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ORIGINAL PAPER

Analysis of Lard's Aroma by an Electronic Nose for Rapid *Halal* Authentication

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Abstract An electronic nose was successfully used to detect and discriminate lard from other types of animal body fats and samples containing lard. The results are presented in the form of VaporPrint™, the image of the polar plot of the odor amplitudes from the surface acoustic wave (SAW) detector frequency. In the VaporPrint™, the radial angles representing the sensor provides individual fingerprints of the aroma of different animal body fats. Principal component analysis (PCA) was used to interpret the data and it provided a good grouping of samples, with 61% of the variation accounted for by PC 1 and 29% accounted for by PC 2. All of the lard-containing samples formed a separate group from the samples that were free from lard. This method can be developed into a rapid method for detecting the presence of lard in food samples for *Halal* authentication.

Keywords Lard · Electronic nose · Adulteration · *Halal* authentication · Principal component analysis (PCA)

Introduction

Oils and fats have been recognized as essential nutrients in the human diet and are concentrated sources of energy [1]. In some countries, food manufacturers choose to blend vegetable oil with animal body fats to be consumed in food production [2]. Animal fats have traditionally been used for the deep frying of many types of foods, and it is known that the flavors imparted through this process have been considered to be desirable for some foods [3]. From a Muslim, Jewish, or Hindu consumer perspective, the origin of fat is a serious issue of concern. Under Jewish and Islamic dietary laws, foods containing porcine-based ingredients such as lard are strictly prohibited from consumption, while in Hinduism, the consumption of beef fats in food is not allowed [4, 5]. Therefore, in order to protect the consumer from fraud and adulteration and to ensure the safety of food, it is important to carry out some rapid and reliable analytical methods for food authentication, especially for *Halal* authentication and species identification of food products. Moreover, it was reported that the *Halal* food trade is worth about $150 billion a year [6]. From the economic standpoint, ensuring the authenticity of *Halal* is significant. In the analytical field, there have been a number of scientific investigations carried out on the adulteration of fats and oils. Aparicio and Aparicio-Ruíz [7] reported that most of the previous studies on edible oil adulteration were based on chromatographic analysis and have been shown to give precise and reliable results. However, chromatography will usually involve a time-consuming sample preparation, and can only be carried out by experts and well-trained operators [8].

About a decade ago, the electronic nose was introduced as a very rapid tool for aroma profile analysis in several fields, including medical diagnosis [9], environmental pollution monitoring [10, 11], cosmetics [12], and the automotive industry [13]. More recently, it has become more popular as a nondestructive technique for food analysis. In food analysis, there are possibly five major categories where the electronic nose can be used for food

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control. These are process monitoring, shelf-life investigation, freshness evaluation, authenticity assessment, and other quality control studies [14, 15]. The advantages that the electronic nose offers in comparison to other techniques are rapid analysis, low cost, broad selectivity, and good reliability [16]. In the food industry, especially in fats and oils analysis, the electronic nose has been widely used to assess a number of fats and oils samples. Marina et al. [17] reported that most of the research studies were based on the analysis of vegetables oils but not animal body fats. Furthermore, some authors also reported positive application of the electronic nose based on principal component analysis (PCA) as the method of pattern recognition in detection and discrimination experiments [9, 18, 19].

Table 1 Fatty acid compositions of lard, chicken fat, beef fat, and mutton fat

Fatty acid	Lard (%)	Chicken fat (%)	Beef fat (%)	Mutton fat (%)
12:0	0.07	0.02	0.07	0.13
14:0	1.10	0.85	2.88	2.61
15:0	0.08	0.17	0.66	0.16
16:0	20.30	22.87	24.19	20.39
16:1	1.48	5.08	3.30	1.10
17:0	0.40	0.04	0.55	1.97
17:1	0.24	0.10	0.82	0.16
18:0	11.21	0.12	0.95	5.15
18:1	23.92	26.40	16.22	26.49
18:2	19.29	14.10	4.90	1.45
18:3	1.31	2.10	3.71	2.96
20:0	0.98	0.16	0.53	1.45
20:1	0.08	0.54	0.87	0.65

Each value in the table represents the mean of triplicate analysis

Therefore, a study to evaluate the potential of an electronic nose combined with PCA for the differentiation and discrimination of various animal body fats such as lard, chicken fats, beef fats, and mutton fats is particularly relevant. The objectives of this study are to assess the potential of an electronic nose to detect and discriminate lard among the other types of fats for *Halal* verification and to demonstrate the sensitivity of the sensor against the adulterated samples.

