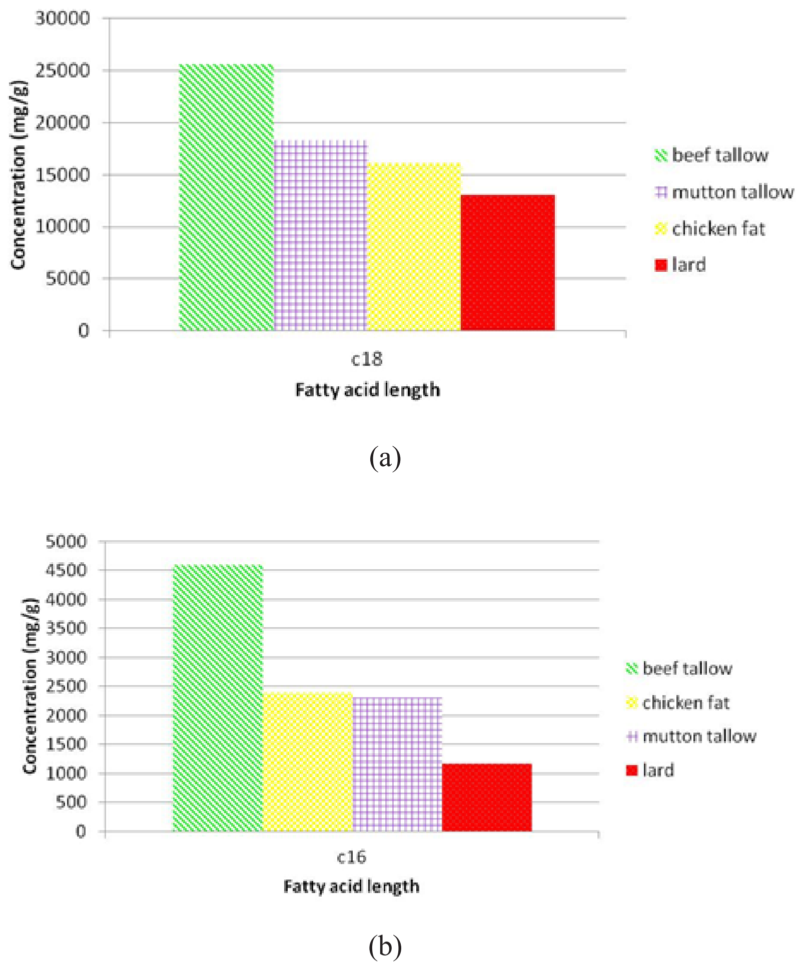


Figure 1. (a) concentration of C18 fatty acid in different animal fats, and (b) concentration of C16 fatty acid in different animal fats.

Fatty acids are classed as short chain, medium chain, or long chain. Short-chain fatty acids are 2-6 carbon atoms long, medium-chain fatty acids are 8-12 carbons long, while long-chain fatty acids are 14-24 carbons long (Brody, 1999). With longer carbon chains, the compounds gradually become less soluble in water, as the hydrophobic hydrocarbon begins to dominate the molecule's properties (Lister and Renshaw, 2014). As water is a polar molecule, longer carbon chains become less soluble in water because they are less polar. Less polar molecule causes resistance for the electric field to polarise the dipoles. Based on the behaviour of different lengths of fatty acid (Lizhi *et al*., 2008), it was reported that longer chain fatty acid resulted in lower dielectric constant, showing that longer chain fatty acid were less polar. C18 is a long-chain fatty acid, making it non-polar. Animal fats that contain higher concentration of C18 fatty acid is expected to have more non-polar molecule, thus having lower dielectric constant. From Figure 1(a), beef tallow was observed to have the most C18 concentration followed by mutton tallow, chicken fat and lard. This is further reflected in the sequence of dielectric spectra in Table 2, where beef tallow depicted the lowest dielectric constant followed by mutton tallow, chicken fat and lard. This was further supported by the concentration of C16, where beef tallow having the highest concentration of C16 while lard having the lowest.

Report from Lizhi *et al*. (2008) showed that the dielectric constant of oils increased with increasing degree of unsaturation of oils suggesting higher unsaturated fatty acid would result in higher dielectric constant and vice versa. Previous work reported higher magnitude of dielectric constant for low-molecular weight of polyvinyl alcohol (PVA) film in comparison to higher molecular weight, which is based on Maxwell-Wagner theory (Joshi and Pawde, 2006). Unsaturated fatty acids have less hydrogen atom, thus having a lower molecular weight. Double bond joint causes bent in unsaturated fatty acid molecule. Due to not having a double bond joint, saturated fatty acids have straight molecular structure and can be easily packed together. This close-intermolecular interactions lead to resistance for the electric field to align the dipoles, thus having low dielectric constant. As observed in Table 2, beef and mutton tallow, which had the higher saturated fatty acid, had lower dielectric spectra. On the other hand, lard and chicken fat, which had lower saturated fatty acid, had higher dielectric spectra.

