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# Media Peternakan

*Journal of Animal Science and Technology*

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*In Vitro* Fertility of Post-thawed Epididymal Ram Spermatozoa after Storage at 5 °C before Cryopreservation. N. W. K. Karja, M. Fahrudin, & M. A. Setiadi.

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## ANNOUNCEMENT FOR THE BEST ARTICLE

As an appreciation for the authors participation in publishing their research results in Media Peternakan, the Editorial Board has selected the best article of Media Peternakan for Volume 35 Year 2012, entitled:

### **A Novel Single Nucleotide Polymorphism in Exon 4 of Insulin-Like Growth Factor-1 Associated with Production Traits in Bali Cattle**

Submitted by:

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The Editorial Board would like to congratulate and thank for your participation and contribution of your best work.

## Application of Electrical Properties to Differentiate Lard from Tallow and Palm Oil

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### ABSTRACT

This study aimed to differentiate lard from tallow and palm oil based on its electrical properties, namely conductance, impedance and capacitance. These properties were measured at spectra frequencies of 4.20 to 5.00 MHz in room temperature (26-27 °C). Statistic multivariate that consist on principal component analysis (PCA) and cluster analysis (CA) were used to evaluate the data. The results showed that lard and tallow can be differentiated using whole parameters electrical properties of materials. On the other hand, lard and palm oil can only be differentiated using part of the material electrical properties. Good performance of differentiation process was obtained using PCA model at 4.91 to 4.98 MHz. The first two components of PCA, which was derived from conductance, impedance and capacitance, contributed more than 90% of the total variances. CA showed that lard and tallow are different groups based on the Euclidean distance of each electrical properties. This technique can be potentially developed as an electrical sensor for differentiation lard to tallow and palm oil.

*Key words: differentiation, lard, tallow, palm oil, electrical properties*

### ABSTRAK

Studi ini bertujuan untuk membedakan lemak babi dari lemak sapi dan minyak goreng sawit berdasar sifat elektriknya, yaitu konduktansi, impedansi, dan kapasitansi. Sifat ini diukur pada frekuensi 4,20 sampai 5,00 MHz di suhu ruang (26-27 °C). Statistik multi variat yang terdiri atas analisis komponen utama (AKU) dan analisis kluster digunakan untuk mengevaluasi data. Hasilnya menunjukkan bahwa lemak babi dan lemak sapi dapat dibedakan menggunakan seluruh parameter sifat elektrik bahan. Di sisi lain, lemak babi dan minyak goreng sawit hanya dapat dibedakan menggunakan sebagian sifat elektrik bahan. Kinerja terbaik proses pembedaan diperoleh menggunakan model AKU pada frekuensi 4,71 sampai 4,98 MHz. Dua komponen utama AKU, yang diturunkan dari konduktansi, impedansi, dan kapasitansi, berkontribusi lebih dari 90% total variansi. Analisis kluster menunjukkan bahwa lemak babi dan lemak sapi berbeda kelompok didasarkan jarak Euclidean masing-masing sifat elektriknya. Teknik ini berpotensi dikembangkan sebagai sensor elektrik untuk pembedaan lemak babi terhadap lemak sapi dan minyak goreng sawit.

*Kata kunci: pembedaan, lemak babi, lemak sapi, minyak goreng sawit, sifat elektrik*

### INTRODUCTION

Adulteration of food products involves the replacement of high cost ingredients with lower grade and cheaper substitutes (Tay *et al.*, 2002). Adulteration of lard to other fats and oils is a serious problem for regulatory agencies, oil suppliers and could also threat

health of consumers (Rashood *et al.*, 1996). The Islamic and Orthodox Jewish prohibits the consumption of both pork and lard in any products (Marikkar *et al.*, 2002). Actually, blended edible oils can be prepared only for suitable products, but if the resulting blend deviates from the mixture proportions given on the label, or if the blend is traded as genuine is considered as adulterated oil (Ulberth *et al.*, 2000).

There are numerous existing methodologies to differentiate lard and other fats and oils. Based on sensing method, lard differentiation and adulteration is divided

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in to two groups. The first detection method is through labeling, for example polymerase chain reaction (PCR). The second method is detection through non-labeling such as Electronic nose (E-Nose) and Fourier transform infrared (FTIR) spectroscopy (Sucipto *et al.*, 2011).

Polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) are employed to analyze pork meat and lard (Aida *et al.*, 2005) and pork authentication in sausage and nugget products (Erwanto *et al.*, 2011). Surface acoustic wave (SAW) sensor electronic nose (zNose™) is used to detect lard adulteration in refined, bleached, deodorized (RBD) palm olein (Che Man *et al.*, 2005<sup>a</sup>). A combination of FTIR spectroscopy and partial least square (PLS) method is used to analyze the adulteration of lard at sheep fat (Jaswir *et al.*, 2003), lard at cake (Syahariza *et al.*, 2005). Combination of FTIR spectroscopy with attenuated total reflectance (ATR) and PLS is used to identify lard at chocolate formulation (Che Man *et al.*, 2005<sup>b</sup>), adulteration fish fat by tallow, sheep fat and lard (Rohman & Che Man, 2009), lard at sheep fat, tallow, and chicken (Rohman *et al.*, 2010). Generally, the methods are instrumentally complex, expensive and time-consuming. Therefore, it is urgently needed to develop a new simple but accurate method for lard differentiation from other edible fats and oils.

Electrical properties includes dielectric properties is used to detect moisture content in food (Toyoda, 2003), analysis and monitor quality of food material (Venkatesh & Raghavan, 2004) and discrimination of olive oil from vegetable oils (Lizhi *et al.*, 2010). Dielectric properties of many kind of foods are needed to understand the behavior of the material when is attached into electromagnetic field, at certain desired frequencies and temperatures (Sosa-Morales *et al.*, 2010). Dielectric properties of material are influenced by frequency, temperature, water content, density, composition and material structure (Castro-Giráldez *et al.*, 2010). Foods have dielectric properties and non-ideal polarization which are involved in dissipation phenomena, energy adsorption, and damage that influence dielectric constant (Toyoda, 2003).

PCA is an unsupervised pattern recognition technique used in multivariate analysis. PCA projects the original data in reduced dimensions which is defined by the principal components (PCs). This technique is useful when there are correlations present among data (Cserha'ti, 2009).

In this research, electrical properties, namely conductance, impedance and capacitance combined with statistic multivariate, PCA and CA methods were investigated to differentiate lard from other fats and oils. This research is a novel application of electrical properties for the lard detection. This study suggests developing dielectric sensing for lard, fats and oils differentiation.

## MATERIALS AND METHODS

### Sample Preparation

In this research, samples of lard and tallow were extracted from adipose tissue of pork and beef by rendering in 90-100 °C for 2 h (Marikkar *et al.*, 2002). Each of

the melted fat was collected and filtered through a filter cloth. Palm oil was obtained from the market. Each fat was dried over anhydrous (sodium sulphate) and stored in a freezer (at -20 °C) before further analysis.

### Measurement of Electrical Properties and Fatty Acid

Each sample was placed in a parallel plate made of copper of 20 x 10 mm size and 5 mm in distance. This parallel plate was connected with 3532-50 LCR HiTESTER (Hioki) that covers the range from 42.00 Hz to 5.00 MHz to measure conductance, impedance, and capacitance of samples. Conductance was measured in nano Siemens (nS), capacitance in nano Farad (nF) and impedance in Mega Ohm (MΩ). Measurements were acquired between 4.20 and 5.00 MHz for 81 levels of frequency at a room temperature of around 26-27 °C. It is the validation and development of previous research that measured the conductance of edible fats and oils at frequency 1.00 to 5.00 MHz for 8 point (Sucipto *et al.*, 2011). The dielectric constant value ( $\epsilon'$ ) of samples was calculated using equation as below:

$$\epsilon' = C_p d / A \epsilon_0$$

where,  $C_p$  is oil capacitance (F),  $d$  is distance of parallel plate (m),  $A$  is area of plate (m<sup>2</sup>) and  $\epsilon_0$  is permittivity of free space = 8.85 e-12 (F/m).

Fatty acid composition of samples was determined by step forming of fatty acid methyl esters (FAMES) according to AOAC 2-66 method (AOAC, 2005). Afterward, resulted FAMES were analyzed by gas-chromatography 1700a (Shimadzu). The column used was DB-23 (30 m x 0.25 mm i.d, film thickness 0.25 μm), a splitless-split injector and a flame ionization detector (FID) and carrier gas is Helium. This is to explain the relationship of electrical properties with the fatty acid composition of material.

### Statistical Analysis

All samples were analyzed using electrical spectra in three replicates and averaged. The statistic analysis of PCA and CA were computed.

