

Detection of lard adulteration in RBD palm olein using an electronic nose

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Abstract

The use of surface acoustic wave (SAW) sensing electronic nose (zNose™) for detection of lard as an adulterant in refined, bleached, deodorized (RBD) palm olein was investigated. Mixing of animal fats, especially lard in any form in food products, is a cause of concern for certain religions. RBD palm olein spiked with lard at levels ranging from 1% to 20% (w/w) was analyzed. The zNose™ produced a two-dimensional olfactory image called VaporPrint™, which could be used for immediate detection (qualitatively) of lard substances in sample admixtures. Lard adulteration could be determined by a few distinct peaks in the zNose™ chromatogram. The best relationship between percentage of lard in adulterated RBD palm olein and SAW detector response was observed in adulterant peak E ($R^2 = 0.906$). Pearson's correlation coefficient (r) was calculated using this parameter. An ideal correlation was observed between the zNose™ data and other chemical tests ($r > 0.90$).

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1. Introduction

The major edible animal fats are lard and tallow. The Code of Federations Regulations of the United States (1994) defines lard as the fat rendered from clean and sound edible swine tissues. Lard is reasonably free from blood and should not include stomachs, livers, spleens, kidneys and brains, or settlings and skimming. Animal fats have traditionally been used for deep frying of many types of foods. Meat fats have a good stability and are economical. The flavours imparted by the meat fats have been considered desirable for some foods (Love, 1996).

However, there is a continued trend for consumers to prefer food products prepared from vegetable oils and avoid those prepared from animal fats (Orthofer,

1996). From a nutritional point of view, animal fats are rejected as they contain a high proportion of saturated fatty acids. Diets rich in lard are known to cause hypercholesterolemia, aortic lesions, aortoiliac atherosclerosis, coronary heart disease, and multiple sclerosis (Imaki et al., 1989; Serpcic, Measros, Materljan, & Sepic-Grahovac, 1993; Thompson, Pyke, Scott, Thompson, & Wood, 1993). Lard is also associated with the risk of breast, pancreas, and colon cancers (Cohen & Chan, 1982; Reddy, 1992) and it initiates and promotes carcinogenesis (Rogers, 1983).

The Islamic and Orthodox Jewish religions prohibit the consumption of both pork and lard in any products (Marikkar, Ghazali, Che Man, & Lai, 2002a). In view of the risk of diseases associated with pork and lard and the restrictions on their consumption by some religions, a reliable method is required for the detection of lard in its various forms. Authenticity covers many aspects,

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including adulteration, mislabelling, characterization and misleading origin. Lard or industrially-modified lard could be effectively blended with other vegetable oils to produce shortenings, margarines and other speciality food oils (Marikkar, Lai, Ghazali, & Che Man, 2002b).

Application and development of new methods to check lard adulteration in fats, oils and fat-based products are of paramount importance in order to protect consumer's rights and food industries. There has been a great deal of scientific investigation of adulteration in fats and oils. Most of the current work on edible oil adulteration is based on chromatographic analysis (Aparicio, 2000). The Codex Alimentarius Specification for Fats and Oils, which lists fatty acid ranges for various fats and oils, has become the international basis for checking adulteration and purity (Abu-Hadeed & Kotb, 1988; Rossell, King, & Downes, 1983). Since there are too many adulterants, comparing the fatty acid composition may not be suitable for authentication purposes.

It is noteworthy that the compositional distributions of phytosterols and non-saponifiable compounds in certain vegetable oils are often used as markers for the assessment of adulterated oils (Gordon & Griffith, 1992; Woodbury & Evershed, 1995; Woodbury, Evershed, & Rossell, 1998). However, extractions of these markers are tedious. As fatty acids are distributed on glycerol molecules according to certain position-specific patterns, triacylglycerols are considered as fingerprints of natural oils. A combination of chemical, physical and/or chromatographic methods (Flor, 1989) has been used to determine the triacylglycerol composition of oils as a means for detecting possible adulteration.