It was reported by Lizhi *et al*. (2008) that the C18 unsaturated fatty acids, namely linolenic acid (C18:3), linoleic acid (C18:2) and oleic acid (C18:1) had decreasing dielectric constant. From the dielectric spectra obtained in the present work, it was observed that lard, which had highest C18:2 and C18:3, had the highest dielectric constant as compared to other animal fats. Based on the fatty acid analysis, it was expected that the PCA would classify beef and mutton closely together while chicken and lard closely as there was a fair similarity.

Effect of temperature on dielectric spectra of animal fats

The effect of different temperatures on dielectric spectral measurements was also used in statistical analysis for higher accuracy. Temperature dependence of dielectric constant of different animal fats can also be observed in Table 2. It was clearly shown that dielectric constant of animal fats decreased as temperature increased ($p < 0.05$). According to Azizian (2014), when the temperature increases, the molecules of the material have a higher kinetic energy and the mechanical amplitudes of motion will also be increased. Therefore, an electric field's ability to align the dipoles in the material will decrease, causing the decrease in dielectric value. In Lizhi *et al*. (2008), it was explained that as the temperature increased, the viscosity decreased, causing the decrease of relaxation time and increase in dipole moment, thus decreasing the dielectric constant.

Table 2. Temperature dependence of dielectric constant of different animal fats.

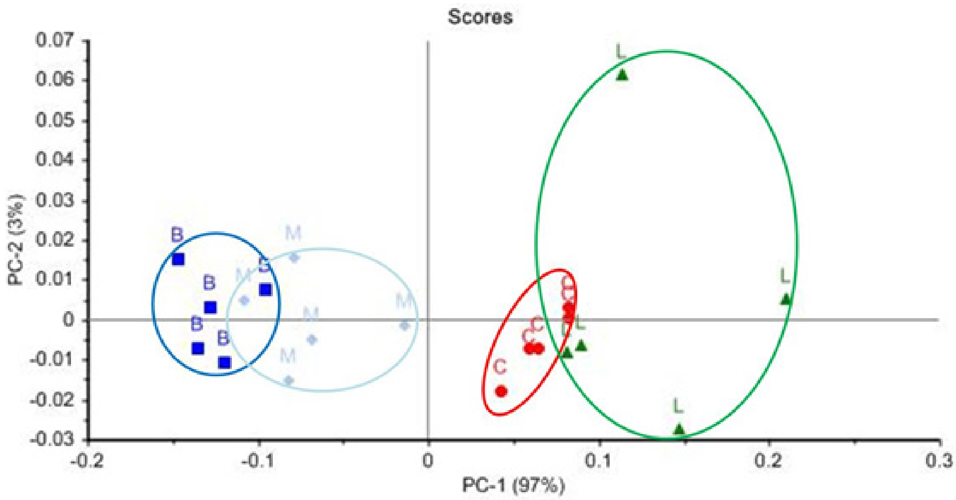
Sample	Frequency (100 Hz - 100 kHz)		
	45°C	50°C	55°C
Lard	3.136 ± 0.001^a	3.115 ± 0.001^a	3.094 ± 0.001^a
Chicken fat	3.055 ± 0.001^b	3.037 ± 0.001^b	3.015 ± 0.001^b
Mutton tallow	3.021 ± 0.001^c	3.005 ± 0.001^c	2.985 ± 0.001^c
Beef tallow	3.010 ± 0.001^c	2.989 ± 0.001^c	2.973 ± 0.001^c

Statistical analysis

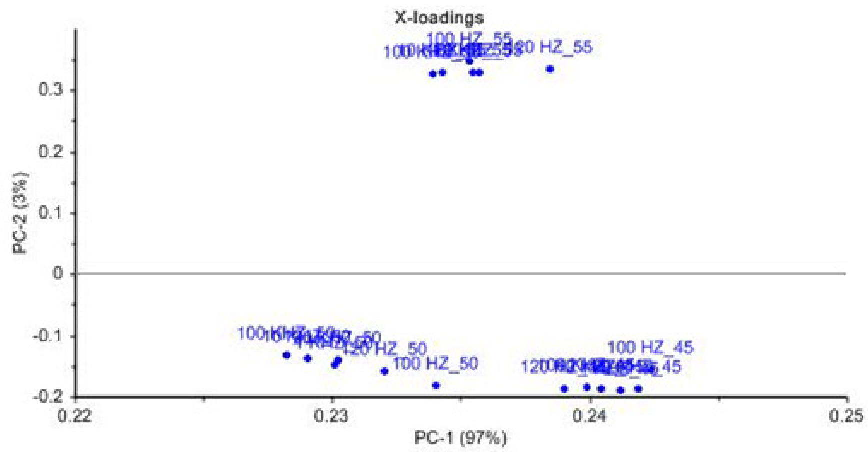
From ANOVA result, a very low *p*-value was obtained, 1.77×10^{-61}, indicated that there was significant difference between the samples, i.e.: types of animal fats. Tukey's range test indicated that different animal fats were being grouped into three separate groups; one group for chicken fat, one group for lard, also one group for mutton and beef. This shows that it is statistically proven that the data of dielectric spectroscopy on the animal fats can be used to distinguish lard from other animal fats.