## RESULTS AND DISCUSSION

### Conductance

Conductance of lard, tallow and palm oil at frequency 4.20 MHz to 5 MHz is shown in Figure 1A. Conductance of samples increased until certain level of frequency and then decreased as frequencies function. The highest conductance of tallow was obtained at 4.40 MHz, whereas lard and palm oil were obtained at 4.60 MHz. Frequency significantly affected the conductance.

Conductance is one of dielectric properties contained in material which vary considerably according to the frequency of the applied electric fields. Thus, an important phenomenon contributing to the frequency dependence of dielectric properties is molecules polar-

ization arising from the orientation with the imposed electric field, which has permanent dipole moments (Venkatesh & Raghavan, 2004).

At low frequencies (less than 200 MHz), ionic conductivity played a major role, whereas both ionic conductivity and dipole rotation of free water were important at microwave frequencies. For instance, ionic conduction had dominant mechanism for dielectric dispersion in whole eggs at frequencies lower than 200 MHz (Ragni *et al.*, 2007). Relatively, changes of conductance value of tallow was greater than lard and palm oil.

Fatty acid composition of samples by gas chromatography is shown at Table 1. Conductance of edible fats was primarily thought to be affected by fatty acid compound. Edible fat which composed of triesters from glycerol and long chain carboxylic acids was often called fatty acids which refer to triglycerides. If one hydrogen atom in fatty acid releases, a single bond carbon atom is replaced by double bond. This fatty acid becomes unsaturated. Unsaturated fat is liquid at room temperature. Lard and palm oil are included as unsaturated oil. Tallow has dominant saturated fat which in room temperature the form is solid.

Conductance of fats is influenced by amount of unsaturated fatty acid composition of each fat. Research of Sucipto *et al.* (2011) was indicated similar results. Tallow had the smaller of unsaturated fatty acids (around 18.78 %), that compared to the lard and palm oil, 42.59% and 45.10%, respectively (Table 1).

In this study, PCA was conducted using conductance value of lard, tallow and palm oil at 81 dielectric spectra frequencies of 4.20 to 5.00 MHz. Figure 1B shows PCA conductance's score plot representing samples projection which is defined as first component (PC1) and second component (PC2). PC1 accounts for the most variation in dielectric spectra, while PC2 accounts for the next largest variation. PC1 accounted for 80.3% of the variation, while PC2 described 14.1% of the variation. Therefore, 94.4% of the variance was described by the first two PCs. From PCA, it was known that frequency regions at 4.91 and 4.98 MHz had more contribution to the PCA model.

In order to divide samples based on conductance into classes, CA with single linkage method was deployed. The main advantage of CA over PCA is that CA can provide numerical values of similarity among evaluated objects. Then, CA enables to reduce dimensionality while retaining required information (Guimet *et al.*, 2004).

Figure 1C shows a dendrogram that illustrates the stages of linkage. If dendrogram cut at horizontal line, conductance analysis found that samples fall in two groups, namely lard and palm oil in the first group, and tallow in the second group. This result indicated that the conductance measurement can differentiate lard and tallow better than lard and palm oil. It is appropriate with fatty acid composition of lard and tallow different greater than lard and palm oil (Table 1).

In this study, conductance is mainly influenced saturated fatty acids, C16:0 and C18:0. The sum of two saturated fatty acids at tallow was 51.02%, while the

lard and palm oil 30.22% and 28.97%, respectively (Table 1). The dominance of saturated fatty acids cause the activation energy for the movement of electrons from one position to another is limited and has small polarity, when it is given a particular frequency on the material. This result is similar according to previous research of electrical properties of natural oils components (Spohner, 2012). Figure 1 shows that the conductance values of tallow was lower than palm oil and lard at frequencies above 4.68 MHz.