Many workers have used differential scanning calorimetry (DSC) to deal with adulteration problems associated with edible fats and oils (Marikkar et al., 2002b). Lambelet and Ganguli (1983) reported that ghee adulterated with animal fat, such as lard and tallow, could be detected by DSC. Kowalski (1989) demonstrated the use of DSC to differentiate lard from lard contaminated with cow tallow.

The electronic nose made its appearance in the market almost a decade ago. It is preferred to routine laboratory analysis because it is rapid, simple and easy-to-handle (Biswas, Heindselmen, Wohltjen, & Staff, 2004). In previous works, the zNose™ was shown to be able to discriminate different types of vegetable oils, as well as to monitor the storage stability of RBD palm olein (Gan, Che Man, Tan, NorAini, & Nazimah, 2005a; Gan, Tan, Che Man, NorAini, & Nazimah, 2005b). The potential use of the electronic nose for detection of lard in RBD palm olein was investigated in this work. The objective of this study was to develop a rapid method for detection of lard adulteration in RBD palm olein using the zNose™.

2. Materials and methods

2.1. Materials

Refined, bleached, deodorized (RBD) palm olein was purchased from a local grocery. Lard was extracted from adipose tissues of pigs collected from a local slaughterhouse according to the method previously reported (Marikkar, Ghazali, Che Man, & Lai, 2001). All chemicals and solvents used were of analytical grade unless otherwise specified.

2.2. Blend preparations

RBD palm olein and lard were mixed in proportions ranging from 1% to 10% of animal fat, in 1% increments (w/w) and from 10% to 20% animal fat, in 5% increments (w/w). Twelve RBD palm olein and lard blends (mass ratio of RBD palm olein to lard) were prepared: 99:1, 98:2, 97:3, 96:4, 95:5, 96:4,95:5, 94:6, 93:7, 92:8, 91:9, 90:10, 85:15, 80:20 (w/w).

2.3. Chemical analyses

The chemical analyses, namely free fatty acid content (FFA), peroxide value (PV), *p*-anisidine value (AV), and iodine value (IV) were carried out by means of AOCS official methods (AOCS Official Methods Ca 5a-40, Cd 8-53, Cd 18-90, and Cd 1b-87, respectively) (AOCS, 1996).

The fatty acid composition of the samples was assayed by gas chromatography (GC) (Hewlett–Packard model 5890 instrument, Palo Alto, CA). Samples were transesterified to convert the fatty acids into relatively volatile methyl ester derivatives (PORIM, 1995). The details of the GC analysis were reported elsewhere (Gan et al., 2005a, 2005b).

2.4. The electronic nose apparatus

A zNose™ (4100 vapour analysis system, Electronic Sensor Technology, Newbury Park, USA) was used throughout this study. Details of this instrument were reported in our previous papers (Gan et al., 2005a, 2005b).

2.5. Electronic nose analysis

Ten grammes of each oil sample was weighed into a septa-sealed screw-cap bottle. After a headspace generation time of 3 min at 60 °C (in a water-bath), the sample's vapour was introduced into the electronic nose. Each sample was measured in triplicate, followed by blanks, and *n*-alkane runs, to ensure quality of measurement. The flow-rate of purified helium was fixed at 30.0 (mL/min), sampling time of 5 s, and tem-

perature programmed from 40 to 160 °C, at a rate of 5 °C/sec.

2.6. Statistical analysis

All measurements were duplicated unless otherwise specified. The results were expressed as means and standard deviations of two replications. All data were subjected to analysis of variance using the SAS Statistical Computer Package Version 6.12 (SAS Institute, Inc., 1989). Duncan's multiple range test was used to compare differences among means. Significance was defined at $P < 0.05$. Electronic nose data were further analysed to obtain the trend line equation as well as the R^2 value using the Microsoft excel software. Pearson's correlation coefficient (r) between the electronic nose method and other chemical tests was calculated using the Microsoft excel software.