Materials and Methods

Materials

Lard and other animal body fats such as chicken fat, beef fat, and mutton fat were collected from a local slaughterhouse and extracted by rendering the adipose tissues according to the method reported by Che Man et al. [2]. All chemicals used in this experiment were of analytical grade.

Blend Preparation

Lard and chicken fats were mixed in various proportions ranging from 1, 5, and 10%, followed by 20–80% of lard in 20% increments (w/w). Seven lard and chicken fat blends (mass ratio of lard to chicken fats) were prepared: 99:1, 95:5, 90:10, 80:20, 60:40, 40:60, and 20:80 (w/w) in duplicate.

Fatty Acid Analysis

Fatty acid methyl esters (FAMEs) were prepared according to the method described by Marina et al. [17]. Fat samples

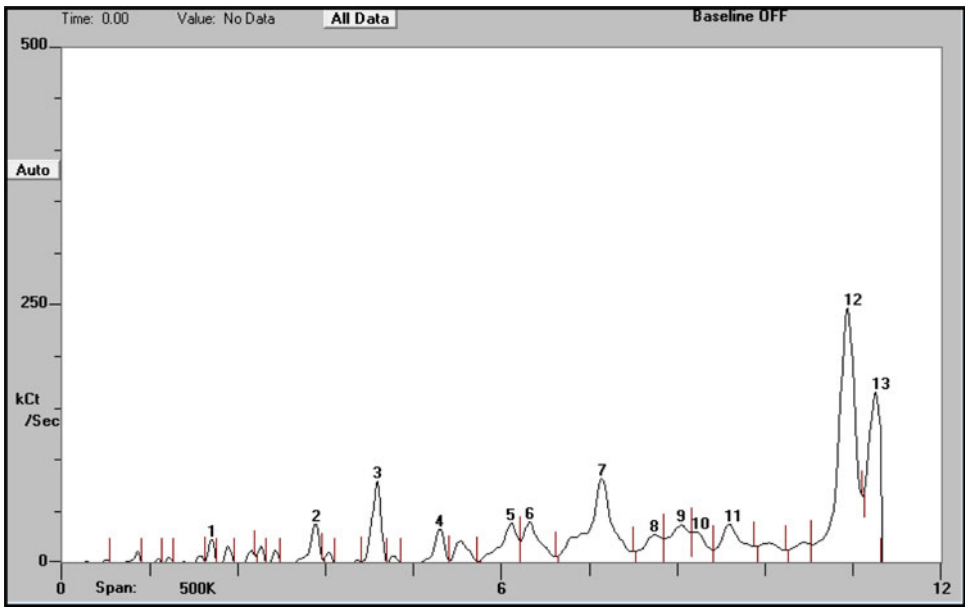


Fig. 1 Typical electronic nose chromatogram of lard from the surface acoustic wave (SAW) detector response. For peak identification, refer to Table 2

(50 mg) were dissolved in 0.8 mL hexane and mixed with a solution of 0.2 mL sodium methoxide (1 M) in anhydrous methanol. The mixture was mixed for 1 min using a vortex mixer. After the sedimentation of sodium glycerolate, 1 μL of the clear supernatant was injected onto the Restex 2330 polar capillary column (0.25 mm internal diameter, 30 m length, and 0.2 μm film thickness; Restex Corp., Bellefonte, PA) and analyzed using a gas chromatograph (GC) (Agilent Technologies model 6890N, Santa Clara, CA) equipped with a flame ionization detector (FID). The oven temperature was programmed as follows: initial temperature of 50 °C (held for 2 min), followed by an increase to 180 °C (5 °C/min), followed by an increase from 180 to 200 °C (8 °C/min), and finally holding at 200 °C for 5 min. Both injector and detector temperatures were maintained at 200 °C throughout the analysis. The carrier gas (helium) flow rate was 6.8 mL/min. Standard FAMEs (Sigma Chemicals, St. Louis, MO) were used as the authentic sample and peak identification was done by comparing the relative retention times. The peak areas were obtained and the percentage of fatty acid was calculated as the ratio of the partial area to the total area.