ANOVA result at individual temperature shows *p*-value at 45°C, 50°C and 55°C of < 0.05 which showed that all temperatures were significantly different. The Tukey's range test groupings from Table 2 showed that all three temperatures showed equal significance in differentiating lard from other animal fats. The result indicated that different animal fats were being grouped into three separate groups, in which mutton and beef tallow were grouped together at all tested temperatures. This can be explained by the close similarity of fatty acid composition of both mutton and beef tallow. Therefore, it was expected that the classification would result in close similarity between mutton and beef.

Figure 2(a) illustrates the PCA score plot and loading plot for chicken fat, lard, beef tallow and mutton tallow. Note that all frequency points for one sample were calculated as one point in PCA score plot, thus, the five points on the plot were represented by five samples of each animal fats. The observation on PCA score plot shows clear performance in the discrimination and identification of lard from other animal fats. Each of the animal fats was grouped in different clusters using dielectric spectra. PC1 describes maximum data variation, while PC2 describes the second largest variation. PC1 contributes 98.91% of the variation; meanwhile PC2 describes 0.54% of the variation, making up of 99.54% for the first two PCs. It was observed that lard was grouped differently from other animal fats in certain pattern. The lard cluster was well-separated along the positive side of PC1 and could be distinguished from other animal fats. From the loading plot in Figure 2(b), all the frequencies were closely and positively correlated. Since lard was located at high PC1, this indicated that lard had high value of the variable, which was a dielectric constant at all different frequency. On the other hand, beef, which was located on the negative side of PC1, indicated that beef has low dielectric constant at different frequencies.



(a)



(b)

Figure 2. (a) PCA score plot for four groups of animal fats, and (b) loading plot for all variables. L: lard, C: chicken fat, B: beef tallow, M: mutton tallow.

It was observed from the PCA score that beef and mutton were well-separated from chicken and lard. This can be explained from their C18 fatty acid composition where beef tallow was the highest thus showed in the PCA plot to be the furthest left. While lard, having the lowest C18 fatty acid composition, was showed to be located furthest on the right on the PCA plot. The saturation factor can also be observed on the overlapping areas of beef tallow and mutton tallow and also of chicken fat and lard. This can be related to C18:0 and C18:2, where beef tallow shared similar composition to mutton tallow, while chicken fat shared similar composition to lard. Beef and mutton tallow both had higher saturated fatty acid (C18:0), while both chicken fat and lard contained higher unsaturated fatty acid (C18:2).

Further classification was done using artificial neural network (ANN) to obtain better classification. From the analysis, it was found that only one out of 15 other animal fats were misclassified as lard, giving 93.3% correct classification. Meanwhile, two out of five lard samples were misclassified into other animal fats giving 60% correct classification. The overall performance of the ANN classification was 85% with three samples misclassified. This explains the overlapped clustering in the PCA score plot.

Conclusion

Dielectric spectroscopy technique in radio frequency range of 100 Hz – 100 kHz has shown potential to discriminate and classify lard from other animal fats. The effect of fatty acid composition was studied using gas chromatography mass spectrometry (GCMS). Statistical analysis RSD, ANOVA, Tukey's range test, PCA and ANN were applied on dielectric data to characterise the animal fats. The study of fatty acid composition shows that animal fats with high C18 composition resulted in low dielectric constant and animal fats with higher unsaturation degree of C18 resulted in higher dielectric constant. The RSD showed that all the measurements done on all the samples had relatively small variation among each group which was less than 0.07%. Statistical analysis showed that different groups of animal fats were classified into different groups. The PCA analysis showed that the data from different animals can be graphically separated by a certain pattern and lard can be clearly differentiated from different animal fats. ANN results showed that dielectric spectra of different animal fats can be classified into each of their own class, and 85% accuracy was achieved. It can be concluded that dielectric spectroscopy in combination with quantitative analysis can provide rapid method to discriminate lard from other animal fats. The method does not require complex sample preparation thus suggesting a low-cost and less chemical usage, and utilisation of radio frequency range indicates low power consumption. Investigation to characterise and discriminate lard from mixed animal fats or plant oils using this method is being carried out and will be optimised in the near future.

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