3. Results and discussion

3.1. Chemical analyses

Table 1 shows the changes in the chemical properties of RBD palm olein due to adulteration with lard. IV measures the degree of unsaturation of oil. From Table 1, IV increased progressively with the percentage of lard added, indicating that the sample has become more and more unsaturated. This result agreed with the GC analysis which indicated that the lard had a higher degree of unsaturated fatty acid than RBD palm olein.

PV and AV are measurements of the level of oxidation occurring in oil, which is determined by the primary oxidation and secondary oxidation, respectively. FFA content measures the degree of hydrolytic rancidity. All three of these quality parameters increased with

the increasing amount of lard spiked (Table 1). The primary reason for this trend was related to the nature of the lard sample used, which was a crude oil. Besides, the content of natural antioxidants, such as tocopherol, in animal fat was generally much lower than in vegetable oil (Love, 1996). As the level of unsaturated fatty acids is quite high in the lard sample, it is expected that lard would be more susceptible to oxidation than RBD palm olein.

The fatty acid compositions of RBD palm olein and lard are shown in Table 2. RBD palm olein was found to contain mainly C16:0, C18:0, C18:1 and C18:2, with C16:0 being the predominant fatty acid. On the other hand, the prevalent fatty acids of lard were C16:0, C18:0, C18:1 and C18:2, with C18:1 being predominant. Moreover, lard showed the presence of odd chain fatty acids, namely C15:0, C17:0 and C17:1. These fatty acids are absent in vegetable oils. On the basis of this, it was possible to detect the presence of animal fat in vegetable oils (Krishnamurthy, 1993).

From Table 2, the saturated to unsaturated fatty acid ratio of RBD palm olein was 1.05 while lard was 0.69. The ratios agreed with the value stated in the literature (Lin, 2002; Love, 1996). The fatty acid composition of animal fat may be influenced by several factors, notably diet. As shown in a study done by Monahan, Buchley, Morrissey, Lynch, and Gray (1992), non-ruminants readily incorporate the unsaturated fatty acid from the diet into depot fat. This demonstrates the effect of feeding unsaturated oils in increasing the unsaturation of the lard.

Changes in FA composition of RBD palm olein samples adulterated with lard are summarized in Table 3. Comparison of fatty acid composition between RBD palm olein and the adulterated RBD palm olein revealed that there was a slow decrease in the amounts of C16:0 and C18:1, while C18:2 increased gradually with the

Table 1
Changes of quality parameters of RBD palm olein adulterated with different percentages of lard^a

Lard (%)	Iodine value (g of I ₂ /100 g oil)	Peroxide value (meq hydroperoxide/kg oil)	Anisidine value	Free fatty acid content (%)
0	56.6 ± 0.15 ^d	6.67 ± 1.15 ^f	1.41 ± 0.39 ^g	0.10 ± 0.00 ^f
1	57.1 ± 0.45 ^{cd}	7.33 ± 1.52 ^{ef}	1.60 ± 0.14 ^{fg}	0.10 ± 0.00 ^f
2	57.2 ± 0.09 ^{cd}	8.32 ± 0.58 ^{def}	2.04 ± 0.34 ^{ef}	0.10 ± 0.00 ^f
3	57.3 ± 0.37 ^{cd}	9.32 ± 1.53 ^{de}	2.21 ± 0.44 ^{de}	0.11 ± 0.01 ^{ef}
4	57.4 ± 0.12 ^{cd}	9.99 ± 1.01 ^d	2.38 ± 0.20 ^{de}	0.11 ± 0.01 ^{de}
5	57.4 ± 0.61 ^{cd}	10.3 ± 0.57 ^{cd}	2.56 ± 0.21 ^{cde}	0.11 ± 0.01 ^{de}
6	57.6 ± 0.07 ^c	12.3 ± 0.58 ^{bc}	2.65 ± 0.20 ^{bcd}	0.12 ± 0.00 ^{cd}
7	57.7 ± 0.99 ^c	12.7 ± 0.57 ^b	2.80 ± 0.23 ^{bcd}	0.12 ± 0.01 ^c
8	59.1 ± 0.30 ^b	13.0 ± 0.01 ^b	3.10 ± 0.27 ^{abc}	0.13 ± 0.01 ^{bc}
9	59.4 ± 0.52 ^b	13.0 ± 2.65 ^b	3.15 ± 0.58 ^{abc}	0.13 ± 0.01 ^{ab}
10	59.9 ± 0.73 ^b	14.0 ± 0.01 ^b	3.21 ± 0.25 ^{ab}	0.14 ± 0.01 ^a
15	60.8 ± 0.11 ^a	18.5 ± 0.73 ^a	3.40 ± 0.28 ^a	0.14 ± 0.00 ^a
20	61.1 ± 0.51 ^a	19.2 ± 0.72 ^a	3.56 ± 0.77 ^a	0.14 ± 0.00 ^a