Table 1. Fatty acid profiles of lard, tallow and palm oil by gas chromatography

Fatty Acid, Formula	Composition (%)		
	Palm oil	Tallow	Lard
Capric Acid, C10:0	0.02	0.05	0.13
Lauric Acid, C12:0	0.15	0.5	2.56
Tridecanoic Acid, C13:0	nd	0.02	nd
Myristic Acid, C14:0	0.72	6.61	4.94
Myristoleic Acid, C14:1	nd	0.35	0.04
Pentadecanoic Acid, C15:0	0.03	0.59	0.08
Palmitic Acid, C16:0	26.05	24.32	20.41
Palmitleic Acid, C16:1	0.16	1.68	1.45
Heptadecanoic Acid, C17:0	0.06	1.3	0.27
Cis-10-Heptadecanoic Acid, C17:1	0.02	0.24	0.12
Stearic Acid, C18:0	2.92	26.7	9.81
Oleic Acid, C18:1n9c	34.7	15.39	27.08
Linoleic Acid, C18:2n6c	9.86	0.84	11.14
Arachidic Acid, C20:0	0.29	0.29	0.13
v-Linolenic Acid, C18:3	nd	nd	0.04
Cis-11-Eicosenoic Acid, C20:1	0.15	0.08	0.52
Linolenic Acid, C18:3n3	0.14	0.17	1.03
Heneicosanoic Acid, C21:0	n.d	0.05	nd
Cis-11,14-Eicosadienoic Acid, C20:2	0.07	0.03	0.41
Behenic Acid, C22:0	0.05	0.05	n.d
Cis-8,11,14-Eicosatrienoic Acid, C20:3n6	nd	nd	0.1
Cis-11,14,17-Eicosatrienoic Acid, C20:3n3	nd	nd	0.11
Arachidonic Acid, C20:4n6	nd	nd	0.32
Tricosanoic Acid, C23:0	nd	0.03	nd
Lignoceric Acid, C24:0	0.05	0.02	nd
Cis-5,8,11,14,17-Eicosapentaenoic Acid, C20:5n3	nd	nd	0.03
Cis-4,7,10,13,16,19-Docosahexaenoic Acid, C22:6n3	nd	nd	0.2
Total fatty acid	75.45	79.34	80.89
The sum of saturated fatty acid (C16:0 and C18:0)	28.97	51.02	30.22
The sum of unsaturated fatty acid (C18:1 and C18:2)	44.56	16.23	38.22
Total unsaturated fatty acid	45.1	18.78	42.59

Note: nd= not detected.