Table 2 Tentative identification of volatile compounds of raw animal body fats from the electronic nose profile

Peak no.	Kovats index	Compounds	Odor description
1	620	Diacetyl	Buttery
2	723	Methyl butanoate	Ethereal
3	806	2-Methylpropanal	Pungent
4	903	Heptanal	Fatty
5	1,000	Trimethyl pyrazine	Roasted
6	1,104	Nonanal	Soapy
7	1,207	Decanal	Soapy
8	1,305	Undecanal	Pungent
9	1,408	Dodecanal	Waxy
10	1,651	Methyl tetraderanoate	Fatty
11	1,798	Ethyl tetradecanoate	Ethereal
12	2,087	Methyl trans-9-octadecanoate	Fatty
13	2,156	Dodecanoic acid	Waxy

Electronic Nose Analysis

The zNose™ (7100 Vapour Analysis System, Electronic Sensor Technology, Newbury Park, CA) was the benchtop analyzer used for this study. It consisted of an uncoated piezoelectric quartz crystal surface acoustic wave (SAW) sensor. This crystal operates by maintaining highly focused and resonant surface acoustic waves of wavelength 500 MHz on its surface. At a given crystal temperature, only those analytes with dew points below the crystal temperature will condense and adsorb on the surface of the sensor, which will, in turn, alter the frequency of the SAW to effect the detection signal and allow the identification of the analytes [20, 21]. For the zNose™ measurements, 5 g of the fat samples was transferred into a 40-mL vial. The samples were equilibrated with the headspace in a water bath maintained at 60 °C for 10 min. To analyze each sample, the sample's vapor was introduced to the electronic nose. The sampling mode was programmed to be 4 s, after which the system was switched to a 20-s data acquisition mode. The vapor was then released from the trap after being rapidly heated and carried over the nonpolar column (DB-5; 85 cm, ID 0.25 mm, film thickness 0.25 μm) which was programmed to rise in temperature from 40 to 160 °C, at a rate of 5 °C/s, in a helium flow of 3.5 cm^3. The SAW sensor was operated at a temperature of 50 °C. The Microsense version 5.29 software (Newbury Park, CA) was used for the data collection. The total time of the analysis was 15 s, including the sampling and analysis time.

Statistical Analysis

All measurements were conducted in duplicate and averaged using Microsoft Excel software. Trend line equations were further added from the frequency data of the electronic nose. Data from the electronic nose were subjected to analysis by Duncan's multiple range tests using the SPSS Statistics 18 software package (SPSS Inc., Chicago, IL). The data were processed with PCA using the Unscrambler version 9.7 software (CAMO Software AS,

Table 3 Profiles of volatile compounds of four types of animal fats

Peak no.													
Animal fat	1	2	3	4	5	6	7	8	9	10	11	12	13
Lard	1,622^{a}	4,489.5^{a}	10,525.5^{a}	4,306.0^{a}	3,701.5^{a}	3,713.5^{a}	17,444.0^{a}	1,308.5^{a}	1,567.0^{a}	449.0^{a}	4,904.0^{a}	35,369.0^{a}	24,432.0^{a}
Chicken fat	1,133^{b}	3,017.0^{a}	5,187.0^{b}	1,939.5^{c}	941.0^{b}	941.0^{b}	586.0^{b}	273.0^{b}	1,796.0^{b}	3,238.0^{a}	948.0^{c}	14,374.5^{b}	14,734.5^{b}
Beef fat	521.5^{c}	4,534.5^{a}	8,364.5^{a}	2,924.5^{b}	491.0^{b}	503.5^{b}	408.5^{b}	ND	ND	12,296.5^{b}	2,072.0^{b}	ND	ND
Mutton fat	544.5^{c}	4,351.0^{a}	8,364.5^{a}	3,669.0^{ab}	537.0^{b}	457.0^{b}	1,251.5^{b}	ND	ND	27,407.5^{c}	ND	169.5^{c}	ND