Means within each column with different superscripts are significantly ($P < 0.05$) different.

Abbreviation: RBD, refined, bleached, and deodorized.

^a Each value in the table represents the means ± SD of four analyses from two replications.

Table 2
Fatty acid composition of RBD palm olein and lard^a

Fatty acid	PO (%)	Lard (%)
12:0	0.60	0.15
14:0	1.62	1.70
15:0	–	0.17
16:0	45.6	26.9
16:1	0.21	1.71
17:0	–	0.65
17:1	–	0.32
18:0	3.29	10.9
18:1	38.8	31.8
18:2	9.57	23.1
18:3	0.19	1.41
20:0	0.12	0.24
20:1	–	0.59
20:2	–	0.32

Abbreviations: PO, RBD palm olein; C12:0, lauric acid; C14:0, myristic acid; C15:0, pentadecenoic acid; C16:0, palmitic acid; C16:1, palmitoleic acid; C17:0, margaric acid; C17:1, margaroleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; C20:0, arachidic acid; C20:1, gadoleic acid; C20:2, ficosadienoic acid. See Table 1 for other abbreviations.

^a Each value in the Table represents the mean of triplicate analyses.

concentration of adulterant. However, the changes in fatty acid composition could not be used as a proof of the occurrence of lard in RBD palm olein since similar changes were also observed for RBD palm oil adulterated with other animal fats, such as chicken fat (Marikkar et al., 2002b).

3.2. Electronic nose analysis

The chromatogram from the electronic nose is a graphical display of the derivative of the frequency change versus time. The peak area was correlated with the compound concentration and was expressed in counts (cts). The zNose™ chromatograms of RBD palm olein and lard were characteristically different. RBD

palm olein contained less volatile compounds than lard. This was due to the different natures of the two oils and processing method. The palm olein used was refined, bleached and deodorised but the lard was a crude oil, with a strong and characteristic swine odour.

Table 4 shows the changes in the peak area of the adulterant peaks with the increase of the concentration of lard. The results show that the peak area of these components significantly ($P < 0.05$) increased with the increase of lard in the samples. There was a significant ($P < 0.05$) difference between detector counts for 0% lard and 1% lard, particularly in adulterant peak C, showing the possibility of detection of lard at the 1% limit.