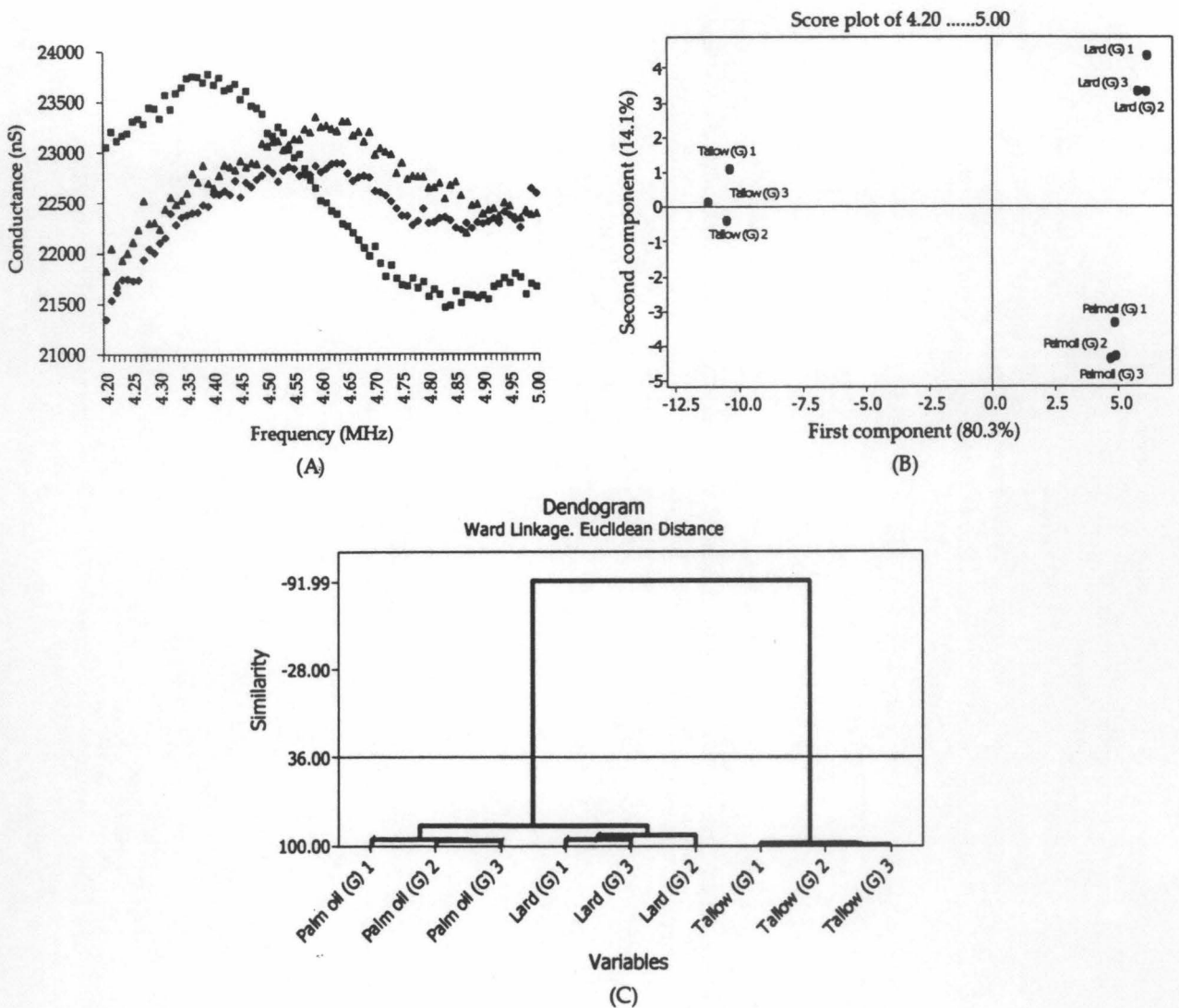


Figure 1. Conductance of samples (lard: -▲-, tallow: -■-, and palm oil: -◆-). (A) The relations frequency with a conductance of samples; (B) The score plot for the first two principal components (PC) for conductance of samples; (C) Dendrogram illustrating the stages of clustering of the evaluated samples based on conductance.

**Impedance**

Impedance of lard, tallow and palm oil at frequency 4.20 MHz to 5.00 MHz is shown in Figure 2A. Impedance of samples tends to increase along with increasing of frequency. Tallow had the lowest initial impedance value but with higher frequency, particularly higher than 4.55 MHz, it reached the highest value. Impedance of lard and palm oil tends to coincide, but impedance of lard and tallow is separate. This result fits with score plot of PCA showed in Figure 2B.

Impedance is a complex of conductance and capacitance which opposes against electric current. Impedance of fats has resistive and capacitive component. Fats acts as an insulator at low frequencies which behaves like a capacitor.

Figure 2B shows the score plot of PCA impedance from 81 dielectric spectra frequencies. PC1 accounts for the most variation in dielectric spectra, while PC2 accounts for the next largest variation. PC1 accounted for 88.1% of the variation, while PC2 described 6.40% of

the variation. From PCA, it was known that frequency regions at 4.71, 4.74, 4.91, 4.92, 4.95, 4.23, 4.30 MHz had more contribution to the PCA model.

Result of CA of impedance samples is show in Figure 2C. The horizontal line in dendrogram separates samples into two groups, namely lard and palm oil in the first group, while tallow in the second group. This result shows that impedance measurement in above specific spectra could differentiate lard from tallow and palm oil by PCA and CA.

Impedance of lard and tallow had greater differences compared with lard and palm oil. Impedance of fats is influenced by unsaturated fatty acid composition of each fat, especially for C18:1 and C18:2. When a certain frequency was given to fat that predominantly unsaturated fatty acids, its activation energy was simple to use for electrons movement from one position to another and it had higher polarity. This result is similar according to research the electrical properties of natural oils components (Spohner, 2012). Table 1 shows the sum of two unsaturated fatty acids of tallow is 16.23%, while the