Means within each column with different superscripts are significantly different at $P < 0.05$

ND not detected

Table 4 Profile of volatile compounds of lard added with different percentages of chicken fat

Peak no.													
Animal fat	1	2	3	4	5	6	7	8	9	10	11	12	13
Lard pure	1,622.0^{bc}	4,489.5^{a}	10,525.5^{a}	4,306.0^{a}	3,701.5^{a}	3,713.5^{a}	17,444.0^{a}	1,308.5^{a}	1,567.0^{b}	449.0^{c}	4,904.0^{a}	35,369.0^{a}	24,432.0^{a}
CF pure	1,133.0^{cd}	3,017.0^{b}	5,187.0^{c}	1,939.5^{d}	941.0^{c}	941.0^{bc}	586.0^{b}	273.0^{d}	1,796.0^{ab}	3,238.0^{a}	948.0^{e}	14,374.5^{b}	14,734.5^{b}
CF 1%	234.5^{f}	2,090.0^{d}	8,240.0^{bc}	2,964.5^{b}	3,866.5^{ab}	3,049.5^{b}	1,095.5^{b}	1,400.0^{a}	1,308.0^{b}	380.0^{c}	4,406.5^{ab}	1,136.5^{b}	10,240.0^{c}
CF 5%	421.0^{ef}	2,226.5^{d}	8,734.5^{b}	2,912.5^{b}	3,997.5^{ab}	2,903.5^{b}	1,161.5^{b}	1,419.5^{a}	1,209.5^{b}	316.5^{c}	4,050.0^{b}	1,175.5^{b}	10,927.5^{c}
CF 10%	829.5^{de}	2,173.0^{d}	7,043.5^{bc}	2,618.0^{b}	3,575.0^{b}	2,866.5^{b}	1,256.0^{b}	1,425.0^{a}	1,315.0^{b}	367.0^{c}	3,425.0^{c}	1,665.0^{b}	11,880.5^{bc}
CF 20%	910.0^{d}	2,259.0^{b}	4,868.5^{c}	2,193.0^{cd}	1,160.0^{c}	1,073.5^{b}	682.5^{b}	542.0^{c}	1,201.0^{b}	ND	1,432.0^{d}	1,913.0^{c}	13,009.0^{b}
CF 40%	2,839.0^{a}	2,766.5^{b}	5,277.0^{c}	2,682.0^{cd}	1,395.0^{bc}	1,355.5^{b}	2,407.0^{b}	791.0^{b}	1,700.5^{ab}	ND	1,929.5^{c}	2,044.5^{c}	17,667.0^{ab}
CF 60%	1,860.0^{b}	4,191.0^{a}	8,569.5^{ab}	3,495.5^{b}	1,757.0^{b}	428.0^{c}	1,168.5^{b}	775.5^{bc}	2,530.0^{a}	ND	1,967.5^{c}	1,800.0^{c}	23,714.5^{a}
CF 80%	1,546.0^{bcd}	3,370.5^{ab}	6,529.5^{bc}	2,841.5^{ab}	1,762.5^{b}	429.5^{c}	1,212.5^{b}	1,392.0^{a}	1,532.0^{b}	2,842.5^{b}	2,997.0^{b}	1,466.5^{c}	24,838.0

Means within each column with different superscripts are significantly different at $P < 0.05$

ND not detected

Trondheim, Norway) in order to discriminate the samples into pure and lard containing chicken fat samples.

Results and Discussion

Volatile Compounds of Animal Body Fats

Several fatty acids which contribute to the aroma profile of different animal body fats analyzed by gas chromatography using a flame ionization detector (GC-FID) are shown in Table 1. The results were all within the range as reported in previous studies [1, 22]. Palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2) were the compounds found to be abundant in all of the tested samples from the different species, while chicken fat and lard were shown to share similarities in the fatty acid compositions. A few other components were noticeably high in other specific species: myristic acid (C14:0) in beef and mutton fat, C18:1 in chicken and mutton fat, and C18:2 in lard. However, GC-FID is a relatively slower technique and also requires a more intensive sample preparation. Therefore, in order to alleviate these concerns, the electronic nose has been used as an alternative analytical method by offering a more rapid method of analysis.