A unique approach of the electronic nose was to use two-dimensional olfactory images, called VaporPrint™. It is produced by a polar plot of the zNose™ chromatogram, using retention time as the angular variable and the SAW detector response as the radial variable. VaporPrint™ provides a high resolution image which translates the olfactory response to a visual response. A gallery of VaporPrint™ for RBD palm olein adulterated with different percentages of lard is shown in Fig. 1

As the RBD palm olein was adulterated with lard (1%), the VaporPrint™ showed subtle differences in the aroma pattern as compared to the unadulterated (0%) sample (Fig. 1). The VaporPrint™ showed that the changes in the strength of the volatile compounds correlated well with the increase of adulterant in RBD palm olein. In this case, the adulterated RBD palm olein was found to produce distinct analyte peaks in the range of 0.7–4.5 s (Fig. 1). The peak area changes were hardly seen for samples containing 1% lard. Therefore, this method may not be applicable for adulteration levels $\leq 1\%$. These peaks dramatically increased in 3% lard, which was obviously an exception to the normal RBD palm olein pattern. The primary usage of VaporPrint™ was to provide the fingerprint of odour concentration and characteristic shape at a glance. Qualitative identifi-

Table 3
Fatty acid composition of RBD palm olein adulterated with lard^a

Lard (%)	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
1	0.65	1.43	43.4	.	3.51	41.0	10.0	.	.
2	0.55	1.62	44.1	0.36	3.69	38.5	10.2	0.33	0.37
3	0.48	1.47	42.7	0.33	4.11	39.9	10.5	0.20	0.38
4	0.58	1.65	45.0	0.38	3.58	38.2	9.8	0.26	0.45
5	0.52	1.51	43.7	0.26	3.71	39.6	10.1	0.26	0.34
6	0.43	1.37	41.9	0.30	4.12	40.6	10.7	0.22	0.39
7	0.45	1.46	42.1	0.31	4.16	40.3	10.7	0.22	0.40
8	0.46	1.47	42.0	0.41	4.11	40.2	10.6	0.31	0.54
9	0.38	1.45	40.5	0.50	4.99	40.0	11.5	0.21	0.49
10	0.39	1.41	41.7	0.33	4.29	40.4	10.8	0.23	0.41
15	0.42	1.49	42.5	0.38	4.29	39.5	10.8	0.27	0.42
20	0.39	1.48	40.7	0.51	4.99	39.7	11.5	0.26	0.50

^a Each value in table represents the means of triplicate analyses. See Tables 1 and 2 for other abbreviations.

Table 4
Electronic nose results of RBD palm olein adulterated with different percentages of lard^a