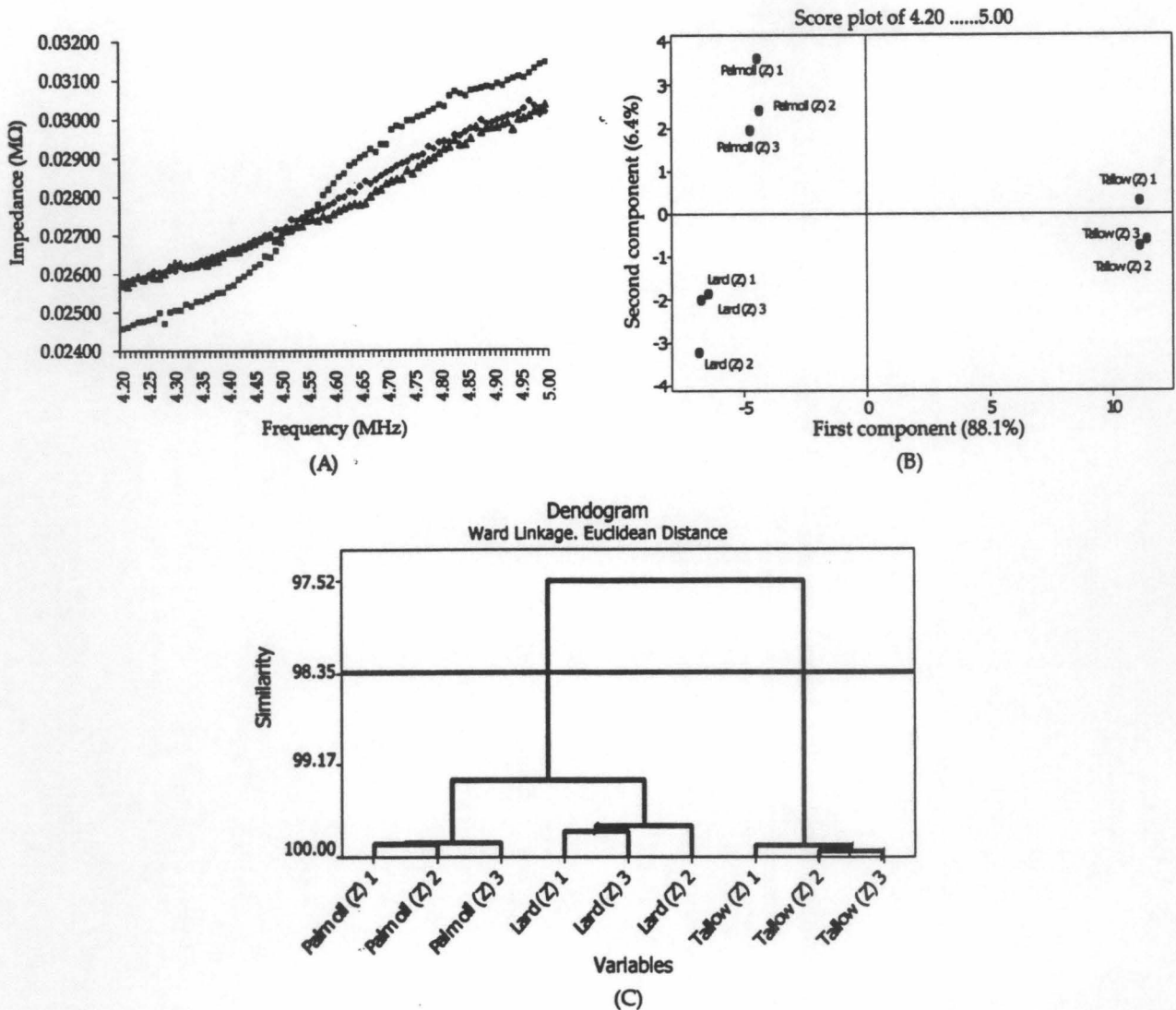


Figure 2. Impedance of samples (lard: -▲-, tallow: -■-, and palm oil: -◆-). (A) The relations frequency with a impedance of samples; (B) The score plot for the first two principal components (PC) for conductance of samples; and (C) Dendrogram illustrating the stages of clustering of the evaluated samples based on impedance.

lard and palm oil 38.22% and 44.56%, respectively. Since the impedance opposes of an electric current, so small unsaturated fatty acid and polarity of tallow causes high impedance. Figure 2 shows at frequency above of 4.68 MHz, the highest impedance values obtained in tallow.

**Capacitance**

Capacitance is the ability of a capacitor to store energy in an electric field. A common form of energy storage device is a parallel-plate capacitor. Capacitance is directly proportional to the surface area of the parallel plates and inversely proportional to the separation distance between the plates. Capacitance is varying considerably with frequency, such as other electrical properties (Toyoda, 2003).

Capacitance of lard, tallow and palm oil at frequency 4.20 MHz to 5 MHz is shown in Figure 3A. The capacitance of samples tended to decrease with the increase of frequency. Capacitance of lard and palm oil are coincided. These shows the characteristics of

lard and palm oil to be difficult separated based on its capacitance.