Figure 1 shows the chromatogram from the electronic nose which is in the form of a graphical display of the derivative of the frequency change versus time. The electronic nose does not resolve the sample's volatiles into its individual components, but responds to a whole set of volatiles in a unique digital pattern [23]. Table 2 shows the set of volatile compounds corresponding to peaks 1–13 and their odor descriptions. The identification of these peaks was tentatively based on a database of Kovats retention indices stored in the substance library of the Microsense software using *n*-alkanes as the standard [17]. Kovats retention indices are retention, relative to those *n*-alkanes, which is useful for the identification of compounds by chromatography techniques [24, 25]. Some of the volatile compounds found in this study, such as methyl tetradecanoate and methyl trans-9-octadecanoate, were also identified in our previous study [22].

Table 3 shows the profile of the volatile compounds of four types of animal fats. There were significant differences ($P < 0.05$) between the peak areas for lard and the other types of animal fats. Table 4 shows the electronic nose data for lard added with different percentages of chicken fats, which also show significant differences ($P < 0.05$) between peak areas for adulterant concentrations of 0 and 1% for most compounds. This indicates that the presence of the adulterant was sensed even at an addition of 1% (wt/wt). Peaks that changed according to the changes in the adulterant concentration were selected and considered to be

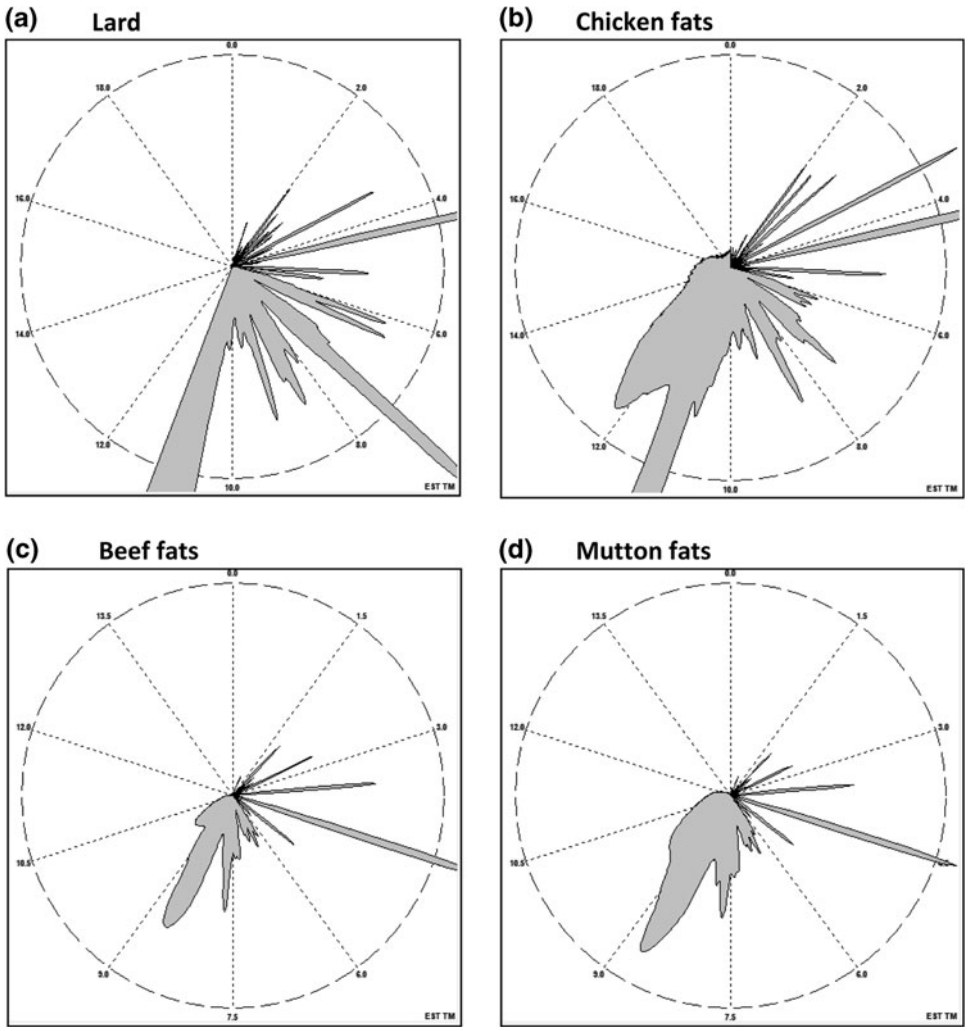


Fig. 2 VaporPrint™ images of different animal body fats

adulterant peaks and plotted versus the lard percentage. The data were fitted to a curve using the linear regression technique and the coefficient of determination (R2) was calculated. Adulterant peaks 12 and 13 with R^2 values of 0.931 and 0.996, respectively, appeared to be the best predictors for quantitative determination of the level adulteration, as the size of these peaks correlated well with increasing level of adulteration. The relationship was found to follow a second-order polynomial. The VaporPrint™ shown in Fig. 2 is a polar plot of the odor amplitude (SAW detector frequency), with the radial angles representing sensors and which can be interpreted as the chemical signature of the aroma of a substance [17, 22]. This image provides individual fingerprints of the aroma of different animal body fats. Qualitative differences between the fingerprints are proposed as a basis for differentiating between pure lard and its blend. However, it must be noted that this study does not focus to identify exactly each of the volatile compounds in the fat samples, but it would be interesting to monitor qualitatively the changes of lard adulterated with chicken fats, as they seem to have similar fatty acid compositions.

The VaporPrint™ image of lard with varying levels of chicken fat changed accordingly, with the aroma pattern slowly resembling the VaporPrint™ of the chicken sample (Fig. 3). The unique feature of the VaporPrint™ is its ability to provide digital evidence that the sample was adulterated even before the data was analyzed. A double-blind study has also been performed as a validation of the method. In this study, the percentage of the precision of the method obtained about 83.3% positive results and 16.7% negative results. This percentage seemed to be sufficient to define a good model, especially for qualitative purposes. However, the use of chemometric approaches using unsupervised pattern recognition techniques such as PCA is needed in order to structure the data matrix.

Principal Component Analysis

PCA was carried out based on the electronic nose data presented in Table 2. PCA is a technique that reduces the dimensions of the data [26]. Reduction of the number of variables can lead to improved performance [20]. Figure 4