Lard (%)	Compound									
	A	B	C	D	E	F	G	H	I	J
0	18.00 ± 3.56 ^{bcd}	18.00 ± 1.00 ^d	11.75 ± 1.71 ^g	11.25 ± 2.06 ^e	10.6 ± 3.21 ^e	58.20 ± 7.05 ^d	88.00 ± 21.35 ^b	6.00 ± 1.41 ^f	37.00 ± 4.00 ^{cde}	48.00 ± 6.82 ^{def}
1	21.00 ± 4.24 ^d	30.67 ± 4.93 ^{abc}	24.29 ± 6.40 ^{ef}	20.29 ± 4.50 ^{cde}	13.67 ± 4.03 ^e	127.33 ± 14.21 ^a	138.80 ± 25.61 ^a	16.00 ± 2.00 ^{def}	30.67 ± 9.29 ^e	43.50 ± 12.55 ^f
2	41.50 ± 3.70 ^{abc}	30.67 ± 9.26 ^{abc}	25.00 ± 4.00 ^{ef}	29.50 ± 6.66 ^{abc}	27.00 ± 6.32 ^{cd}	81.50 ± 9.25 ^{bc}	63.33 ± 11.02 ^d	10.00 ± 1.41 ^{ef}	33.33 ± 4.73 ^{de}	36.80 ± 9.28 ^f
3	43.50 ± 13.41 ^{ab}	25.20 ± 4.44 ^{bcd}	27.00 ± 5.34 ^{def}	17.20 ± 3.56 ^{de}	14.83 ± 3.06 ^{de}	76.57 ± 14.00 ^{cd}	77.00 ± 14.11 ^{bc}	21.00 ± 0.00 ^{cde}	41.20 ± 6.10 ^{bcd}	47.14 ± 7.90 ^{ef}
4	30.00 ± 0.00 ^{abcd}	31.40 ± 5.03 ^{abc}	23.20 ± 4.32 ^f	18.50 ± 3.54 ^{de}	20.60 ± 3.78 ^{de}	72.83 ± 16.57 ^{cd}	49.00 ± 8.46 ^d	31.00 ± 1.00 ^{abc}	45.50 ± 11.68 ^{abc}	54.50 ± 15.69 ^{cdef}
5	27.00 ± 0.11 ^{cd}	33.17 ± 4.67 ^{abc}	30.67 ± 7.61 ^{cdef}	22.75 ± 6.50 ^{bcd}	43.43 ± 10.67 ^b	100.13 ± 25.10 ^b	132.67 ± 27.21 ^a	36.75 ± 11.15 ^{ab}	31.57 ± 9.09 ^e	66.63 ± 15.09 ^{bc}
6	44.33 ± 8.02 ^{ab}	34.67 ± 7.74 ^{abc}	32.80 ± 3.27 ^{bcd}	27.50 ± 2.12 ^{abcd}	43.83 ± 7.52 ^b	80.50 ± 13.35 ^{bc}	54.60 ± 13.03 ^{cd}	25.60 ± 7.89 ^{bcd}	44.17 ± 8.91 ^{bcd}	45.00 ± 10.12 ^{ef}
7	40.00 ± 4.58 ^{abc}	34.50 ± 7.50 ^{abc}	29.60 ± 5.68 ^{abcd}	26.83 ± 5.04 ^{bcd}	35.86 ± 10.33 ^{bc}	77.00 ± 17.09 ^{cd}	40.00 ± 10.95 ^d	23.14 ± 6.67 ^{cd}	37.17 ± 8.01 ^{cde}	62.57 ± 16.98 ^{abc}
8	40.50 ± 10.28 ^{abc}	36.17 ± 10.78 ^{ab}	31.60 ± 8.56 ^{bcd}	19.83 ± 5.38 ^{cde}	43.71 ± 9.78 ^b	82.43 ± 17.60 ^{bc}	38.00 ± 4.97 ^d	26.17 ± 5.67 ^{bcd}	38.14 ± 7.56 ^{cde}	64.83 ± 13.53 ^{bcd}
9	42.00 ± 9.90 ^{abc}	42.14 ± 11.91 ^a	44.83 ± 5.31 ^a	37.40 ± 10.21 ^a	45.86 ± 10.82 ^b	72.71 ± 9.10 ^{cd}	38.80 ± 9.76 ^d	22.80 ± 4.32 ^{dc}	50.17 ± 9.15 ^{ab}	70.14 ± 15.45 ^{cd}
10	29.33 ± 4.51 ^{abcd}	40.13 ± 11.71 ^a	36.14 ± 6.34 ^{abcd}	31.88 ± 9.70 ^{ab}	62.90 ± 18.81 ^a	98.50 ± 27.27 ^b	119.57 ± 24.53 ^a	26.38 ± 5.85 ^{bcd}	38.90 ± 7.85 ^{bcd}	79.00 ± 19.73 ^{ab}
15	45.33 ± 8.24 ^a	35.43 ± 8.77 ^{abc}	39.00 ± 10.43 ^{abc}	37.50 ± 9.61 ^a	73.57 ± 13.38 ^a	85.00 ± 11.69 ^{bc}	45.80 ± 11.08 ^d	39.00 ± 8.43 ^a	55.43 ± 5.32 ^{de}	92.00 ± 7.35 ^a
20	26.67 ± 4.73 ^{cd}	24.17 ± 5.49 ^{cd}	40.50 ± 7.14 ^{ab}	21.00 ± 4.47 ^{cde}	75.00 ± 9.38 ^a	73.75 ± 10.28 ^{cd}	45.86 ± 12.43 ^d	31.50 ± 8.96 ^{abc}	44.00 ± 8.89 ^{bcd}	77.38 ± 19.26 ^{ab}

Means within each column with different superscripts are significantly ($P < 0.05$) different.