Figure 3B shows the result of PCA which related to the capacitance data set of samples. The two principal components (PC) explain 90.5% (PC1 86.5% + PC2 4.0%) of the total variance. It is known that frequency regions at 4.67, 4.70, 4.71, 4.73, 4.74, 4.75, 4.76, 4.94, 4.23, 4.38 MHz had more contribution to the PCA model.

CA of capacitance value is shown in Figure 3C. The dendrogram illustrates the stage of linkage. Horizontal line divides samples into two groups, namely lard and palm oil in the first group, while tallow in the second group.

Visually, capacitance value of lard and palm oil are still coincided, but the two of fat is separated from tallow. Using PCA and CA of capacitance lard and tallow could be differentiate, while lard and palm oil is one group. This means lard more difficult to distinguish from palm oil, because both of them had a similar fatty acids. Table 1 shows the number of each fatty acid, particularly C18:0, C18:1, C18:2 for lard and palm oil is

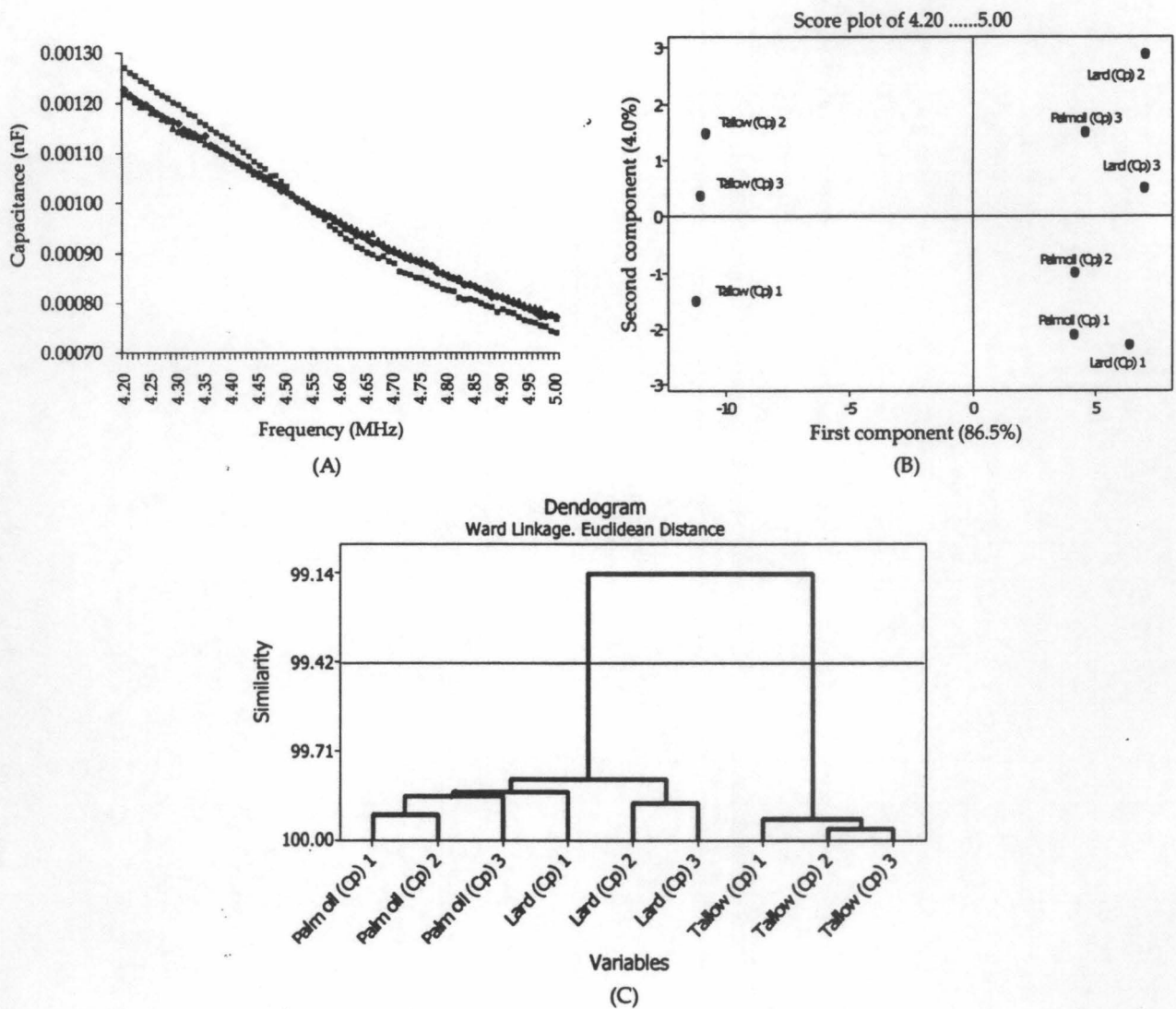


Figure 3. Capacitance of samples (lard: -▲-, tallow: -■-, and palm oil: -◆-). (A) The relations frequency with a capacitance of samples; (B) The score plot for the first two principal components (PC) for capacitance of samples; and (C) Dendrogram illustrating the stages of clustering of the evaluated samples based on capacitance.

almost similar compared with tallow. The proportion of these fatty acids caused the ability to motion electrons of lard and palm oil was almost similar, so its capacitance value was also almost similar. This result agreed the previous research results which differentiated lard from other edible fats, included tallow by FTIR spectroscopy and chemometrics (Che Man, *et al.* 2011).

**Dielectric Constant**

Dielectric constant of samples in some frequencies is shown in Figure 4. Dielectric constant of samples decreased along with the increase of frequency. This pattern is similar with previous results found in dielectric constant of edible oils and fatty acids at frequency 100 Hz to 1 MHz (Lizhi *et al.*, 2008). Similar result of lard and tallow dielectric constant was obtained at 300, 1000 and 3000 MHz (Pace *et al.*, 2005).