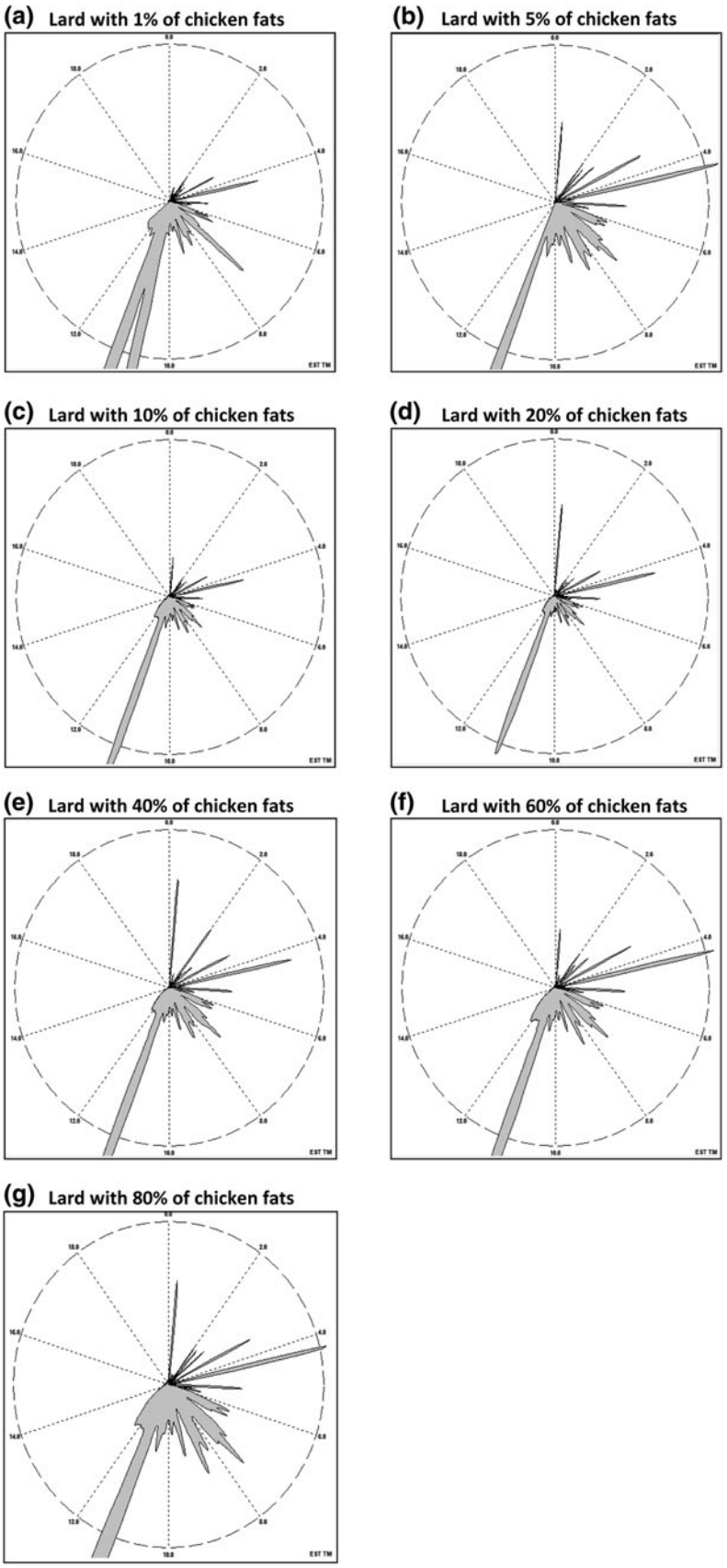


Fig. 3 VaporPrint™ images of lard added with varying amounts