^a Each value in the table represents the means ± SD of six analyses from two replications.

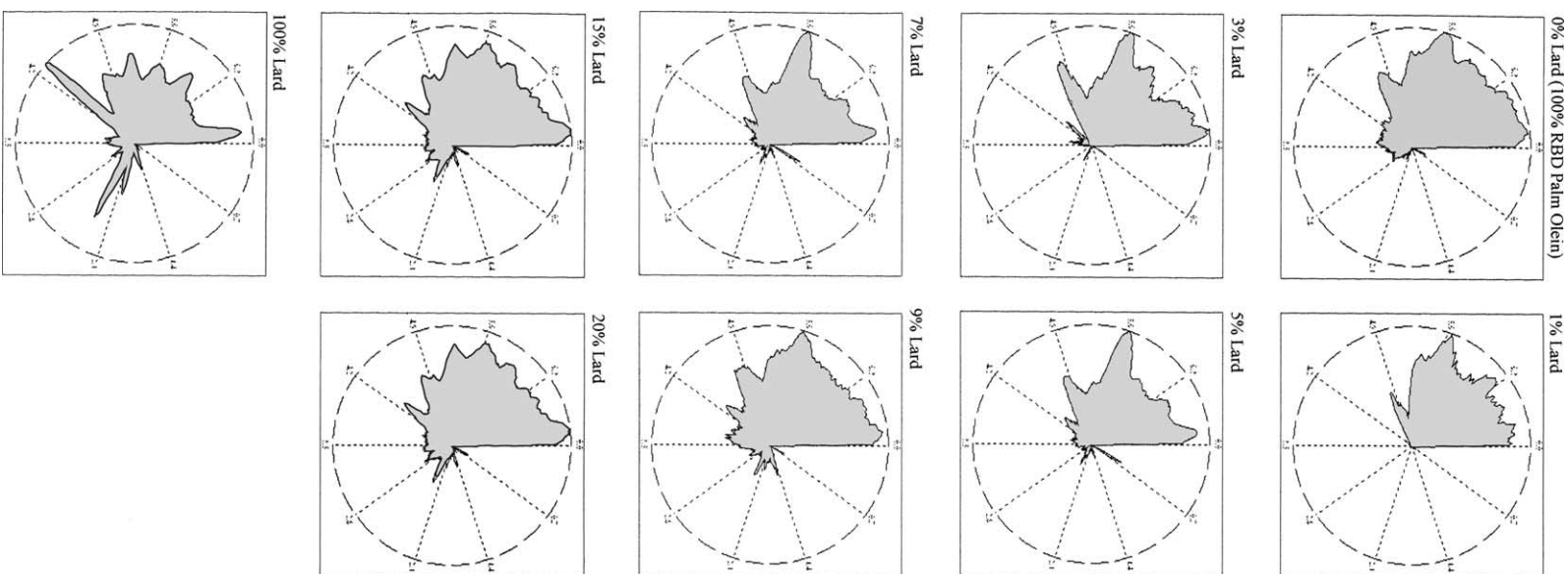


Fig. 1. VaporPrint™ of RBD palm olein adulterated with different percentages of lard.
cation and rapid detection of RBD palm olein samples adulterated with lard could be achieved through monitoring VaporPrint™.