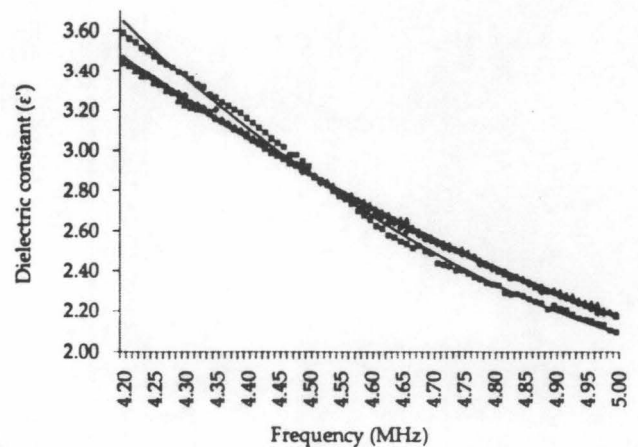


Figure 4. The relations frequency with a dielectric constant of lard (-▲-), tallow (-■-), and palm oil (-◆-).

In mathematical equation, relationship between dielectric constant and frequency was written as follow:

$$\text{Lard } (\epsilon') = 6E-05x^2 - 0.020x + 3.466, R^2 = 0,999$$

$$\text{Palm oil } (\epsilon') = 7E-05x^2 - 0.021x + 3.495, R^2 = 0,999$$

$$\text{Tallow } (\epsilon') = 9E-05x^2 - 0.029x + 3.675, R^2 = 0,996$$

Dielectric constant of lard and palm oil are coincided, but both of them could be separated from tallow. The dielectric constant was a function of capacitance and related material composition. Therefore, the predominant fatty acids composition, especially C16:0, C:18:0, C18:1, 18:2 (Table 1) affect the dielectric constant of the material. This is based on its ability of electrons motion from one position to another, when is given a specific frequency. The dielectric constant of fats is mainly affected by their unsaturated fatty acids composition. This statement is similar with previous research of dielectric constant in edible oils and fatty acids at frequency 100 Hz - 1 MHz (Lizhi *et al.*, 2008).

This result indicated that three components of dielectric properties, i.e. conductance, impedance, capacitance had the role for lard differentiation. In general, electrical properties and statistic multivariate by PCA and CA could well differentiate lard from tallow, but lard and palm oil can be differentiated using part the dielectric properties of materials. Based on PCA model of conductance, impedance and capacitance, the frequencies at 4.71–4.98 MHz had significant role for differentiation. Further research in data preprocessing and other classification technique is required to enable separation of lard from fats and oil more accurately base on their electrical properties.

## CONCLUSION

Electrical properties combined with the statistic multivariate of PCA and CA enable to differentiate lard from tallow and palm oil. Frequencies at 4.71–4.98 MHz has significant contribution to differentiation using PCA Model. Electrical properties of fats influence by fatty acid composition.

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