of chicken fats

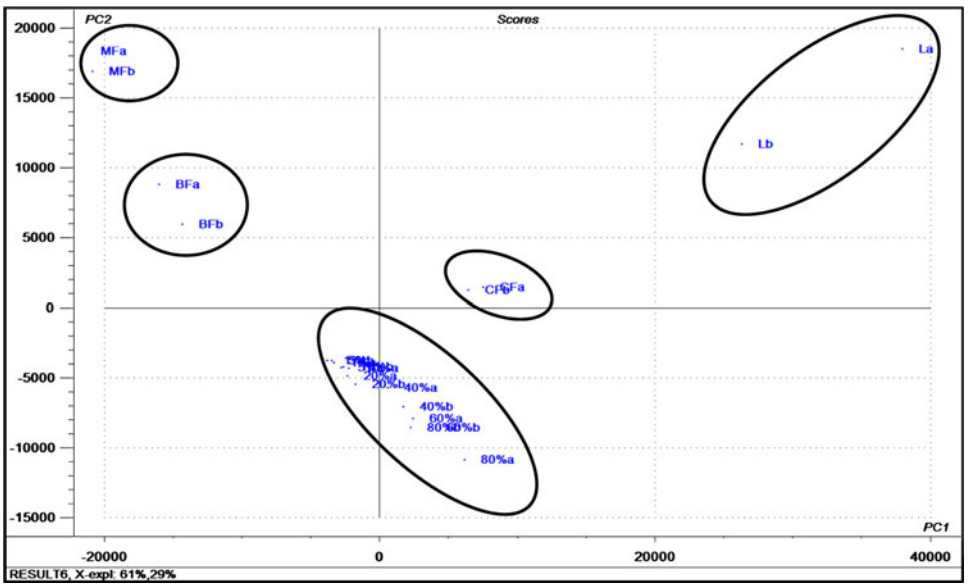
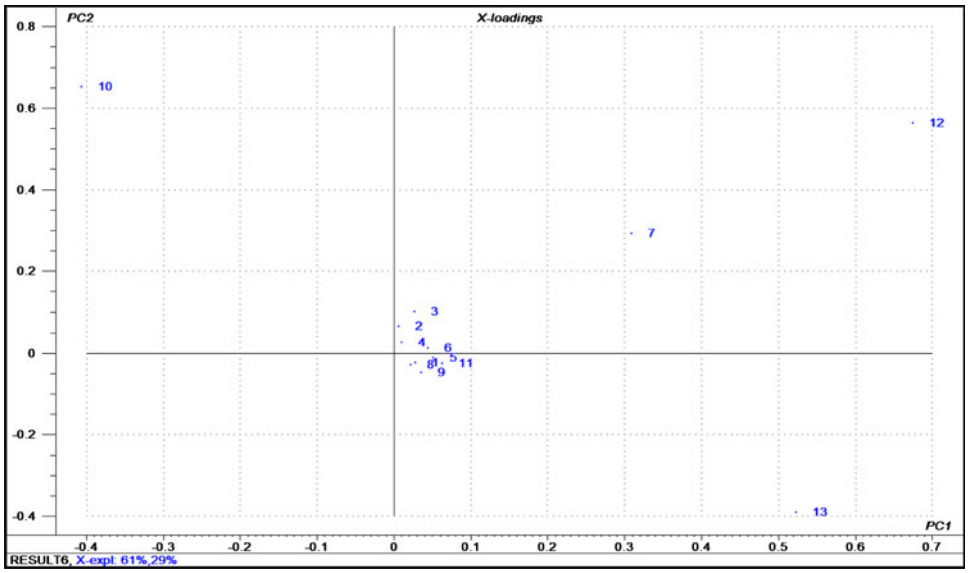