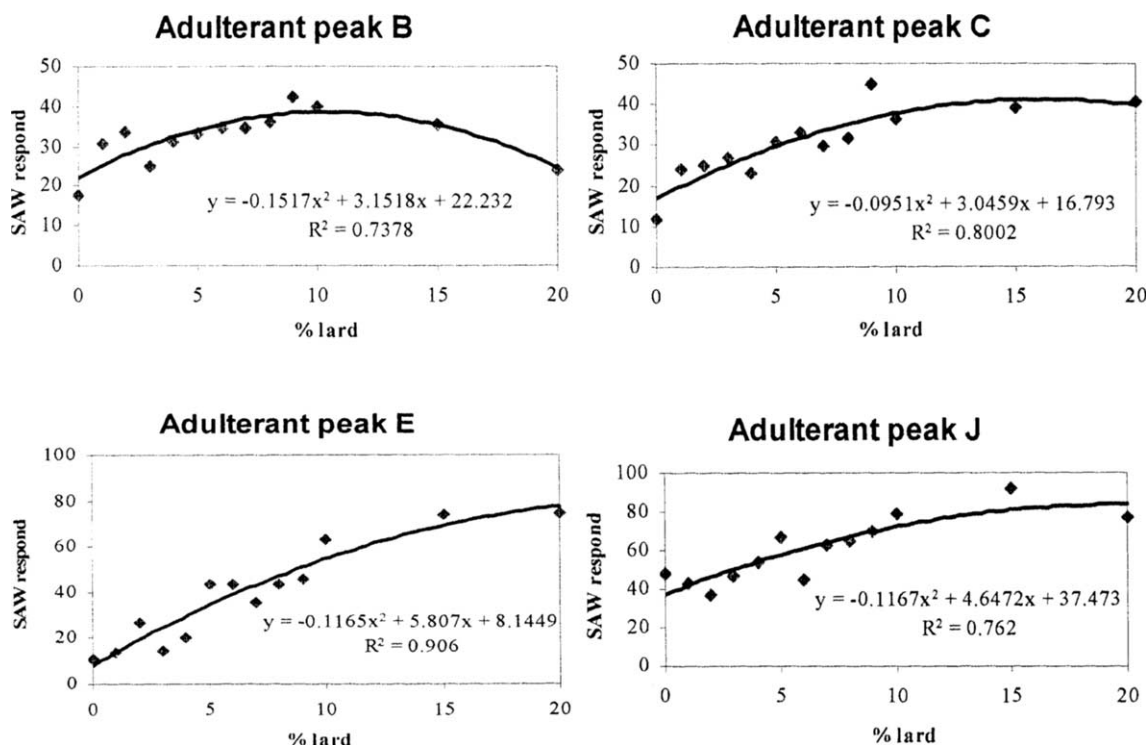


Fig. 2. SAW response versus different percentages of lard.

Furthermore, the adulterant peaks were plotted versus the lard percentage (Fig. 2). The data were fitted to a curve using the linear regression technique and the coefficient of determination (R^2) was calculated. The results showed that the relationship between the amount of lard added and the parameters was not linear but curvilinear. A second order polynomial fit appeared to present a good relationship. Fig. 2 shows plots of adulterant peaks B, C, E, and J, which have good correlation between percentage of lard with the detector response. The best relationship (highest R^2) occurred between percent of lard and adulterant peak E ($R^2 = 0.906$). To investigate the correlation between the electronic nose method and the chemical test, Pearson's correlation coefficients (r) were separately determined between peak E and each of IV/PV/AV/FFA (Table 5). Good correlations were observed between the electronic nose response and each of the chemical test ($r > 0.90$).

Table 5

Pearson correlation coefficients (r)

	Adulterant peak E
Peroxide value	0.920
FFA content	0.994
Iodine value	0.912
Anisidine value	0.916

4. Conclusion

Although GC and chemical analyses were capable of providing the fine details of fatty acid composition and quality parameters of adulterated RBD palm olein, they are of very little use for qualitative identification and quantitative determination of adulterants such as lard in RBD palm olein. On the other hand, the use of the zNose™ offered a more sensitive method for lard detection in RBD palm olein. It has been shown that detection of RBD palm olein samples adulterated with lard (as low as 1%) using the zNose™ was possible. Another useful advantage of the electronic nose is that it does not require any sample pre-treatment or chemicals for analysis. In addition, accuracy and speed of the electronic nose method for the detection and determination of lard in RBD palm olein made it ideal for quality control purposes.

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