Fig. 4 Score plot of four different animal body fats and lard samples added with chicken fat in principal component analysis (PCA) of the electronic nose data: chicken fat a (CFa), chicken fat b (CFb), lard a (La), lard b (Lb), mutton fat a (Mfa), mutton fat b (MFb), beef fat a (Bfa), beef fat b (BFb), chicken fat 1% a (1%a), chicken fat 1% b (1%b), chicken fat 10% a (10%a), chicken fat 10% b (10%b), chicken fat 20% a (20% a), chicken fat 20% b (20% b), chicken fat 40% a (40%a), chicken fat 40% b (40%b), chicken fat 60% a (60%a), chicken fat 60% b (60%b), chicken fat 80% a (80%a), chicken fat 80% b (80%b)

Fig. 5 Loading plot of ten electronic nose variables (loading plot) in PCA



illustrates the score plot of PC 1 versus PC 2 from the electronic nose analysis. PC 1 described 61% of the variation, while PC 2 accounted for 29% of the variation, which resulted in a model that described 80% of the total variance in the data. With 61% of the peak variation along the first PC, it is clear that the four types of animal body fat samples and adulterated samples were formed according to their own group and showed five well-defined and well-separated groups: pure lard with high positive scores, chicken fat and some adulterated lard samples with low positive scores, while mutton and beef fats had low negative scores.

Figure 5 shows the loading plot of PC 1 versus PC 2. The loading plot is used to determine which variables influence the separation of the samples. The absolute value of the loading in a component describes the importance of the contribution of the particular component. Thus, the further a variable is away from the origin, the greater the contribution of that particular variable to the model. As shown in Fig. 5, the main compound that caused the separation of the samples based on PC 1 were components 10 (methyl tetradecanoate), 12 (methyl trans-9-octadecanoate), and 13 (dodecanoic acid). The high positive correlation

between components 12 and 13 along PC 1 indicated that the volatile profile of lard indicated a higher proportion of components 12 and 13 [22], while the high negative score of 10 indicated that component 10 had a major influence on the differentiation of the ruminant animal body fats (mutton and beef).

Conclusion

In this work, the electronic nose was used as a new potential rapid technique to analyze the aroma of various animal's body fats. The aroma fingerprints of four different animal fats and lard containing chicken fat were sufficiently specific to differentiate these fats based on their aroma composition. With principal component analysis (PCA), pure lard, pure chicken fats, beef fats, mutton fats, and adulterated samples were discriminated from each other. This work clearly shows the potential of the electronic nose as a rapid aroma profiling technique. Future works on the optimization of the experimental conditions need to be carried out. This study also demonstrated that the method developed has the potential for practical implementation in *Halal* authentication.

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